(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2012 (01.03.2012)

PCT

(10) International Publication Number
WO 2012/027546 A2

(51) International Patent Classification:
C12N 5/074 (2010.01)

(21) International Application Number:
PCT/US2011/049097

(22) International Filing Date:
25 August 2011 (25.08.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/377,556 27 August 2010 (27.08.2010) US
61/479,492 27 April 2011 (27.04.2011) US

(71) Applicant *(for all designated States except US)*: **TEMPERO PHARMACEUTICALS, INC.** [US/US]; 200 Technology Square, Suite 602, Cambridge, MA 02139 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **SUNDRUD, Mark, S.** [US/US]; 200 Technology Square, Cambridge, MA 02139 (US).

(74) Agents: **LISSY, Natalie, A** et al.; Glaxosmithkline, Corporate Intellectual Property, Uw2220, 709 Swedeland Road, P.o. Box 1539, King Of Prussia, PA 19406-0939 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2012/027546 A2

(54) Title: POISED TH17 CELLS

(57) Abstract: Isolated poised Thl7 memory T cells, and methods of preparing and using the same are described.

POISED TH17 CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/377,556, filed on August 27, 2010 and U.S. Application Serial No. 61/479,492, filed on April 27, 2011. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

BACKGROUND

Effector T helper 17 (Th17) cells are a subset of T helper cells that produce interleukin-17 (IL-17). They are considered developmentally distinct from Th1 and Th2 cells. Excessive amounts of effector Th17 cells or increased cytokine production by these cells is thought to play a prominent role in autoimmune disease. The cells may play a role in inflammation and tissue injury. Effector Th17 cells also function in anti-microbial immunity at epithelial/mucosal barriers.

SUMMARY

The development of interleukin (IL)-17-secreting effector Th17 cells from naïve precursor cells is implicated in autoimmune pathogenesis. Strategies to block the development of these Th17 effector cells are predicted to have broad efficacy against inflammatory and autoimmune disorders. Immune pathology *in vivo* is largely controlled by established, antigen-specific effector and memory T cells; rather than involving further differentiation of naïve T cells. Novel "poised" Th17 cell subsets are memory T cells that can convert into pro-inflammatory, IL-17 producing effector Th17 cells. Poised Th17 cells have been identified in, and isolated from, the peripheral blood of healthy adult human donors. Poised Th17 cells can also be isolated from secondary lymphoid organs of mice. Poised Th17 cells are IL-17- (e.g., they do not produce or secrete IL-17 (i.e., IL-17A)), and are CD25- (they do not express CD25), (i.e., they are not T regulatory (Treg) cells) and CCR6+ (they express CCR6 (Chemokine (C-C motif) receptor 6)). Effector Th17 cells are also CCR6+, but these cells are distinguished from poised Th17 cells at steady-state based on their active secretion of IL-17 (i.e., IL-17A). Poised Th17 cells, e.g., under conditions that promote conversion to effector Th17 cells, can be used to screen for compounds that

regulate the conversion of poised Th17 cells into effector Th17 cells. *In vitro* culture of poised Th17 cells with IL-2, IL-7, or IL-15 (e.g., a common gamma chain (γc or gc)-utilizing cytokine) is necessary to drive their conversion into effector Th17 cells, and can (e.g., concomitantly) increase expression the Treg-signature transcription factor, FOXP3.

In some aspects, the disclosure is drawn to an isolated human poised Th17 cell, wherein the cell is CD4+ (expresses CD4), CD25- (does not express CD25), CD45RO+ (expresses CD45RO), CCR6+ (expresses CCR6 (Chemokine (C-C motif) receptor 6)) and IL-17- (does not express interleukin (IL)-17).

In some embodiments, the cell is CD4+ CD25- CD45RO+ CCR6+ IL-17- CRTH2-.

In some embodiments, the cell is CRTH2+ (expresses CRTH2).

In some embodiments, the cell is CRTH2- (does not express CRTH2).

In some embodiments, the cell is CXCR3+ (expresses CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cell is CXCR3- (does not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cell is IFN-g+ (expresses IFN-g (interferon-gamma; IFN-γ)).

In some embodiments, the cell is IFN-g- (does not express IFN-g).

In some embodiments, the cell is CD161+ (expresses CD161).

In some embodiments, the cell is CD161- (does not express CD161).

In some aspects, the disclosure is drawn to an isolated population of human poised Th17 cells, wherein the cells are CD4+ (express CD4), CD25- (do not express CD25), CD45RO+ (express CD45RO), CCR6+ (express CCR6 (Chemokine (C-C motif) receptor 6)) and IL-17- (do not express interleukin (IL)-17).

In some embodiments, the cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- CRTH2-.

In some embodiments, the cells are CRTH2+ (express CRTH2).

In some embodiments, the cells are CRTH2- (do not express CRTH2).

In some embodiments, the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are IFN-g+ (express IFN-g (interferon-gamma; IFN-γ)).

In some embodiments, the cells are IFN-g- (do not express IFN-g).

In some embodiments, the cells are CD161+ (express CD161).

In some embodiments, the cells are CD161- (do not express CD161).

In some aspects, the disclosure is drawn to a method of isolating human poised Th17 cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ CD25- CCR6+ and IL-17- cells from a mixture of cells containing the same (e.g., peripheral blood mononuclear cells (PBMCs) or a buffy coat);

thereby isolating human poised Th17 cells, wherein the poised Th17 cells are CD4+ CD45RO+ CD25- CCR6+ and IL-17-.

In some embodiments, the method further comprises isolating or having isolated CRTH2+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CRTH2- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CXCR3+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CXCR3- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated IFN-g+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated IFN-g- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CD161+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CD161- cells from the human poised Th17 cells.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some embodiments, the CD25- CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ CD45RO+ cells with antibodies that specifically bind CD25 and CCR6 (respectively) and gating and sorting CD25- CCR6+ cells).

In some aspects, the disclosure is drawn to a method of isolating human poised Th17 cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ cells from peripheral blood mononuclear cells (PBMCs);

isolating or having isolated CD25- CCR6+ cells from the isolated CD4+ CD45RO+ cells; and

isolating or having isolated IL-17- cells from the isolated CD25- CCR6+ cells;

thereby isolating human poised Th17 cells, wherein the poised Th17 cells are CD4+ CD45RO+ CD25- CCR6+ IL-17-.

In some embodiments, the PBMCs are obtained from a buffy coat.

In some embodiments, the method further comprises isolating or having isolated CRTH2+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CRTH2- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CXCR3+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CXCR3- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated IFN-g+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated IFN-g- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CD161+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CD161- cells from the human poised Th17 cells.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some embodiments, the CD25- CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ CD45RO+ cells with antibodies that specifically bind CD25, and CCR6 (respectively) and gating and sorting CD25- CCR6+ cells).

In some aspects, the disclosure is drawn to a method of isolating human poised Th17 cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ cells from peripheral blood mononuclear cells (PBMCs);

isolating or having isolated CD25- CRTH2- CCR6+ cells from the isolated CD4+ CD45RO+ cells; and

isolating or having isolated IL-17- cells from the isolated CD25- CRTH2- CCR6+ cells;

thereby isolating human poised Th17 cells, wherein the poised Th17 cells are CD4+ CD45RO+ CD25- CRTH2- CCR6+ IL-17-.

In some embodiments, the PBMCs are obtained from a buffy coat.

In some embodiments, the method further comprises isolating or having isolated CXCR3+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CXCR3- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated IFN-g+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated IFN-g- cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CD161+ cells from the human poised Th17 cells.

In some embodiments, the method further comprises isolating or having isolated CD161- cells from the human poised Th17 cells.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some embodiments, the CD25- CRTH2- CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ CD45RO+ cells with antibodies that specifically bind CD25, CRH2, and CCR6 (respectively) and gating and sorting CD25- CRTH2- CCR6+ cells).

In some aspects, the disclosure is drawn to a method of converting a human poised Th17 cell (e.g., an isolated population thereof) (e.g., a population of human poised Th17 cells obtained by a method described herein), wherein the poised Th17 cell is CD4+ CD25- CD45RO+ CCR6+ IL-17-, to a human effector Th17 cell, the method comprising:

culturing the human poised Th17 cell in a culture medium that comprises interleukin (IL)-2, IL-7, or IL-15, wherein the human effector Th17 cell is IL-17+.

In some embodiments, the method further comprises stimulating the cell through the T cell receptor (TCR), e.g., prior to culturing (e.g., with anti-CD3 and anti-CD28 stimulation, e.g., with anti CD3/CD28 beads).

In some embodiments, the culture medium comprises IL-2.

In some embodiments, the culture medium comprises IL-7.

In some embodiments, the culture medium comprises IL-15.

In some embodiments, the human poised Th17 cell is CRTH2+.

In some embodiments, the human poised Th17 cell is CRTH2-.

In some embodiments, the human poised Th17 cell is CXCR3+.

In some embodiments, the human poised Th17 cell is CXCR3-.

In some embodiments, the human poised Th17 cell is IFN-g+.

In some embodiments, the human poised Th17 cell is IFN-g-.

In some embodiments, the human poised Th17 cell is CD161+.

In some embodiments, the human poised Th17 cell is CD161-.

In some embodiments, the cell is cultured for at least 3 days. In some embodiments, the cell is cultured for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cell is cultured for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cell is cultured for 6 days. In some embodiments, the cell is cultured for 7 days. In some embodiments, the cell is cultured for 8 days.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) conversion of human poised Th17 cells (e.g., an isolated population thereof), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, to human effector Th17 cells, wherein the human Th17 effector cells are IL-17+, the method comprising:

incubating the human poised Th17 cells with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the human poised Th17 cells to human effector Th17 cells, e.g., as compared to a control. For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- CRTH2-.

In some embodiments, the cells are CRTH2+ (express CRTH2).

In some embodiments, the cells are CRTH2- (do not express CRTH2).

In some embodiments, the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are IFN-g+ (express IFN-g (interferon-gamma; IFN-γ)).

In some embodiments, the cells are IFN-g- (do not express IFN-g).

In some embodiments, the cells are CD161+ (express CD161).

In some embodiments, the cells are CD161- (do not express CD161).

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) IL-17 expression, the method comprising:

incubating human poised Th17 cells (e.g., an isolated population thereof) with a test compound, wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- and wherein the incubating occurs in conditions that promote IL-17 expression by the cells in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the cells, e.g., as compared to a control. For example, the control can be the amount (e.g., percentage) of cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., percentage) of cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the cells under identical conditions.

In some embodiments, the cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- CRTH2-.

In some embodiments, the cells are CRTH2+ (express CRTH2).

In some embodiments, the cells are CRTH2- (do not express CRTH2).

In some embodiments, the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are IFN-g+ (express IFN-g (interferon-gamma; IFN-γ)).

In some embodiments, the cells are IFN-g- (do not express IFN-g).

In some embodiments, the cells are CD161+ (express CD161).

In some embodiments, the cells are CD161- (do not express CD161).

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human poised Th17 cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, and evaluating if the test compound modulates (e.g., increases or decreases) conversion of the human poised Th17 cells to human effector Th17 cells, wherein the human effector Th17 cells are IL-17+ (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- CRTH2-.

In some embodiments, the cells are CRTH2+ (express CRTH2).

In some embodiments, the cells are CRTH2- (do not express CRTH2).

In some embodiments, the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are IFN-g+ (express IFN-g (interferon-gamma; IFN-γ)).

In some embodiments, the cells are IFN-g- (do not express IFN-g).

In some embodiments, the cells are CD161+ (express CD161).

In some embodiments, the cells are CD161- (do not express CD161).

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to evaluating. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some

embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human poised Th17 cells (e.g., an isolated population thereof) with a test compound (e.g., *in vitro*) (e.g., in conditions that promote IL-17 expression by the cells in the absence of the test compound), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, and determining if the test compound modulates (e.g., increases or decreases) IL-17 expression by the cells (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., number or percentage) of cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the cells under identical conditions.

In some embodiments, the cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- CRTH2-.

In some embodiments, the cells are CRTH2+ (express CRTH2).

In some embodiments, the cells are CRTH2- (do not express CRTH2).

In some embodiments, the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

In some embodiments, the cells are IFN-g+ (express IFN-g (interferon-gamma; IFN-γ)).

In some embodiments, the cells are IFN-g- (do not express IFN-g).

In some embodiments, the cells are CD161+ (express CD161).

In some embodiments, the cells are CD161- (do not express CD161).

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to determining. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing by the cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing by the cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In addition to the isolation and use of human poised Th17 cells (as described herein), resting (i.e., not stimulated through TCR) CCR6+ memory T cells (CD4+ CD45RO+ CCR6+ CD25-) can be isolated and used in the methods described herein.

In some aspects, the disclosure is drawn to an isolated human CCR6+ memory T cell, wherein the cell is CD4+ (expresses CD4), CD25- (does not express CD25), CD45RO+ (expresses CD45RO), and CCR6+ (expresses CCR6 (Chemokine (C-C motif) receptor 6)).

In some aspects, the disclosure is drawn to an isolated population of human CCR6+ memory T cells, wherein the cells are CD4+ (express CD4), CD25- (do not express CD25), CD45RO+ (express CD45RO), and CCR6+ (express CCR6 (Chemokine (C-C motif) receptor 6)).

In some aspects, the disclosure is drawn to a method of isolating human CCR6+ memory T cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ CD25- CCR6+ cells from a mixture of cells containing the same (e.g., peripheral blood mononuclear cells (PBMCs) or a buffy coat);

thereby isolating human CCR6+ memory T cells, wherein the CCR6+ memory T cells are CD4+ CD45RO+ CD25- CCR6+.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some embodiments, the CD25- CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ CD45RO+ cells with antibodies that specifically bind CD25 and CCR6 (respectively) and gating and sorting CD25- CCR6+ cells).

In some aspects, the disclosure is drawn to a method of isolating human CCR6+ memory T cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ cells from peripheral blood mononuclear cells (PBMCs);

and

isolating or having isolated CD25- CCR6+ cells from the isolated CD4+ CD45RO+ cells;

thereby isolating human CCR6+ memory T cells, wherein the CCR6+ memory T cells are CD4+ CD45RO+ CD25- CCR6+.

In some embodiments, the PBMCs are obtained from a buffy coat.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some embodiments, the CD25- CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ CD45RO+ cells with antibodies that specifically bind CD25, and CCR6 (respectively) and gating and sorting CD25- CCR6+ cells).

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) conversion of human poised Th17 cells (e.g., an isolated population thereof), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, to human effector Th17 cells, wherein the human Th17 effector cells are IL-17+, and wherein the human poised Th17 cells are comprised in a population of human CCR6+ memory T cells, the method comprising:

incubating the human CCR6+ memory T cells with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the human poised Th17 cells to human effector Th17 cells, e.g., as compared to a control. For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells (i.e., in the population of CCR6+ memory T cells).

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells (i.e., in the population of CCR6+ memory T cells).

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17). (i.e., in the population of CCR6+ memory T cells)

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) IL-17 expression, the method comprising:

incubating human CCR6+ memory T cells with a test compound, wherein the human CCR6+ memory T cells are CD4+ CD25- CD45RO+ CCR6+, and wherein the incubating occurs in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human CCR6+ memory T cells in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the human CCR6+ memory T cells, e.g., as compared to a control. For example, the control can be the amount (e.g., percentage) of human CCR6+ memory T cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., percentage) of human CCR6+ memory T cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human CCR6+ memory T cells under identical conditions. Contained within the human CCR6+ memory T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing and/or secreting IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human CCR6+ memory T cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that are favorable for conversion of human poised Th17 cells comprised in the human CCR6+ memory T cells to human effector Th17 cells), wherein the human CCR6+ memory T cells are CD4+ CD25- CD45RO+ CCR6+, and evaluating if the test compound modulates (e.g., increases or decreases) conversion of the human poised Th17 cells to human effector Th17 cells, wherein the human effector Th17 cells are IL-17+ (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions. Contained within the human CCR6+ memory T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of converting to effector Th17 cells under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to evaluating. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound

antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human CCR6+ memory T cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human CCR6+ memory T cells in the absence of the test compound), wherein the human CCR6+ memory T cells are CD4+ CD25- CD45RO+ CCR6+, and determining if the test compound modulates (e.g., increases or decreases) IL-17 expression by the human CCR6+ memory T cells (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of human CCR6+ memory T cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., number or percentage) of human CCR6+ memory T cells secreting IL-17 in the absence of a

test compound under identical conditions, or the total amount of IL-17 secreted from the human CCR6+ memory T cells under identical conditions. Contained within the human CCR6+ memory T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to determining. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing by the cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing by the cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In addition to the isolation and use of human poised Th17 cells (as described herein), total memory T cells (CD4+ CD45RO+) can be isolated and used in the methods described herein.

In some aspects, the disclosure is drawn to an isolated population of human total memory T cells, wherein the cells are CD4+ (express CD4) and CD45RO+ (express CD45RO).

In some aspects, the disclosure is drawn to a method of isolating human total memory T cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ cells from a mixture of cells containing the same (e.g., peripheral blood mononuclear cells (PBMCs) or a buffy coat);

thereby isolating human total memory T cells, wherein the total memory T cells are CD4+ CD45RO+.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some aspects, the disclosure is drawn to a method of isolating human total memory T cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ cells from peripheral blood mononuclear cells (PBMCs);

thereby isolating human total memory T cells, wherein the total memory T cells are CD4+ CD45RO+.

In some embodiments, the PBMCs are obtained from a buffy coat.

In some embodiments, the CD4+ CD45RO+ cells are isolated by magnetic separation.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) conversion of human poised Th17 cells (e.g., an isolated population thereof), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, to human effector Th17 cells, wherein the human Th17 effector cells are IL-17+, and wherein the human poised Th17 cells are comprised in a population of human total memory T cells, the method comprising:

incubating the human total memory T cells with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the human poised Th17 cells to human effector Th17 cells, e.g., as compared to a control. For example, the

control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells (i.e., in the population of total memory T cells).

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells (i.e., in the population of total memory T cells).

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17) (i.e., in the population of total memory T cells).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) IL-17 expression, the method comprising:

incubating human total memory T cells with a test compound, wherein the human total memory T cells are CD4+ CD45RO+, and wherein the incubating occurs in conditions

that promote IL-17 expression by human poised Th17 cells comprised in the human total memory T cells in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the human total memory T cells, e.g., as compared to a control. For example, the control can be the amount (e.g., percentage) of human total memory T cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., percentage) of human total memory T cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human total memory T cells under identical conditions. Contained within the human total memory T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing and/or secreting IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human total memory T cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that are favorable for conversion of human poised Th17 cells comprised in the human total memory T cells to human effector Th17 cells), wherein the human total memory T cells are CD4+ CD45RO+, and evaluating if the test compound modulates (e.g., increases or decreases) conversion of the human poised Th17 cells to human effector Th17 cells, wherein the human effector Th17 cells are IL-17+ (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions. Contained within the human total memory T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of converting to effector Th17 cells under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to evaluating. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human total memory T cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human total memory T cells in the absence of the test compound), wherein the human total memory T cells are CD4+ CD45RO+, and determining if the test compound modulates (e.g., increases or decreases) IL-17 expression by the human total memory T cells (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of human total memory T cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., number or percentage) of human total memory T cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human total memory T cells under identical conditions. Contained within the human total memory T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to determining. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing by the cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing by the cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In addition to the isolation and use of human poised Th17 cells (as described herein), human CD4+ T cells can be isolated and used in the methods described herein.

In some aspects, the disclosure is drawn to an isolated population of human cells, wherein the cells are CD4+ (express CD4).

In some aspects, the disclosure is drawn to a method of isolating human CD4+ T cells, the method comprising:

isolating or having isolated CD4+ cells from a mixture of cells containing the same (e.g., peripheral blood mononuclear cells (PBMCs) or a buffy coat);

thereby isolating human CD4+ T cells.

In some embodiments, the CD4+ cells are isolated by magnetic separation.

In some aspects, the disclosure is drawn to a method of isolating human CD4+ T cells, the method comprising:

isolating or having isolated CD4+ cells from peripheral blood mononuclear cells (PBMCs);

thereby isolating CD4+ T cells.

In some embodiments, the PBMCs are obtained from a buffy coat.

In some embodiments, the CD4+ cells are isolated by magnetic separation.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) conversion of human poised Th17 cells (e.g., an isolated population thereof), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, to human effector Th17 cells, wherein the human Th17 effector cells are IL-17+, and wherein the

human poised Th17 cells are comprised in a population of human CD4+ T cells, the method comprising:

incubating the human CD4+ T cells with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the human poised Th17 cells to human effector Th17 cells, e.g., as compared to a control. For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells (i.e., in the population of CD4+ T cells).

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells (i.e., in the population of CD4+ T cells).

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17) (i.e., in the population of CD4+ T cells).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) IL-17 expression, the method comprising:

incubating human CD4+ T cells with a test compound, wherein the incubating occurs in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human CD4+ T cells in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the human CD4+ T cells, e.g., as compared to a control. For example, the control can be the amount (e.g., percentage) of human CD4+ T cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., percentage) of human CD4+ T cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human CD4+ T cells under identical conditions. Contained within the human CD4+ T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing and/or secreting IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human CD4+ T cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that are favorable for conversion of human poised Th17 cells comprised in the human CD4+ T cells to human effector Th17 cells), and evaluating if the test compound modulates (e.g., increases or decreases) conversion of the human poised Th17 cells to human effector Th17 cells, wherein the human effector Th17 cells are IL-17+ (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions. Contained within the human CD4+ T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of converting to effector Th17 cells under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to evaluating. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human CD4+ T cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human CD4+ T cells in the absence of the test compound), and determining if the test compound modulates (e.g., increases or decreases) IL-17 expression by the human CD4+ T cells (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of human CD4+ T cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., number or percentage) of human CD4+ T cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human CD4+ T cells under identical conditions. Contained within the human CD4+ T cells are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to determining. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some

embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing by the cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing by the cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In addition to the use of human poised Th17 cells (as described herein), human peripheral blood mononuclear cells (PBMCs) can used in the methods described herein.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) conversion of human poised Th17 cells (e.g., an isolated population thereof), wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, to human effector Th17 cells, wherein the human Th17 effector cells are IL-17+, and wherein the human poised Th17 cells are comprised in a population of human PBMCs, the method comprising:

incubating the PBMCs with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the human poised Th17 cells to human effector Th17 cells, e.g., as compared to a control. For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells (i.e., in the population of PBMCs).

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells (i.e., in the population of PBMCs).

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17) (i.e., in the population of PBMCs).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) IL-17 expression, the method comprising:

incubating human PBMCs with a test compound, wherein the incubating occurs in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human PBMCs in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the human PBMCs, e.g., as compared to a control. For example, the control can be the amount (e.g., percentage) of human PBMCs expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., percentage) of human PBMCs secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human PBMCs under identical conditions. Contained within the human PBMCs are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing and/or secreting IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human PBMCs (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that are favorable for conversion of human poised Th17 cells comprised in the human PBMCs to human effector Th17 cells), and evaluating if the test compound modulates (e.g., increases or decreases) conversion of the human poised Th17 cells to human effector Th17 cells, wherein the human effector Th17 cells are IL-17+ (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions. Contained within the human PBMCs are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of converting to effector Th17 cells under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to evaluating. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of human poised Th17 cells to human effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting human PBMCs (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that promote IL-17 expression by human poised Th17 cells comprised in the human PBMCs in the absence of the test compound), and determining if the test compound modulates (e.g., increases or decreases) IL-17

expression by the human PBMCs (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of human PBMCs expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., number or percentage) of human PBMCs secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the human PBMCs under identical conditions. Contained within the human PBMCs are human poised Th17 cells (which are CD4+ CD25- CD45RO+ CCR6+ IL-17-). It is these poised Th17 cells that are capable of expressing IL-17 under appropriate conditions, as described herein.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to determining. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing by the cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing by the cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

As poised Th17 cells are present in mice as well as in humans, mouse (murine) poised Th17 cells can be isolated and used in the same types of assays as human poised Th17 cells, e.g., as described herein.

In some aspects, the disclosure is drawn to an isolated mouse poised Th17 cell, wherein the cell is CD4+ (expresses CD4), CD62Llo (does not express CD62L or expresses low levels of CD62L), CCR6+ (expresses CCR6 (Chemokine (C-C motif) receptor 6)) and IL-17- (does not express interleukin (IL)-17).

In some aspects, the disclosure is drawn to an isolated population of mouse poised Th17 cells, wherein the cells are CD4+ (express CD4), CD62Llo (do not express CD62L or express low levels of CD62L), CCR6+ (express CCR6 (Chemokine (C-C motif) receptor 6)) and IL-17- (do not express interleukin (IL)-17).

In some aspects, the disclosure is drawn to a method of isolating mouse poised Th17 cells, the method comprising:

isolating or having isolated CD4+ CD62Llo CCR6+ and IL-17- cells from a mixture of cells containing the same (e.g., peripheral blood mononuclear cells (PBMCs));

thereby isolating mouse poised Th17 cells, wherein the poised Th17 cells are CD4+ CD62Llo CCR6+ and IL-17-.

In some embodiments, the CD4+ cells are isolated by magnetic separation.

In some embodiments, the CD62Llo CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ cells with antibodies that specifically bind CD62L and CCR6 (respectively) and gating and sorting CD62Llo CCR6+ cells).

In some aspects, the disclosure is drawn to a method of isolating mouse poised Th17 cells, the method comprising:

isolating or having isolated CD4+ cells from peripheral blood mononuclear cells (PBMCs);

isolating or having isolated CD62Llo CCR6+ cells from the isolated CD4+ cells; and

isolating or having isolated IL-17- cells from the isolated CD62Llo CCR6+ cells;

thereby isolating mouse poised Th17 cells, wherein the poised Th17 cells are CD4+ CD62Llo CCR6+ and IL-17-.

In some embodiments, the CD4+ cells are isolated by magnetic separation.

In some embodiments, the CD62Llo CCR6+ cells are isolated by fluorescence activated cell sorting (e.g., by incubating isolated CD4+ cells with antibodies that specifically bind CD62L and CCR6 (respectively) and gating and sorting CD62Llo CCR6+ cells).

In some aspects, the disclosure is drawn to a method of converting a mouse poised Th17 cell (e.g., an isolated population thereof) (e.g., a population of mouse poised Th17 cells obtained by a method described herein), wherein the poised Th17 cell is CD4+ CD62Llo CCR6+ and IL-17-, to a mouse effector Th17 cell, the method comprising:

culturing the mouse poised Th17 cell in a culture medium that comprises interleukin (IL)-2, IL-7, or IL-15, wherein the mouse effector Th17 cell is IL-17+.

In some embodiments, the method further comprises stimulating the cell through the T cell receptor (TCR), e.g., prior to culturing (e.g., with anti-CD3 and anti-CD28 stimulation, e.g., with anti CD3/CD28 beads).

In some embodiments, the culture medium comprises IL-2.

In some embodiments, the culture medium comprises IL-7.

In some embodiments, the culture medium comprises IL-15.

In some embodiments, the cell is cultured for at least 3 days. In some embodiments, the cell is cultured for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cell is cultured for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cell is cultured for 6 days. In some embodiments, the cell is cultured for 7 days. In some embodiments, the cell is cultured for 8 days.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) conversion of mouse poised Th17 cells (e.g., an isolated population thereof), wherein the mouse poised Th17 cells are CD4+ CD62Llo CCR6+ and IL-17-, to mouse effector Th17 cells, wherein the mouse Th17 effector cells are IL-17+, the method comprising:

incubating the mouse poised Th17 cells with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the mouse poised Th17 cells to mouse effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the mouse poised Th17 cells to mouse effector Th17 cells, e.g., as compared to a control. For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating (e.g., *in vitro*) a test compound for its ability to modulate (e.g., decrease (e.g., inhibit) or increase (e.g., promote)) IL-17 expression, the method comprising:

incubating mouse poised Th17 cells (e.g., an isolated population thereof) with a test compound, wherein the mouse poised Th17 cells are CD4+ CD62Llo CCR6+ and IL-17-

and wherein the incubating occurs in conditions that promote IL-17 expression by the cells in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the cells, e.g., as compared to a control. For example, the control can be the amount (e.g., percentage) of cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., percentage) of cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the cells under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the incubating is for at least 3 days. In some embodiments, the incubating is for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the incubating is for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the incubating is for 6 days. In some embodiments, the incubating is for 7 days. In some embodiments, the incubating is for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 producing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting mouse poised Th17 cells (e.g., an isolated population thereof) with the test compound (e.g., *in vitro*) (e.g., in conditions that are favorable for conversion of the mouse poised Th17 cells to mouse effector Th17 cells), wherein the mouse poised Th17 cells are CD4+ CD62Llo CCR6+ and IL-17-, and evaluating if the test compound modulates (e.g., increases or decreases) conversion of the mouse poised Th17 cells to mouse effector

Th17 cells, wherein the mouse effector Th17 cells are IL-17+ (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of conversion in the absence of a test compound under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to evaluating. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17+ cells.

In some embodiments, a decrease in the percentage of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, an increase in the percentage of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, the percentage of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17+ cells.

In some embodiments, a decrease in the number of IL-17+ cells indicates that the test compound decreases (e.g., inhibits) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, an increase in the number of IL-17+ cells indicates that the test compound increases (e.g., promotes) conversion of mouse poised Th17 cells to mouse effector Th17 cells.

In some embodiments, the number of IL-17+ cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the cell-bound antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

In some aspects, the disclosure is drawn to a method of evaluating a test compound, the method comprising:

contacting mouse poised Th17 cells (e.g., an isolated population thereof) with a test compound (e.g., *in vitro*) (e.g., in conditions that promote IL-17 expression by the cells in the absence of the test compound), wherein the mouse poised Th17 cells are CD4+ CD62Llo CCR6+ and IL-17-, and determining if the test compound modulates (e.g., increases or decreases) IL-17 expression by the cells (e.g., as compared to a control). For example, the control can be the amount (e.g., number or percentage) of cells expressing IL-17 (e.g., intracellularly) and/or the amount (e.g., number or percentage) of cells secreting IL-17 in the absence of a test compound under identical conditions, or the total amount of IL-17 secreted from the cells under identical conditions.

In some embodiments, the method is performed in the presence of interleukin (IL)-2, IL-7, or IL-15.

In some embodiments, the method is performed in the presence of interleukin (IL)-2.

In some embodiments, the method is performed in the presence of interleukin (IL)-7.

In some embodiments, the method is performed in the presence of interleukin (IL)-15.

In some embodiments, the cells are incubated with the test compound for at least 3 days prior to determining. In some embodiments, the cells are incubated with the test compound for 3-21 days, e.g., 3-10 days, e.g., 5-8 days. In some embodiments, the cells are incubated with the test compound for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In some embodiments, the cells are incubated with the test compound for 6 days. In some embodiments, the cells are incubated with the test compound for 7 days. In some embodiments, the cells are incubated with the test compound for 8 days.

In some embodiments, the evaluating comprises determining the percentage of IL-17 expressing by the cells.

In some embodiments, a decrease in the percentage of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the percentage of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the percentage of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises determining the number of IL-17 expressing by the cells.

In some embodiments, a decrease in the number of IL-17 expressing cells indicates that the test compound decreases (e.g., inhibits) IL-17 expression.

In some embodiments, an increase in the number of IL-17 expressing cells indicates that the test compound increases (e.g., promotes) IL-17 expression.

In some embodiments, the number of IL-17 expressing cells is determined by flow cytometry (e.g., incubating the cells with antibody specific to IL-17 and detecting the antibody (e.g., the anti-IL-17 antibody itself or a secondary antibody that binds the anti-IL-17 antibody is detectably (e.g., fluorescently) labeled).

In some embodiments, the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

In some embodiments, a decrease in the amount of IL-17 (e.g., secreted IL-17) indicates that the test compound decreases (e.g., inhibits) IL-17 expression by the cells.

In some embodiments, an increase in amount of IL-17 (e.g., secreted IL-17) indicates that the test compound increases (e.g., promotes) IL-17 expression by the cells.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by ELISA.

In some embodiments, the amount of IL-17 (e.g., secreted IL-17) is determined by flow cytometry.

In some embodiments, the test compound is comprised in a compound library/ members of a compound library are evaluated.

In some embodiments, the test compound comprises a nucleic acid, a protein, or a small molecule.

In some embodiments, the test compound comprises RNAi.

In some embodiments, the RNAi is selected from the group consisting of: miRNA, siRNA, esiRNA, and shRNA.

In some embodiments, the test compound comprises a chemical compound.

An isolated cell refers to a cell that has been removed from the environment in which it naturally occurs. An isolated population of cells refers to a population that is enriched in a cell type of interest. For example, at least about 30%, about 40%, about 50%, about 60%,

about 70%, about 80%, about 90%, about 95% or more of which is made up of the cell of interest. For example, human poised Th17 cells can make up at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of an isolated population of such cells. As another example, mouse poised Th17 cells can make up at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of an isolated population of such cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is two dot plots showing the human naïve T cell (T_N) and memory T cell (T_{MEM}) populations based on CCR7 and CD45RO (RO) staining. Naïve CCR7hi and CD45RO- T cells were sorted and activated. Cells were stained for IL-17A and IFN-g.

FIG. 2 is five dot plots showing the human naïve T cell (T_N) and memory T cell (T_{MEM}) populations based on CCR7 and CD45RO (RO) staining. Memory CD45RO+ T cells were sorted and activated. CCR6- IL-17A-, CCR6+ IL-17A-, and CCR6+ IL-17A+ cells were sorted and cultured. Cells were stained for IL-17A and IFN-g.

FIG. 3 is three dot plots showing the staining for CCR6 and IL-17A following *in vitro* differentiation of human naïve T cells in the presence of Th17-polarizing cytokines. Expanded cells were sorted into CCR6- IL-17- and CCR6+ IL-17- populations and cultured. The cells of each population were stained for IL-17A and IFN-g.

FIG. 4 is a line graph showing CCR6- IL-17A- (R6- 17-) and CCR6+ IL-17A- (R6+ 17-) cells sorted as in FIG. 2 and cultured. Cells were harvested at the indicated times and restimulated with PMA and ionomycin to measure IL-17 production over time. Data are presented as the mean percentage of IL-17A+ T cells +/- SD from triplicate samples.

FIG. 5 is a bar graph showing CCR6- IL-17A- or CCR6+ IL-17A- T cells sorted as in FIG. 2 and cultured in the presence of the indicated recombinant cytokines and neutralizing antibodies. IL-17A production was determined after restimulation with PMA and ionomycin. Data are presented as the mean percentage of IL-17A+ T cells +/- SD from triplicate samples.

FIG. 6 is a bar graph showing CCR6- IL-17A- (R6- 17-) or CCR6+ IL-17A- (R6+ 17-) T cells sorted as in FIG. 2 and cultured under the conditions indicated (first line under horizontal axis (Wk 1)). Cells were restimulated and cultured under the conditions indicated

(second line under horizontal axis (Wk 2)). Cells were then restimulated to determine IL-17 production.

FIG. 7 is a dot plot and four histograms showing human CD4+ memory T cells pre-stained with CXCR3 and then stimulated with PMA and ionomycin in the presence of brefeldin A. Following stimulation, the cells were further stained for CCR6, CD161, IL-17A and IFN-g. Percent positive of CD161 and IFN-g within each of CCR6+ CXCR3- (R6+ X3-) and CCR6+ CXCR3+ (R6+ X3+) is shown.

FIG. 8 is a series of twelve bar graphs showing relative gene expression of Th1- (top row), Th2- (middle row), or Th17-associated (bottom row) transcripts measured by quantitative nuclease protection assay in FACS-sorted human memory T cell subsets (as indicated on the horizontal axis).

FIG. 9 is a series of sixteen dot plots showing FACS sorted CCR6- IL-17A-, CCR6+ CXCR3- IL-17A-, CCR6+ CXCR3+ IL-17A-, or CCR6+ IL-17A+ T cells cultured for 6 days in IL-2-supplemented medium. Cells were restimulated with PMA and ionomycin in the presence of brefeldin A and cytokine production was determined.

FIG. 10 is a dot plot showing human CCR6- (R6-), CCR6+ CXCR3- IL-17A- (R6+ X3- TH2-), or CCR6+ CXCR3+ IL-17A- (R6+ X3+ TH2-) T cells FACS sorted from 5 individual donors and cultured in IL-2-supplemented medium for 6-7 days. The percentages of IL-17 positive cells in each expanded population were determined following restimulation with PMA and ionomycin.

FIG. 11 is three dot plots showing IL-17A and FOXP3 expression in poised Th17 cells after IL-2, anti-IL-2 antibody (anti-IL-2), or IL-2 and IL-2 plus rapamycin (IL-2/Rapa) treatment.

FIG. 12 is a series of six histograms showing levels of phospho-Stat3 (Y705) or phospho-Stat5 (Y694) staining in naïve T cells (CD45RA+); CCR6- IL-17A-, CCR6+ CXCR3- IL-17A- memory cells; and CCR6+ CXCR3+ IL-17A- memory cells.

FIGS. 13A and **13B** are a series of dot plots showing the isolation of CD62Lhi CCR6- IL-17- (naïve T cells) (panel iii), CD62Llo CCR6- IL-17- (panel iv), and CD62Llo CCR6+ IL-17- and CD62Llo CCR6+ IL-17+ (panel v) cells in mice (FIG. 13A). IL-17A and IFNg intracellular staining was performed on isolated populations of CCR6- IL17A-, CCR6+ IL17A-, and CCR6+ IL17A+ cells (FIG. 13B) following culture in IL-2-supplemented for 6 days and restimulation with PMA and ionomycin.

FIGS. 14A and **14B** are a pair of line graphs showing IL-17A concentrations in supernatants (FIG. 14 A) and ATP levels (FIG. 14B) in CCR6- cells stimulated with IL-15 (R6- IL15), CCR6+ cells with no IL-15 stimulation (R6+ None), and CCR6+ cells stimulated with IL-15 (R6+ IL15).

FIG. 15 is a dot plot showing IL-17 production by CCR6- or CCR6+ memory T cells isolated from the peripheral blood of rheumatoid arthritis (RA) patients (n= 4). The percentage of IL-17-producing T cells was determined in *ex vivo* PMA and ionomycin stimulated cells (*left two "Ex vivo" results*), or in PMA and ionomycin-restimulated $CCR6^-$ $IL17^-$ or $CCR6^+$ $IL17^-$ cells following 7 days in culture with IL-2 (*right two "Day 7" results*).

FIGS. 16A, 16B, and **16C** are a series of dot plots showing IL-17 induction in response to γc-cytokine stimulation is a conserved feature shared by heterogenous populations of CCR6+ memory T cells. FIG. 16A: *Ex vivo* (day 0) isolated CD4+ TM cells were co-stained with CCR6, CD161, and CXCR3. FACS sorted CCR6- IL-17-, CCR6+ CD161+/- IL-17- (FIG. 16B) or CCR6+ CXCR3+/- IL-17- (FIG. 16C) cells were cultured in IL-2-supplemented medium for 7 days and restimulated with PMA and ionomycin.

FIG. 17 is a scatter plot showing that *ex vivo*-isolated CCR6+ IL-17+ and CCR6+ IL-17- memory T cell subsets express similarly high levels of numerous Th17-associated cytokines following culture with γc-cytokines in comparison to CCR6- IL-17- memory T cells.

FIGS. 18A and **18B** are a line graph (FIG. 18A) and a pair of histograms (FIG. 18B) showing that CCR6+IL-17+ and CCR6+IL-17- T cells proliferate similarly in response to IL-2 stimulation (FIG. 18A), as judged by CFSE of eFluor 670 dye dilution. Fold expansion was determined by dividing the mean fluorescence intensity (MFI) of CFSE or eFluor 670 at day 0 (i.e., the start of the culture) by the CFSE or eFluor 670 MFI observed after 48 or 96 hours in culture with IL-2. IL-17 expression in the two populations of cells after IL-2 stimulation for 6 days was determined by intracellular cytokine staining in PMA and ionomycin-restimulated T cells (FIG. 18B).

FIG. 19 is three dot plots showing IL-17A (antibody stain) and IL-17 (GFP) levels in CCR6- GFP-; CCR6+ GFP-; and CCR6+ GFP+ cells after 6 days in the presence of anti-CD3/ anti-CD28 coated beads and IL-2, followed by 4 hours of restimulation with PMA and ionomycin in the presence of Brefeldin A.

DETAILED DESCRIPTION

The disclosure provides, *inter alia*, isolated human poised Th17 memory T cells, and methods of preparing, converting, and using the same.

The inefficiency of human naïve T cell differentiation into effector Th17 cells *in vitro* has largely precluded a comprehensive molecular understanding of this process, in contrast to our understanding of murine Th17 cell biology. This disclosure provides a method for *ex vivo* isolation of human poised Th17 memory T cells that can lead to ~ 30-40% of the cells acquiring IL-17 expression (e.g., converting into effector Th17 cells), as compared to ~ 1-5% of cells acquiring IL-17 expression (e.g., differentiating into effector Th17 cells) when starting from naïve T cells. The use of poised cells is amenable to the study of Th17 biology (e.g., the conversion of poised to effector Th17 cells) and for use in screening assays, such as high-content screens (e.g., using libraries of compounds, such as siRNA, shRNA, small molecules, or peptides, etc.).

Standard human Th17 differentiation protocols using naïve T cells as starting material can require the use of combinations of neutralizing antibodies and recombinant human cytokines (e.g., one or more of IL-6, TGFβ, IL-23, IL-21, IL-1, IL-2, α-IL-4, and α-IFNγ). As described herein, the conversion of human poised Th17 memory T cells to effector Th17 cells can be achieved by using only IL-2, IL-7, or IL-15, thus limiting assay expense and improving inter-assay variability.

Because human poised Th17 memory T cells express CCR6, they have the necessary homing receptor that can allow them to traffic to inflamed tissues. Our data indicate that these poised CCR6+ cells rapidly convert into pro-inflammatory IL-17-secreting effector Th17 cells, suggesting that this conversion may take place locally at the site of ongoing inflammation, rather than in regional lymph nodes where naïve T cells are initially primed. Because T cell-driven inflammation is generally already established in patients seeking treatment for inflammatory conditions such as autoimmune disorders, our data suggest that compounds that regulate the conversion of poised Th17 memory cells into effector Th17 cells will be more predictive of pharmacological approaches to reverse or reduce established inflammation. Using the methods provided herein, such compounds can be identified. The compounds can be used therapeutically to reverse or decrease established inflammation. In addition, the compounds can be used to prevent or delay the onset of an inflammatory (e.g.,

autoimmune) condition, e.g., in subjects at risk of developing such a condition, e.g., a subject with a predisposition thereto.

The disclosure also provides *inter alia*, isolated mouse poised Th17 memory T cells, and methods of preparing, converting, and using the same.

Th17 Cells

Effector Th17 cells. Human effector Th17 cells are characterized by the production and secretion of IL-17 (i.e., IL-17A) (the cells are IL-17+). The cells also express CCR6 (Chemokine (C-C motif) receptor 6) (the cells are CCR6+). At present, if a human memory T cell does not produce IL-17 upon stimulation with antigen, T cell receptor (TCR)-crosslinking antibodies, or chemical agonists (i.e., phorbol myristate acetate (PMA) and ionomycin), these cells are not considered effector Th17 cells. Effector Th17 cells have pro-inflammatory functions by virtue of the cytokines they express (e.g., IL-17A, IL-17F, IL-22), lead to progressive tissue injury, and are often found within affected tissues of patients with chronic inflammatory and autoimmune disorders.

Poised Th17 cells. Human poised Th17 cells are IL-17- (they do not produce or secrete IL-17 (i.e., IL-17A)). The cells are also CCR6+ (express CCR6), CD4+ (express CD4), CD25- (do not express CD25), and CD45RO+ (express CD45RO). The cells can be CRTH2- or CRTH2+ and/or can be CD161+ or CD161- . These cells can be CXCR3+ or CXCR3-; and can be IFNγ+ or IFNγ-. This indicates that several functionally and phenotypically distinct poised Th17 cell subsets exist at steady-state in human peripheral blood. A poised Th17 cell converts to (i.e., becomes) an effector Th17 cell when it becomes IL-17+ (begins producing IL-17).

Distinct types of adaptive immune responses affording protection against different classes of pathogens are facilitated by the differentiation of CD4+ T cells into the corresponding types of effector T cells, which currently comprise Th1, Th2, and Th17 subsets. Through elaboration of distinct sets of cytokines and other soluble and cell-bound products, these cells act as immune effectors eliminating cells infected by pathogens. Importantly, such differentiated CD4+ T cells act as principal amplifiers and inducers of the appropriate inflammatory and effector responses in cells of the innate immune system and "nonimmune" cells. As a collective unit, the adaptive and innate immune responses generally lead to efficient clearance or containment of pathogens. The downside of such

powerful immune mechanisms is inflammation associated with the "unwanted" immune responses against "self," i.e., in autoimmune disorders, and environmental antigens and commensal microorganisms, i.e., in allergic and atopic disorders, as well as "collateral" damage to the host as a side effect of immune responses against pathogens. These side effects can be, at times, more devastating than the infection itself. Effector Th17 cells have been implicated in most known autoimmune diseases and other inflammatory conditions, and are most abundant at mucosal surfaces, particularly the intestinal lamina propria.

Following infection with diverse microbes, naïve CD4+ T cells undergo differentiation when their TCRs are triggered in the presence of particular combinations of cytokines produced by innate immune cells. Infection of myeloid cells with intracellular bacteria and viruses typically elicits production of IL-12, which induces differentiation of IFN-γ producing Th1 cells and cytotoxic CD8+ T cells that are best suited to clear such pathogens. Infection with parasitic worms, in contrast, induces production of IL-4 by cells of the innate immune system, and this, in turn, stimulates CD4+ T cells to differentiate into Th2 cells that produce more IL-4, as well as IL-5 and IL-13, cytokines involved in parasite expulsion. The third subset of CD4 T helper cells, effector Th17 cells, are abundant at mucosal interfaces, where they contain infection by certain species of pathogenic bacteria and most fungi. These cells produce IL-17A (also referred to as IL-17), IL-17F, and IL-22, cytokines involved in neutrophilia, tissue remodeling and repair, and production of antimicrobial proteins.

Naïve T cells differentiate into effector Th17 cells in response to the STAT3-activating cytokines IL-6, IL-21, and IL-23, along with TGF-β and IL-1β. Effector Th17 cells have been shown to differentiate *in vitro* from naive CD4+ T cells in response to TCR signaling in the presence of IL-6 and TGF-β, but not IL-2. As described herein, human poised Th17 cells convert to effector Th17 cells after TCR stimulation and IL-2 treatment; eliminating any or all of the cytokines that control effector Th17 differentiation from naïve T cells (i.e., IL-6, IL-21, IL-23, TGF-β, IL-1β have no impact on the conversion of poised Th17 cells into effector Th17 cells). Human poised Th17 cells can convert to effector Th17 cells after IL-7 or IL-15 treatment, with or without TCR stimulation.

IL-17

Interleukin-17 (IL-17 or IL-17A) is the founding member of the IL-17 family of cytokines. IL-17 shows high homology to viral IL-17 encoded by an open reading frame of the T lymphotropic rhadinovirus *Herpesvirus saimiri*. To elicit its functions, IL-17 binds to a type I cell surface receptor called IL-17R of which there are at least three variants: IL17RA, IL17RB, and IL17RC. IL-17 is produced by effector Th17 cells, which also secrete IL-17F and IL-22. Expression of the NK cell marker CD161 is also enriched within the effector Th17 cell compartment, though it is not an exclusive marker of such cells (i.e., CD161- cells can also express, or be induced to express IL-17). IL-17 secretion has also been described for other cell types, such as CD8+ memory T cells. Effector Th17 cells are involved in the recruitment of neutrophils to control early stages of infection by a number of pathogens, such as extracellular bacteria and fungi. IL-17 and effector Th17 cells play an important role in many immune-mediated inflammatory diseases, such as rheumatoid arthritis, psoriasis, multiple sclerosis, asthma, and inflammatory bowel disease.

In addition to IL-17A, members of the IL-17 family include IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F. All members of the IL-17 family have a similar protein structure, with four highly conserved cysteine residues critical to their 3-dimensional shape, yet they have no sequence similarity to any other known cytokines. These cytokines are all well conserved in mammals, with as much as 62–88% of amino acids conserved between the human and mouse homologs.

Numerous immune regulatory functions have been reported for the IL-17 family of cytokines. The most notable role of IL-17 is it involvement in inducing and mediating proinflammatory responses. IL-17 is commonly associated with allergic responses. IL-17 induces the production of many other cytokines (such as IL-6, G-CSF, GM-CSF, IL-1β, TGF-β, TNF-α), chemokines (including IL-8, GRO-α, and MCP-1), and prostaglandins (e.g., PGE_2) from many cell types (such as fibroblasts, endothelial cells, epithelial cells, keratinocytes, and macrophages). The increased expression of chemokines attracts other cells including neutrophils. IL-17 function is also essential to the effector Th17 subset of CD4+ T cells. As a result of these roles, the IL-17 family has been linked to many immune/autoimmune related diseases including rheumatoid arthritis, asthma, lupus, allograft rejection and anti-tumour immunity.

Each member of the IL-17 family has a distinct pattern of cellular expression. The expression of IL-17A and IL-17F appears to be restricted to a small group of activated T cells (e.g., effector Th17 cells), and is upregulated during inflammation. IL-17B is expressed in several peripheral tissues and immune tissues. IL-17C is also highly upregulated in inflammatory conditions, although in resting conditions is low in abundance. IL-17D is highly expressed in the nervous system and in skeletal muscle and IL-17E is found at low levels in various peripheral tissues.

IL-17 (i.e., IL-17A) is a 155-amino acid protein that is a disulfide-linked, homodimeric, secreted glycoprotein with a molecular mass of 35 kDa. Each subunit of the homodimer is approximately 15-20 KDa. IL-17 has a signal peptide of 23 amino acids (aa) followed by a 123-aa chain region characteristic of the IL-17 family. An N-linked glycosylation site is present. Comparison of different members of the IL-17 family revealed four conserved cysteines that form two disulfide bonds. IL-17 is unique in that it bears no resemblance to other known interleukins. Furthermore, IL-17 bears no resemblance to any other known proteins or structural domains. See, e.g., Yao et al., J. Immunol. 155:5483-5486 (1995).

The crystal structure of IL-17F, which is 50% homologous to IL-17A, revealed that IL-17F is structurally similar to the cysteine knot family of proteins that includes the neurotrophins. The cysteine knot fold is characterized by two sets of paired β-strands stabilized by three disulfide interactions. However, in contrast to the other cysteine knot proteins, IL-17F lacks the third disulfide bond. Instead, a serine replaces the cysteine at this position. This unique feature is conserved in the other IL-17 family members. IL-17F also dimerizes in a fashion similar to nerve growth factor (NGF) and other neurotrophins.

A nucleotide (gene) sequence of human IL-17A is:

```
  1 gcaggcacaa actcatccat ccccagttga ttggaagaaa caacgatgac tcctgggaag
 61 acctcattgg tgtcactgct actgctgctg agcctggagg ccatagtgaa ggcaggaatc
121 acaatcccac gaaatccagg atgcccaaat tctgaggaca agaacttccc ccggactgtg
181 atggtcaacc tgaacatcca taaccggaat accaatacca atcccaaaag gtcctcagat
241 tactacaacc gatccacctc accttggaat ctccaccgca atgaggaccc tgagagatat
301 ccctctgtga tctgggaggc aaagtgccgc cacttgggct gcatcaacgc tgatgggaac
361 gtggactacc acatgaactc tgtccccatc cagcaagaga tcctggtcct gcgcagggag
421 cctccacact gccccaactc cttccggctg gagaagatac tggtgtccgt gggctgcacc
481 tgtgtcaccc cgattgtcca ccatgtggcc taagagctct ggggagccca cactccccaa
541 agcagttaga ctatggagag ccgacccagc ccctcaggaa ccctcatcct tcaaagacag
```

601 cctcatttcg gactaaactc attagagttc ttaaggcagt ttgtccaatt aaagcttcag
661 aggtaacact tggccaagat atgagatctg aattaccttt ccctctttcc aagaaggaag
721 gtttgactga gtaccaattt gcttcttgtt tacttttta agggctttaa gttatttatg
781 tatttaatat gccctgagat aactttgggg tataagattc cattttaatg aattacctac
841 tttatttgt ttgtcttttt aaagaagata agattctggg cttgggaatt ttattattta
901 aaaggtaaaa cctgtattta tttgagctat ttaaggatct atttatgttt aagtatttag
961 aaaaaggtga aaaagcacta ttatcagttc tgcctaggta aatgtaagat agaattaaat
1021 ggcagtgcaa aatttctgag tctttacaac atacggatat agtatttcct cctctttgtt
1081 tttaaaagtt ataacatggc tgaaaagaaa gattaaacct actttcatat gtattaattt
1141 aaattttgca atttgttgag gttttacaag agatacagca agtctaactc tctgttccat
1201 taaaccctta taataaaatc cttctgtaat aataaagttt caaaagaaaa tgtttatttg
1261 ttctcattaa atgtatttta gcaaactcag ctcttcccta ttgggaagag ttatgcaaat
1321 tctcctataa gcaaaacaaa gcatgtcttt gagtaacaat gacctggaaa tacccaaaat
1381 tccaagttct cgatttcaca tgccttcaag actgaacacc gactaaggtt ttcatactat
1441 tagccaatgc tgtagacaga agcattttga taggaataga gcaaataaga taatggccct
1501 gaggaatggc atgtcattat taaagatcat atggggaaaa tgaaaccctc cccaaaatac
1561 aagaagttct gggaggagac attgtcttca gactacaatg tccagtttct cccctagact
1621 caggcttcct ttggagatta aggcccctca gagatcaaca gaccaacatt tttctcttcc
1681 tcaagcaaca ctcctagggc ctggcttctg tctgatcaag gcaccacaca acccagaaag
1741 gagctgatgg ggcagaacga actttaagta tgagaaaagt tcagcccaag taaaataaaa
1801 actcaatcac attcaattcc agagtagttt caagtttcac atcgtaacca ttttcgccc

(SEQ ID NO:1)

GenBank ACCESSION NM_002190

VERSION NM_002190.2 GI:27477085

An amino acid sequence of human IL-17A is:

1 mtpgktslvs lllllsleai vkagitiprn pgcpnsedkn fprtvmvnln ihnrntntnp
61 krssdyynrs tspwnlhrne dperypsviw eakcrhlgci nadgnvdyhm nsvpiqqeil
121 vlrrepphcp nsfrlekilv svgctcvtpi vhhva

(SEQ ID NO:2)

GenBank ACCESSION Q16552

VERSION Q16552.1 GI:2498481

A nucleotide (cDNA) sequence of human IL-17F is:

1 gaacacaggc atacacagga agatacattc acagaaagag cttcctgcac aaagtaagcc
61 accagcgcaa catgacagtg aagaccctgc atggcccagc catggtcaag tacttgctgc
121 tgtcgatatt ggggcttgcc tttctgagtg aggcggcagc tcggaaaatc cccaaagtag

```
181 gacatacttt tttccaaaag cctgagagtt gcccgcctgt gccaggaggt agtatgaagc
241 ttgacattgg catcatcaat gaaaaccagc gcgtttccat gtcacgtaac atcgagagcc
301 gctccacctc cccctggaat tacactgtca cttgggaccc caaccggtac ccctcggaag
361 ttgtacaggc ccagtgtagg aacttgggct gcatcaatgc tcaaggaaag gaagacatct
421 ccatgaattc cgttcccatc cagcaagaga ccctggtcgt ccggaggaag caccaaggct
481 gctctgtttc tttccagttg gagaaggtgc tggtgactgt tggctgcacc tgcgtcaccc
541 ctgtcatcca ccatgtgcag taagaggtgc atatccactc agctgaagaa gctgtagaaa
601 tgccactcct tacccagtgc tctgcaacaa gtcctgtctg acccccaatt ccctccactt
661 cacaggactc ttaataagac ctgcacggat ggaaacagaa aatattcaca atgtatgtgt
721 gtatgtacta cactttatat ttgatatcta aaatgttagg agaaaaatta atatattcag
781 tgctaatata ataaagtatt aataattt
```

(SEQ ID NO:3)

GenBank ACCESSION NM_052872

VERSION NM_052872.3 GI:57863305

An amino acid sequence of human IL-17F is:

```
 1 mtvktlhgpa mvkylllsil glaflseaaa rkipkvghtf fqkpescppv pggsmkldig
61 iinenqrvsm srniesrsts pwnytvtwdp nrypsevvqa qcrnlgcina qgkedismns
121 vpiqqetlvv rrkhqgcsvs fqlekvlvtv gctcvtpvih hvq
```

(SEQ ID NO:4)

GenBank ACCESSION Q96PD4

VERSION Q96PD4.3 GI:239938888

Levels of IL-17A (IL-17) and/or IL-17F can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular levels), and other methods known in the art. Reagents for measuring IL-17 and/or IL-17F levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA). In addition, an IL-17 secretion assay detection kit, for detecting viable IL-17–secreting cells, is available from Miltenyi Biotec (Bergisch Gladbach, DE). An IL-17-specific Catch Reagent is attached to the surface of cells. Secreted IL-17 binds to the Catch Reagent on IL-17-secreting cells. The cells are then labeled with an IL-17-specific antibody that is conjugated

to a fluorescent label (flourochrome) to allow detection and sorting of the IL-17+ cells by flow cytometry.

CCR6

CC chemokine receptors (or beta chemokine receptors) are integral membrane proteins that specifically bind and respond to cytokines of the CC chemokine family. To date, at least ten true members of the CC chemokine receptor subfamily (CCR1 to CCR10) have been described.

CCR6 (Chemokine (C-C motif) receptor 6), a receptor for CCL20 (also known as macrophage inflammatory protein 3 alpha (MIP-3 alpha)), is expressed on unactivated memory T-cells, regulatory T cells and some dendritic cells. CCR6 is also expressed on both poised and effector Th17 cells. CCR6 is down-regulated in T-cells upon activation through TCR. CCR6 has is also designated CD196 (cluster of differentiation 196).

The CCR6 gene encodes a protein of 374 amino acids. The CCR6 gene is preferentially expressed by immature dendritic cells and memory T cells. This receptor has been shown to be important for B-lineage maturation and antigen-driven B-cell differentiation, and it may regulate the migration and recruitment of dentritic and T cells during inflammatory and immunological responses.

A nucleotide (cDNA) sequence of human CCR6 is:

```
  1 caaacgttcc caaatcttcc cagtcggctt gcagagactc cttgctccca ggagataacc
 61 agaagctgca tcttattgac agatggtcat cacattggtg agctggagtc atcagattgt
121 ggggcccgga gtgaggctga agggagtgga tcagagcact gcctgagagt cacctctact
181 ttcctgctac cgctgcctgt gagctgaagg ggctgaacca tacactcctt tttctacaac
241 cagcttgcat tttttctgcc cacaatgagc ggggaatcaa tgaatttcag cgatgttttc
301 gactccagtg aagattattt tgtgtcagtc aatacttcat attactcagt tgattctgag
361 atgttactgt gctccttgca ggaggtcagg cagttctcca ggctatttgt accgattgcc
421 tactccttga tctgtgtctt tggcctcctg gggaatattc tggtggtgat caccttgct
481 ttttataaga aggccaggtc tatgacagac gtctatctct tgaacatggc cattgcagac
541 atcctctttg ttcttactct cccattctgg gcagtgagtc atgccactgg tgcgtgggtt
601 ttcagcaatg ccacgtgcaa gttgctaaaa ggcatctatg ccatcaactt taactgcggg
661 atgctgctcc tgacttgcat tagcatggac cggtacatcg ccattgtaca ggcgactaag
721 tcattccggc tccgatccag aacactaccg cgcacgaaaa tcatctgcct tgttgtgtgg
781 gggctgtcag tcatcatctc cagctcaact tttgtcttca accaaaaata caacacccaa
841 ggcagcgatg tctgtgaacc caagtaccag actgtctcgg agcccatcag gtggaagctg
901 ctgatgttgg ggcttgagct actctttggt ttctttatcc ctttgatgtt catgatattt
```

961 tgttacacgt tcattgtcaa aaccttggtg caagctcaga attctaaaag gcacaaagcc
1021 atccgtgtaa tcatagctgt ggtgcttgtg tttctggctt gtcagattcc tcataacatg
1081 gtcctgcttg tgacggctgc aaatttgggt aaaatgaacc gatcctgcca gagcgaaaag
1141 ctaattggct atacgaaaac tgtcacagaa gtcctggctt tcctgcactg ctgcctgaac
1201 cctgtgctct acgcttttat tgggcagaag ttcagaaact actttctgaa gatcttgaag
1261 gacctgtggt gtgtgagaag gaagtacaag tcctcaggct tctcctgtgc cgggaggtac
1321 tcagaaaaca tttctcggca gaccagtgag accgcagata acgacaatgc gtcgtccttc
1381 actatgtgat agaaagctga gtctccctaa ggcatgtgtg aaacatactc atagatgtta
1441 tgcaaaaaaa agtctatggc caggtatgca tggaaaatgt gggaattaag caaaatcaag
1501 caagcctctc tcctgcggga cttaacgtgc tcatgggctg tgtgatctct tcagggtggg
1561 gtggtctctg ataggtagca ttttccagca ctttgcaagg aatgttttgt agctctaggg
1621 tatatatccg cctggcattt cacaaaacag cctttgggaa atgctgaatt aaagtgaatt
1681 gttgacaaat gtaaacattt tcagaaatat tcatgaagcg gtcacagatc acagtgtctt
1741 ttggttacag cacaaaatga tggcagtggt ttgaaaaact aaaacagaaa aaaaaatgga
1801 agccaacaca tcactcattt taggcaaatg tttaaacatt tttatctatc agaatgttta
1861 ttgttgctgg ttataagcag caggattggc cggctagtgt ttcctctcat ttccctttga
1921 tacagtcaac aagcctgacc ctgtaaaatg gaggtggaaa gacaagctca agtgttcaca
1981 acctggaagt gcttcgggaa gaaggggaca atggcagaac aggtgttggt gacaattgtc
2041 accaattgga taaagcagct caggttgtag tgggccatta ggaaactgtc ggtttgcttt
2101 gatttccctg ggagctgttc tctgtcgtga gtgtctcttg tctaaacgtc cattaagctg
2161 agagtgctat gaagacagga tctagaataa tcttgctcac agctgtgctc tgagtgccta
2221 gcggagttcc agcaaacaaa atggactcaa gagagatttg attaatgaat cgtaatgaag
2281 ttggggttta ttgtacagtt taaaatgtta gatgttttta atttttaaa taaatggaat
2341 actttttttt tttttaaaga aagcaacttt actgagacaa tgtagaaaga agttttgttc
2401 cgtttcttta atgtggttga agagcaatgt gtggctgaag acttttgtta tgaggagctg
2461 cagattagct aggggacagc tggaattatg ctggcttctg ataattattt taaaggggtc
2521 tgaaatttgt gatggaatca gattttaaca gctctcttca atgacataga aagttcatgg
2581 aactcatgtt tttaaagggc tatgtaaata tatgaacatt agaaaaatag caacttgtgt
2641 tacaaaaata caaacacatg ttaggaaggt actgtcatgg gctaggcatg gtggctcaca
2701 cctgtaatcc cagcattttg ggaagctaag atgggtggat cacttgaggt caggagtttg
2761 agaccagcct ggccaacatg gcgaaacccc tctctactaa aaatacaaaa atttgccagg
2821 cgtggtggcg ggtgcctgta atcccagcta cttgggaggc tgaggcaaga gaatcgcttg
2881 aacccaggag gcagaggttg cagtgagccg agatcgtgcc attgcactcc agcctgggtg
2941 acagagcgag actccatctc aaaaaaaaaa aaaaaaaa

(SEQ ID NO:5)

GenBank ACCESSION U68030

VERSION U68030.1 GI:1870665

An amino acid sequence of human CCR6 is:

```
 1 msgesmnfsd vfdssedyfv svntsyysvd semllcslqe vrqfsrlfvp iayslicvfg
61 llgnilvvit fafykkarsm tdvyllnmai adilfvltlp fwavshatga wvfsnatckl
121 lkgiyainfn cgmllltcis mdryiaivqa tksfrlrsrt lprtkiiclv vwglsviiss
181 stfvfnqkyn tqgsdvcepk yqtvsepirw kllmlglell fgffiplmfm ifcytfivkt
241 lvqaqnskrh kairviiavv lvflacqiph nmvllvtaan lgkmnrscqs ekligytktv
301 tevlaflhcc lnpvlyafig qkfrnyflki lkdlwcvrrk ykssgfscag rysenisrqt
361 setadndnas sftm
```

(SEQ ID NO:6)

GenBank ACCESSION AAC51124

VERSION AAC51124.1 GI:1870666

Levels of CCR6 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CCR6 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), BioLegend (San Diego, CA, USA), and eBioscience (San Diego, CA, USA).

CXCR3

Chemokine receptor CXCR3 (chemokine (C-X-C motif) receptor 3) is a Gαi protein-coupled receptor in the CXC chemokine receptor family. Other names for CXCR3 are G protein-coupled receptor 9 (GPR9) and CD183. There are two variants of CXCR3: (1) CXCR3-A binds to the CXC chemokines CXCL9 (MIG), CXCL10, and CXCL11 (IP-10, I-TAC), and (2) CXCR3-B can also bind to CXCL4 in addition to CXCL9, CXCL10, and CXCL11.

CXCR3 is expressed primarily on activated T lymphocytes and NK cells, and some epithelial cells and some endothelial cells. CXCR3 and CCR5 are preferentially expressed on IFN-g-expressing Th1 cells, in contrast to Th2 cells, which favor the expression of CCR3 and CCR4, and poised and effector Th17 cells that predominantly express CCR6 (see above). CXCR3 ligands that attract Th1 cells can concomitantly block the migration of Th2

cells in response to CCR3 ligands, thus enhancing the polarization of effector T cell recruitment.

Binding of CXCL9, CXCL10, and CXCL11 to CXCR3 is able to elicit increases in intracellular Ca2++ levels and activate phosphoinositide 3-kinase and mitogen-activated protein kinase (MAPK).

CXCR3 is able to regulate leukocyte trafficking. Binding of chemokines to CXCR3 induces various cellular responses, including integrin activation, cytoskeletal changes and chemotactic migration. CXCR3-ligand interaction attracts Th1 cells and promotes Th1 cell maturation.

CXCR3 is expressed in *in vitro* cultured effector/memory T cells, and in T cells present in many types of inflamed tissues. In addition, CXCL9, CXCL10 and CXCL11 are commonly produced by local cells in inflammatory lesions, suggesting that CXCR3 and its chemokines participate in the recruitment of inflammatory cells. CXCR3 has also been implicated in wound healing.

A nucleotide (cDNA) sequence of human CXCR3-A is:

```
   1 ccaaccacaa gcaccaaagc agaggggcag gcagcacacc acccagcagc cagagcacca
  61 gcccagccat ggtccttgag gtgagtgacc accaagtgct aaatgacgcc gaggttgccg
 121 ccctcctgga gaacttcagc tcttcctatg actatggaga aaacgagagt gactcgtgct
 181 gtacctcccc gccctgccca caggacttca gcctgaactt cgaccgggcc ttcctgccag
 241 ccctctacag cctcctcttt ctgctggggc tgctgggcaa cggcgcggtg gcagccgtgc
 301 tgctgagccg gcggacagcc ctgagcagca ccgacacctt cctgctccac ctagctgtag
 361 cagacacgct gctggtgctg acactgccgc tctgggcagt ggacgctgcc gtccagtggg
 421 tctttggctc tggcctctgc aaagtggcag gtgccctctt caacatcaac ttctacgcag
 481 gagccctcct gctggcctgc atcagctttg accgctacct gaacatagtt catgccaccc
 541 agctctaccg ccgggggccc ccggcccgcg tgaccctcac ctgcctggct gtctgggggc
 601 tctgcctgct tttcgccctc ccagacttca tcttcctgtc ggcccaccac gacgagcgcc
 661 tcaacgccac ccactgccaa tacaacttcc cacaggtggg ccgcacggct ctgcgggtgc
 721 tgcagctggt ggctggcttt ctgctgcccc tgctggtcat ggcctactgc tatgcccaca
 781 tcctggccgt gctgctggtt tccaggggcc agcggcgcct gcgggccatg cggctggtgg
 841 tggtggtcgt ggtggccttt gccctctgct ggacccccta tcacctggtg gtgctggtgg
 901 acatcctcat ggacctgggc gctttggccc gcaactgtgg ccgagaaagc agggtagacg
 961 tggccaagtc ggtcacctca ggcctgggct acatgcactg ctgcctcaac ccgctgctct
1021 atgcctttgt aggggtcaag ttccgggagc ggatgtggat gctgctcttg cgcctgggct
1081 gccccaacca gagagggctc cagaggcagc catcgtcttc ccgccgggat tcatcctggt
1141 ctgagacctc agaggcctcc tactcgggct tgtgaggccg gaatccgggc tccccttttcg
1201 cccacagtct gacttccccg cattccaggc tcctccctcc ctctgccggc tctggctctc
```

```
1261 cccaatatcc tcgctcccgg gactcactgg cagccccagc accaccaggt ctcccgggaa
1321 gccaccctcc cagctctgag gactgcacca ttgctgctcc ttagctgcca agccccatcc
1381 tgccgcccga ggtggctgcc tggagcccca ctgcccttct catttggaaa ctaaaacttc
1441 atcttcccca agtgcgggga gtacaaggca tggcgtagag ggtgctgccc catgaagcca
1501 cagcccaggc ctccagctca gcagtgactg tggccatggt ccccaagacc tctatatttg
1561 ctcttttatt tttatgtcta aaatcctgct taaaactttt caataaacaa gatcgtcagg
1621 accaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
```

(SEQ ID NO:7)

GenBank ACCESSION NM_001504

VERSION NM_001504.1 GI:4504098

An amino acid sequence of human CXCR3-A is:

```
 1 mvlevsdhqv lndaevaall enfsssydyg enesdsccts ppcpqdfsln fdraflpaly
61 sllfllgllg ngavaavlls rrtalsstdt fllhlavadt llvltlplwa vdaavqwvfg
121 sglckvagal fninfyagal llacisfdry lnivhatqly rrgpparvtl tclavwglcl
181 lfalpdfifl sahhderlna thcqynfpqv grtalrvlql vagfllpllv maycyahila
241 vllvsrgqrr lramrlvvvv vvafalcwtp yhlvvlvdil mdlgalarnc gresrvdvak
301 svtsglgymh cclnpllyaf vgvkfrermw mlllrlgcpn qrglqrqpss srrdsswset
361 seasysgl
```

(SEQ ID NO:8)

GenBank ACCESSION NP_001495

VERSION NP_001495.1 GI:4504099

A nucleotide (cDNA) sequence of human CXCR3-B is:

```
 1 ccaaccacaa gcaccaaagc agaggggcag gcagcacacc acccagcagc cagagcacca
61 gcccagccat ggtccttgag gggtccctgg gccgatggga tcacgcagaa gaatgcgaga
121 gaagcagcct ttgagaaggg aagtcactat cccagagccc aggctgagcg gatggagttg
181 aggaagtacg gccctggaag actggcgggg acagttatag gaggagctgc tcagagtaaa
241 tcacagacta aatcagactc aatcacaaaa gagttcctgc caggccttta cacagcccct
301 tcctccccgt tcccgccctc acaggtgagt gaccaccaag tgctaaatga cgccgaggtt
361 gccgccctcc tggagaactt cagctcttcc tatgactatg gagaaaacga gagtgactcg
421 tgctgtacct ccccgccctg cccacaggac ttcagcctga acttcgaccg ggccttcctg
481 ccagccctct acagcctcct ctttctgctg gggctgctgg gcaacggcgc ggtggcagcc
541 gtgctgctga gccggcggac agccctgagc agcaccgaca ccttcctgct ccacctagct
601 gtagcagaca cgctgctggt gctgacactg ccgctctggg cagtggacgc tgccgtccag
661 tgggtctttg gctctggcct ctgcaaagtg gcaggtgccc tcttcaacat caacttctac
721 gcaggagccc tcctgctggc ctgcatcagc tttgaccgct acctgaacat agttcatgcc
```

781 acccagctct accgccgggg gcccccggcc cgcgtgaccc tcacctgcct ggctgtctgg
841 gggctctgcc tgcttttcgc cctcccagac ttcatcttcc tgtcggccca ccacgacgag
901 cgcctcaacg ccacccactg ccaatacaac ttcccacagg tgggccgcac ggctctgcgg
961 gtgctgcagc tggtggctgg ctttctgctg cccctgctgg tcatggccta ctgctatgcc
1021 cacatcctgg ccgtgctgct ggtttccagg ggccagcggc gcctgcgggc catgcggctg
1081 gtggtggtgg tcgtggtggc ctttgccctc tgctggaccc cctatcacct ggtggtgctg
1141 gtggacatcc tcatggacct gggcgctttg gcccgcaact gtggccgaga aagcagggta
1201 gacgtggcca agtcggtcac ctcaggcctg ggctacatgc actgctgcct caacccgctg
1261 ctctatgcct ttgtaggggt caagttccgg gagcggatgt ggatgctgct cttgcgcctg
1321 ggctgcccca accagagagg gctccagagg cagccatcgt cttcccgccg ggattcatcc
1381 tggtctgaga cctcagaggc ctcctactcg ggcttgtgag gccggaatcc gggctccct
1441 ttcgcccaca gtctgacttc cccgcattcc aggctcctcc ctccctctgc cggctctggc
1501 tctccccaat atcctcgctc ccgggactca ctggcagccc cagcaccacc aggtctcccg
1561 ggaagccacc ctcccagctc tgaggactgc accattgctg ctccttagct gccaagcccc
1621 atcctgccgc ccgaggtggc tgcctggagc cccactgccc ttctcatttg gaaactaaaa
1681 cttcatcttc cccaagtgcg gggagtacaa ggcatggcgt agagggtgct gccccatgaa
1741 gccacagccc aggcctccag ctcagcagtg actgtggcca tggtccccaa gacctctata
1801 tttgctcttt tatttttatg tctaaaatcc tgcttaaaac ttttcaataa acaagatcgt
1861 caggaccaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa

(SEQ ID NO:9)

GenBank ACCESSION NM_001142797

VERSION NM_001142797.1 GI:218563729

An amino acid sequence of human CXCR3-B is:

1 melrkygpgr lagtviggaa qsksqtksds itkeflpgly tapsspfpps qvsdhqvlnd
61 aevaallenf sssydygene sdscctsppc pqdfslnfdr aflpalysll fllgllgnga
121 vaavllsrrt alsstdtfll hlavadtllv ltlplwavda avqwvfgsgl ckvagalfni
181 nfyagallla cisfdrylni vhatqlyrrg pparvtltcl avwglcllfa lpdfiflsah
241 hderlnathc qynfpqvgrt alrvlqlvag fllpllvmay cyahilavll vsrgqrrlra
301 mrlvvvvvva falcwtpyhl vvlvdilmdl galarncgre srvdvaksvt sglgymhccl
361 npllyafvgv kfrermwmll lrlgcpnqrg lqrqpsssrr dsswsetsea sysgl

(SEQ ID NO:10)

GenBank ACCESSION NP_001136269

VERSION NP_001136269.1 GI:218563730

Levels of CXCR3 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on

cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CXCR3 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA). In the examples provided herein, an antibody that recognizes both CXCR3A and CXCR3B is used.

IFN-g

Interferon-gamma (IFN-g; IFNg; IFN-γ) is a dimerized soluble cytokine that is the only member of the type II class of interferons. IFN-g is important for innate and adaptive immunity against viral and intracellular bacterial infections and for tumor control. Aberrant IFN-g expression is associated with a number of autoinflammatory and autoimmune diseases. The importance of IFN-g in the immune system stems in part from its ability to inhibit viral replication directly, but, most important, derives from its immunostimulatory and immunomodulatory effects. IFN-g is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops.

A nucleotide (cDNA) sequence of human IFN-g is:

```
  1 cacattgttc tgatcatctg aagatcagct attagaagag aaagatcagt taagtccttt
 61 ggacctgatc agcttgatac aagaactact gatttcaact tctttggctt aattctctcg
121 gaaacgatga aatatacaag ttatatcttg gcttttcagc tctgcatcgt tttgggttct
181 cttggctgtt actgccagga cccatatgta aaagaagcag aaaaccttaa gaaatatttt
241 aatgcaggtc attcagatgt agcggataat ggaactcttt tcttaggcat tttgaagaat
301 tggaaagagg agagtgacag aaaaataatg cagagccaaa ttgtctcctt ttacttcaaa
361 ctttttaaaa actttaaaga tgaccagagc atccaaaaga gtgtggagac catcaaggaa
421 gacatgaatg tcaagttttt caatagcaac aaaaagaaac gagatgactt cgaaaagctg
481 actaattatt cggtaactga cttgaatgtc caacgcaaag caatacatga actcatccaa
541 gtgatggctg aactgtcgcc agcagctaaa acagggaagc gaaaaaggag tcagatgctg
601 tttcgaggtc gaagagcatc ccagtaatgg ttgtcctgcc tgcaatattt gaattttaaa
661 tctaaatcta tttattaata tttaacatta tttatatggg gaatatattt ttagactcat
721 caatcaaata agtatttata atagcaactt ttgtgtaatg aaaatgaata tctattaata
781 tatgtattat ttataattcc tatatcctgt gactgtctca cttaatcctt tgttttctga
```

841 ctaattaggc aaggctatgt gattacaagg ctttatctca ggggccaact aggcagccaa
901 cctaagcaag atcccatggg ttgtgtgttt atttcacttg atgatacaat gaacacttat
961 aagtgaagtg atactatcca gttactgccg gtttgaaaat atgcctgcaa tctgagccag
1021 tgctttaatg gcatgtcaga cagaacttga atgtgtcagg tgaccctgat gaaaacatag
1081 catctcagga gatttcatgc ctggtgcttc caaatattgt tgacaactgt gactgtaccc
1141 aaatggaaag taactcattt gttaaaatta tcaatatcta atatatatga ataaagtgta
1201 agttcacaac aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

(SEQ ID NO:11)

GenBank ACCESSION P01579

VERSION P01579.1 GI:124479

An amino acid sequence of human IFN-g is:

1 mkytsyilaf qlcivlgslg cycqdpyvke aenlkkyfna ghsdvadngt lflgilknwk
61 eesdrkimqs qivsfyfklf knfkddqsiq ksvetikedm nvkffnsnkk krddfekltn
121 ysvtdlnvqr kaiheliqvm aelspaaktg krkrsqmlfr grrasq

(SEQ ID NO:12)

GenBank ACCESSION Q96AZ6

VERSION Q96AZ6.2 GI:57012967

Levels of IFN-g can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring IFN-g levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

CD45

CD45 antigen (cluster of differentiation 45) is also known as leukocyte common antigen, and Protein tyrosine phosphatase, receptor type, C (PTPRC). The CD45 protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including

cell growth, differentiation, mitotic cycle, and oncogenic transformation. CD45 contains an extracellular domain, a single transmembrane segment and two tandem intracytoplasmic catalytic domains, and thus belongs to receptor type PTP. The *CD45* gene is specifically expressed in hematopoietic cells. This PTP has been shown to be an essential regulator of T- and B-cell antigen receptor signaling. It functions through either direct interaction with components of the antigen receptor complexes or by activating various Src family kinases required for the antigen receptor signaling. CD45 also suppresses JAK kinases, and, thus, functions as a regulator of cytokine receptor signaling. Four alternatively spliced transcripts variants of the gene, which encode distinct isoforms, have been reported.

CD45 is a type I transmembrane protein that is present in various forms on all differentiated hematopoietic cells except erythrocytes and plasma cells and assists in the activation of those cells. It is expressed in lymphomas, B-cell chronic lymphocytic leukemia, hairy cell leukemia, and acute nonlymphocytic leukemia.

The CD45 family consists of multiple members that are all products of a single complex gene. This gene contains 34 exons and three exons of the primary transcripts are alternatively spliced to generate up to eight different mature mRNAs and after translation eight different protein products.

Various isoforms of CD45 exist: CD45RA, CD45RB, CD45RC, CD45RAB, CD45RAC, CD45RBC, CD45RO, and CD45R (ABC). CD45RA is located on naive T cells and CD45RO is located on memory T cells. CD45 is also highly glycosylated. Naive T lymphocytes express large CD45 isoforms and are usually positive for CD45RA. Activated and memory T lymphocytes express the shortest CD45 isoform, CD45RO, which lacks RA, RB and RC exons. This shortest isoform facilitates T cell activation. Because poised cells are memory T cells, they are CD45RO+.

The cytoplasmic domain of CD45 is one of the largest known and it has an intrinsic phosphatase activity that removes an inhibitory phosphate group on Lck (in T cells) or Lyn/Fyn/Lck (in B cells) and activates it.

A nucleotide (cDNA) sequence of human CD45RO is:

```
  1 agtatttttg gagaagttag taaaaccgaa tctgacatca tcacctagca gttcatgcag
 61 ctagcaagtg gtttgttctt agggtaacag aggaggaaat tgttcctcgt ctgataagac
121 aacagtggag aaaggacgca tgctgtttct tagggacacg gctgacttcc agatatgacc
181 atgtatttgt ggcttaaact cttggcattt ggctttgcct ttctggacac agaagtattt
241 gtgacagggc aaagcccaac accttccccc actggattga ctacagcaaa gatgcccagt
```

301 gttccacttt caagtgaccc cttacctact cacaccactg cattctcacc cgcaagcacc
361 tttgaaagag aaaatgactt ctcagagacc acaacttctc ttagtccaga caatacttcc
421 acccaagtat ccccggactc tttggataat gctagtgctt ttaataccac aggtgtttca
481 tcagtacaga cgcctcacct tcccacgcac gcagactcgc agacgccctc tgctggaact
541 gacacgcaga cattcagcgg ctccgccgcc aatgcaaaac tcaaccctac cccaggcagc
601 aatgctatct cagatgtccc aggagagagg agtacagcca gcacctttcc tacagaccca
661 gtttccccat tgacaaccac cctcagcctt gcacaccaca gctctgctgc cttacctgca
721 cgcacctcca acaccaccat cacagcgaac acctcagatg cctaccttaa tgcctctgaa
781 acaaccactc tgagcccttc tggaagcgct gtcatttcaa ccacaacaat agctactact
841 ccatctaagc caacatgtga tgaaaaatat gcaaacatca ctgtggatta cttatataac
901 aaggaaacta aattatttac agcaaagcta aatgttaatg agaatgtgga atgtggaaac
961 aatacttgca caaacaatga ggtgcataac cttacagaat gtaaaaatgc gtctgtttcc
1021 atatctcata attcatgtac tgctcctgat aagacattaa tattagatgt gccaccaggg
1081 gttgaaaagt ttcagttaca tgattgtaca caagttgaaa aagcagatac tactatttgt
1141 ttaaaatgga aaaatattga aacctttact tgtgatacac agaatattac ctacagattt
1201 cagtgtggta atatgatatt tgataataaa gaaattaaat tagaaaacct tgaacccgaa
1261 catgagtata agtgtgactc agaaatactc tataataacc acaagtttac taacgcaagt
1321 aaaattatta aaacagattt tgggagtcca ggagagcctc agattatttt ttgtagaagt
1381 gaagctgcac atcaaggagt aattacctgg aatccccctc aaagatcatt tcataatttt
1441 accctctgtt atataaaaga gacagaaaaa gattgcctca atctggataa aaacctgatc
1501 aaatatgatt tgcaaaattt aaaaccttat acgaaatatg ttttatcatt acatgcctac
1561 atcattgcaa aagtgcaacg taatggaagt gctgcaatgt gtcatttcac aactaaaagt
1621 gctcctccaa gccaggtctg gaacatgact gtctccatga catcagataa tagtatgcat
1681 gtcaagtgta ggcctcccag ggaccgtaat ggcccccatg aacgttacca tttggaagtt
1741 gaagctggaa atactctggt tagaaatgag tcgcataaga attgcgattt ccgtgtaaaa
1801 gatcttcaat attcaacaga ctacactttt aaggcctatt ttcacaatgg agactatcct
1861 ggagaaccct ttattttaca tcattcaaca tcttataatt ctaaggcact gatagcattt
1921 ctggcatttc tgattattgt gacatcaata gccctgcttg ttgttctcta caaaatctat
1981 gatctacata agaaaagatc ctgcaattta gatgaacagc aggagcttgt tgaaagggat
2041 gatgaaaaac aactgatgaa tgtggagcca atccatgcag atattttgtt ggaaacttat
2101 aagaggaaga ttgctgatga aggaagactt tttctggctg aatttcagag catcccgcgg
2161 gtgttcagca agtttcctat aaaggaagct cgaaagccct ttaaccagaa taaaaaccgt
2221 tatgttgaca ttcttcctta tgattataac cgtgttgaac tctctgagat aaacggagat
2281 gcagggtcaa actacataaa tgccagctat attgatggtt tcaaagaacc caggaaatac
2341 attgctgcac aaggtcccag ggatgaaact gttgatgatt tctggaggat gatttgggaa
2401 cagaaagcca cagttattgt catggtcact cgatgtgaag aaggaaacag gaacaagtgt
2461 gcagaatact ggccgtcaat ggaagagggc actcgggctt ttggagatgt tgttgtaaag
2521 atcaaccagc acaaaagatg tccagattac atcattcaga aattgaacat tgtaaataaa
2581 aaagaaaaag caactggaag agaggtgact cacattcagt tcaccagctg gccagaccac

2641 ggggtgcctg aggatcctca cttgctcctc aaactgagaa ggagagtgaa tgccttcagc
2701 aatttcttca gtggtcccat tgtggtgcac tgcagtgctg gtgttgggcg cacaggaacc
2761 tatatcggaa ttgatgccat gctagaaggc ctggaagccg agaacaaagt ggatgtttat
2821 ggttatgttg tcaagctaag gcgacagaga tgcctgatgg ttcaagtaga ggcccagtac
2881 atcttgatcc atcaggcttt ggtggaatac aatcagtttg gagaaacaga agtgaatttg
2941 tctgaattac atccatatct acataacatg aagaaaaggg atccacccag tgagccgtct
3001 ccactagagg ctgaattcca gagacttcct tcatatagga gctggaggac acagcacatt
3061 ggaaatcaag aagaaaataa aagtaaaaac aggaattcta atgtcatccc atatgactat
3121 aacagagtgc cacttaaaca tgagctggaa atgagtaaag agagtgagca tgattcagat
3181 gaatcctctg atgatgacag tgattcagag gaaccaagca aatacatcaa tgcatctttt
3241 ataatgagct actggaaacc tgaagtgatg attgctgctc agggaccact gaaggagacc
3301 attggtgact tttggcagat gatcttccaa agaaaagtca aagttattgt tatgctgaca
3361 gaactgaaac atggagacca ggaaatctgt gctcagtact ggggagaagg aaagcaaaca
3421 tatggagata ttgaagttga cctgaaagac acagacaaat cttcaactta tacccttcgt
3481 gtctttgaac tgagacattc caagaggaaa gactctcgaa ctgtgtacca gtaccaatat
3541 acaaactgga gtgtggagca gcttcctgca gaacccaagg aattaatctc tatgattcag
3601 gtcgtcaaac aaaaacttcc ccagaagaat tcctctgaag ggaacaagca tcacaagagt
3661 acacctctac tcattcactg cagggatgga tctcagcaaa cgggaatatt ttgtgctttg
3721 ttaaatctct tagaaagtgc ggaaacagaa gaggtagtgg atatttttca agtggtaaaa
3781 gctctacgca aagctaggcc aggcatggtt tccacattcg agcaatatca attcctatat
3841 gacgtcattg ccagcaccta ccctgctcag aatggacaag taaagaaaaa caaccatcaa
3901 gaagataaaa ttgaatttga taatgaagtg gacaaagtaa agcaggatgc taattgtgtt
3961 aatccacttg gtgccccaga aaagctccct gaagcaaagg aacaggctga aggttctgaa
4021 cccacgagtg gcactgaggg gccagaacat tctgtcaatg gtcctgcaag tccagcttta
4081 aatcaaggtt cataggaaaa gacataaatg aggaaactcc aaacctcctg ttagctgtta
4141 tttctatttt tgtagaagta ggaagtgaaa ataggtatac agtggattaa ttaaatgcag
4201 cgaaccaata tttgtagaag ggttatattt tactactgtg gaaaaatatt taagatagtt
4261 ttgccagaac agtttgtaca gacgtatgct tattttaaaa ttttatctct tattcagtaa
4321 aaaacaactt ctttgtaatc gttatgtgtg tatatgtatg tgtgtatggg tgtgtgtttg
4381 tgtgagagac agagaaagag agagaattct ttcaagtgaa tctaaaagct tttgcttttc
4441 ctttgttttt atgaagaaaa aatacatttt atattagaag tgttaactta gcttgaagga
4501 tctgttttta aaaatcataa actgtgtgca gactcaataa aatcatgtac atttctgaaa
4561 tgacctcaag atgtcctcct tgttctactc atatatatct atcttatata gtttactatt
4621 ttacttctag agatagtaca taaaggtggt atgtgtgtgt atgctactac aaaaaagttg
4681 ttaactaaat taacattggg aaatcttata ttccatatat tagcatttag tccaatgtct
4741 ttttaagctt atttaattaa aaaatttcca gtgagcttat catgctgtct ttacatgggg
4801 ttttcaattt tgcatgctcg attattccct gtacaatatt taaaatttat tgcttgatac
4861 ttttgacaac aaattaggtt ttgtacaatt gaacttaaat aaatgtcatt aaaataaata
4921 aatgcaatat gtattaatat tcattgtata aaaatagaag aatacaaaca tatttgttaa

```
4981 atatttacat atgaaattta atatagctat ttttatggaa tttttcattg atatgaaaaa
5041 tatgatattg catatgcata gttcccatgt taaatcccat tcataacttt cattaaagca
5101 tttactttga atttctccaa tgcttagaat gtttttacca ggaatggatg tcgctaatca
5161 taataaaatt caaccattat ttttttcttg tttataatac attgtgttat atgttcaaat
5221 atgaaatgtg tatgcaccta ttgaaatatg tttaatgcat ttattaacat ttgcaggaca
5281 cttttacagg ccccaattat ccaatagtct aataattgtt taagatctag
```

(SEQ ID NO:13)

GenBank ACCESSION NM_002838

VERSION NM_002838.3 GI:115385975

An amino acid sequence of human CD45RO is:

```
 1 mylwlkllaf gfafldtevf vtgqsptpsp tglttakmps vplssdplpt httafspast
61 ferendfset ttslspdnts tqvspdsldn asafnttgvs svqtphlpth adsqtpsagt
121 dtqtfsgsaa naklnptpgs naisdvpger stastfptdp vspltttlsl ahhssaalpa
181 rtsnttitan tsdaylnase tttlspsgsa vistttiatt pskptcdeky anitvdylyn
241 ketklftakl nvnenvecgn ntctnnevhn ltecknasvs ishnsctapd ktlildvppg
301 vekfqlhdct qvekadttic lkwknietft cdtqnityrf qcgnmifdnk eiklenlepe
361 heykcdseil ynnhkftnas kiiktdfgsp gepqiifcrs eaahqgvitw nppqrsfhnf
421 tlcyiketek dclnldknli kydlqnlkpy tkyvlslhay iiakvqrngs aamchfttks
481 appsqvwnmt vsmtsdnsmh vkcrpprdrn gpheryhlev eagntlvrne shkncdfrvk
541 dlqystdytf kayfhngdyp gepfilhhst synskaliaf lafliivtsi allvvlykiy
601 dlhkkrscnl deqqelverd dekqlmnvep ihadillety krkiadegrl flaefqsipr
661 vfskfpikea rkpfnqnknr yvdilpydyn rvelseingd agsnyinasy idgfkeprky
721 iaaqgprdet vddfwrmiwe qkatvivmvt rceegnrnkc aeywpsmeeg trafgdvvvk
781 inqhkrcpdy iiqklnivnk kekatgrevt hiqftswpdh gvpedphlll klrrrvnafs
841 nffsgpivvh csagvgrtgt yigidamleg leaenkvdvy gyvvklrrqr clmvqveaqy
901 ilihqalvey nqfgetevnl selhpylhnm kkrdppseps pleaefqrlp syrswrtqhi
961 gnqeenkskn rnsnvipydy nrvplkhele mskesehdsd essdddsdse epskyinasf
1021 imsywkpevm iaaqgplket igdfwqmifq rkvkvivmlt elkhgdqeic aqywgegkqt
1081 ygdievdlkd tdksstytlr vfelrhskrk dsrtvyqyqy tnwsveqlpa epkelismiq
1141 vvkqklpqkn ssegnkhhks tpllihcrdg sqqtgifcal lnllesaete evvdifqvvk
1201 alrkarpgmv stfeqyqfly dviastypaq ngqvkknnhq edkiefdnev dkvkqdancv
1261 nplgapeklp eakeqaegse ptsgtegpeh svngpaspal nqgs
```

(SEQ ID NO:14)

GenBank ACCESSION P08575

VERSION P08575.2 GI:33112650

Levels of CD45RO can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CD45RO levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

CRTH2

CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells; also referred to as GPR44) is a member of the G protein-coupled leukocyte chemoattractant receptor family and it is expressed on Th2, but not Th1 lineage cells. It is detectable on CD4+ T cells in fresh peripheral blood mononuclear cells (PBMC) and no significant expression is detected on B cells and NK cells. CRTH2 is also expressed on peripheral blood basophils and eosinophils.

CRTH2 is a Prostanoid Receptor that binds prostaglandin D2 (PGD2). PGD2 signals through CRTH2 to induce Th2, eosinophil, and basophil chemotaxis in a G-alpha(i) dependent manner. CRTH2 may play a role in mast cell-mediated immune responses such as those involved in allergic reactions. This receptor is upregulated in activated T helper-2 (Th2) cells, peripheral blood basophils, and eosinophils, but not in Th1 lineage cells. It is a useful marker for Th2 cells.

A nucleotide (cDNA) sequence of human CRTH2 is:

```
  1 cagcctccct ctcccacctc tgtctgcccg ctgcctcttg tctagctgct gtcaggagct
 61 gactgcctcc agggctggaa tcctgtgctc cctctgtgcc cagagcccca cgatgtcggc
121 caacgccaca ctgaagccac tctgccccat cctggagcag atgagccgtc tccagagcca
181 cagcaacacc agcatccgct acatcgacca cgcggccgtg ctgctgcacg ggctggcctc
241 gctgctgggc ctggtggaga atggagtcat cctcttcgtg gtgggctgcc gcatgcgcca
301 gaccgtggtc accacctggg tgctgcacct ggcgctgtcc gacctgttgg cctctgcttc
361 cctgcccttc ttcacctact tcttggccgt gggccactcg tgggagctgg gcaccacctt
421 ctgcaaactg cactcctcca tcttctttct caacatgttc gccagcggct tcctgctcag
481 cgccatcagc ctggaccgct gcctgcaggt ggtgcggccg gtgtgggcgc agaaccaccg
541 caccgtggcc gcggcgcaca aagtctgcct ggtgctttgg gcactagcgg tgctcaacac
601 ggtgccctat ttcgtgttcc gggacaccat ctcgcggctg gacgggcgca ttatgtgcta
661 ctacaatgtg ctgctcctga acccggggcc tgaccgcgat gccacgtgca actcgcggca
```

721 ggtggccctg gccgtcagca agttcctgct ggccttcctg gtgccgctgg cgatcatcgc
781 ctcgagccac gcggccgtga gcctgcggtt gcagcaccgc ggccgccggc ggccaggccg
841 cttcgtgcgc ctggtggcgg ccgtcgtggc cgccttcgcg ctctgctggg ggccctacca
901 cgtgttcagc ctgctggagg cgcgggcgca cgcaaacccg gggctgcggc cgctcgtgtg
961 gcgcgggctg cccttcgtca ccagcctggc cttcttcaac agcgtggcca acccggtgct
1021 ctacgtgctc acctgccccg acatgctgcg caagctgcgg cgctcgctgc gcacggtgct
1081 ggagagcgtg ctggtggacg acagcgagct gggtggcgcg ggaagcagcc gccgccgccg
1141 cacctcctcc accgcccgct cggcctcccc tttagctctc tgcagccgcc cggaggaacc
1201 gcggggcccc gcgcgtctcc tcggctggct gctgggcagc tgcgcagcgt ccccgcagac
1261 gggccccctg aaccgggcgc tgagcagcac ctcgagttag aacccggccc acgtagggcg
1321 gcactcacac gcgaaagtat caccagggtg ccgcggttca attcgatatc cggactcctg
1381 ccgcagtgat caaagtccga ggggcgggac ccaggcacct gcattttaaa gcgccccggg
1441 agactctgaa tctttttcag aaacagtgag ttaaagcagt gcttctcaaa ccttgatgtg
1501 cctgtgaatc acctaggggt cttgttaagt gcagtctgat ccaggaggcc ggggccgggt
1561 actgagagtc tgcacttaac aagctcccag gccgagaagc cagtgcggca ggttcacagg
1621 cgaggcctgg agtaacacaa agtgaaactc ataatagact tcccactcta gggcagtgga
1681 gtcggaaggg cacacggggt gcgtctcccc ggagttcagt tttaccagat gatgggggag
1741 gggggaagga gttttatgtt aaaccatcca tgtatttttg gagaagagag aggaaaggtt
1801 tgagaagcac tgttccagcc tgccctcttc atttagccaa tgcttactgc gctagacgct
1861 tcatcccaca atcttaaggg gcagcttcta ttagccagtc tttacagctg agcacattct
1921 ggctcaggga ggttaagtga cttgcccagt ttcagggcta acgaccacag ggtctgcact
1981 ctaaccctag gcatcacatg ctcaatgact ctctggtgag cgaggacatt ctctgaccta
2041 ctcgagggac ttaagatgct accttgtgac ccagcactgc ccaaagtgct tccaaggcag
2101 aagcagcagg ggatggcgtg gtcaagcact cgggaaacct ggggctaatc aaatccaatg
2161 ggggaaatga ctaaaagtct tcggtcgtta gaagttgaat gggcacagca actctaagac
2221 tacagcacac gtcatttctt agctaagcgg accagcctcc ctgtcggcct ggtgttctgt
2281 gggatccctc tgggcactgg taatcccaag atctgtgcag ccccgcctcc aggccacatg
2341 gggctgggca gctaccattt ccctttgcg gatgggaggg gtaacttgca cctctgacct
2401 atcacttcca ctgcaccccg tctcattcct ccacctgccg tggacttggg gtcagagact
2461 gctgtgtttg agctctgcag cccagggacc gaaaagttgg tgtcaatgaa ttttgcttgg
2521 tggatgaaat gtcagtggaa gaagcagatg agaaactctt gagatcttgg tcctgtgttt
2581 tttctgccac caaaggccag ggtcactgaa ggcctggccc acagcaggtg ctgagcaaag
2641 ggaacagtga ggtgcccagc tagctgcaga gccaccctgt gttgacacct cgcccctgct
2701 ccctcccatc ccttcccccc ttactcatag cacttccccc attggacacg tggtgcattt
2761 tgcttgttta ttatgttttc tctccatcag aatgaaagct cctcgagggc agggactttg
2821 gtctattgtc tgtatttgcc ggtgcctagg attgtgcctg tatgcaacag gcactcaata
2881 aatatttttg ctgtagactg gacaggcatg

(SEQ ID NO:15)

GenBank ACCESSION NM_004778

VERSION NM_004778.2 GI:153791423

An amino acid sequence of human CRTH2 is:

```
 1 msanatlkpl cpileqmsrl qshsntsiry idhaavllhg lasllglven gvilfvvgcr
61 mrqtvvttwv lhlalsdlla saslpfftyf lavghswelg ttfcklhssi fflnmfasgf
121 llsaisldrc lqvvrpvwaq nhrtvaaahk vclvlwalav lntvpyfvfr dtisrldgri
181 mcyynvllln pgpdrdatcn srqvalavsk fllaflvpla iiasshaavs lrlqhrgrrr
241 pgrfvrlvaa vvaafalcwg pyhvfsllea rahanpglrp lvwrglpfvt slaffnsvan
301 pvlyvltcpd mlrklrrslr tvlesvlvdd selggagssr rrrtsstars asplalcsrp
361 eeprgparll gwllgscaas pqtgplnral sstss
```

(SEQ ID NO:16)

GenBank ACCESSION Q9Y5Y4

VERSION Q9Y5Y4.3 GI:296439334

Levels of CRTH2 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CRTH2 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

CD4

CD4 (cluster of differentiation 4) is a glycoprotein expressed on the surface of T helper cells, regulatory T cells, monocytes, macrophages, and dendritic cells. CD4 is a co-receptor that assists the T cell receptor (TCR) to activate its T cell following an interaction with an antigen-presenting cell. CD4 also interacts directly with MHC class II molecules on the surface of the antigen-presenting cell using its extracellular domain.

Like many cell surface receptors, CD4 is a member of the immunoglobulin superfamily. It has four immunoglobulin domains (D1 to D4) that are exposed on the extracellular surface of the cell. CD4 uses its D1 domain to interact with the β2-domain of

MHC class II molecules. T cells expressing CD4 molecules on their surface are MHC class II-restricted.

A nucleotide (cDNA) sequence of human CD4 is:

```
   1 ccaggcctag ggtgtggagg agccttgcca tcgggcttcc tgtctctctt catttaagca
  61 cgactctgca gaaggaacaa agcaccctcc ccactgggct cctggttgca gagctccaag
 121 tcctcacaca gatacgcctg tttgagaagc agcgggcaag aaagacgcaa gcccagaggc
 181 cctgccattt ctgtgggctc aggtccctac tggctcaggc ccctgcctcc ctcggcaagg
 241 ccacaatgaa ccggggagtc ccttttaggc acttgcttct ggtgctgcaa ctggcgctcc
 301 tcccagcagc cactcaggga aagaaagtgg tgctgggcaa aaaaggggat acagtggaac
 361 tgacctgtac agcttcccag aagaagagca tacaattcca ctggaaaaac tccaaccaga
 421 taaagattct gggaaatcag ggctccttct taactaaagg tccatccaag ctgaatgatc
 481 gcgctgactc aagaagaagc ctttgggacc aaggaaactt tcccctgatc atcaagaatc
 541 ttaagataga agactcagat acttacatct gtgaagtgga ggaccagaag gaggaggtgc
 601 aattgctagt gttcggattg actgccaact ctgacaccca cctgcttcag gggcagagcc
 661 tgaccctgac cttggagagc ccccctggta gtagcccctc agtgcaatgt aggagtccaa
 721 ggggtaaaaa catacagggg gggaagaccc tctccgtgtc tcagctggag ctccaggata
 781 gtggcacctg gacatgcact gtcttgcaga accagaagaa ggtggagttc aaaatagaca
 841 tcgtggtgct agctttccag aaggcctcca gcatagtcta taagaaagag ggggaacagg
 901 tggagttctc cttcccactc gcctttacag ttgaaaagct gacgggcagt ggcgagctgt
 961 ggtggcaggc ggagagggct tcctcctcca agtcttggat caccttgac ctgaagaaca
1021 aggaagtgtc tgtaaaacgg gttacccagg accctaagct ccagatgggc aagaagctcc
1081 cgctccacct caccctgccc caggccttgc ctcagtatgc tggctctgga aacctcaccc
1141 tggcccttga agcgaaaaca ggaaagttgc atcaggaagt gaacctggtg gtgatgagag
1201 ccactcagct ccagaaaaat ttgacctgtg aggtgtgggg acccacctcc cctaagctga
1261 tgctgagttt gaaactggag aacaaggagg caaaggtctc gaagcgggag aaggcggtgt
1321 gggtgctgaa ccctgaggcg gggatgtggc agtgtctgct gagtgactcg ggacaggtcc
1381 tgctggaatc caacatcaag gttctgccca catggtccac cccggtgcag ccaatggccc
1441 tgattgtgct ggggggcgtc gccggcctcc tgcttttcat tgggctaggc atcttcttct
1501 gtgtcaggtg ccggcaccga aggcgccaag cagagcggat gtctcagatc aagagactcc
1561 tcagtgagaa gaagacctgc cagtgtcctc accggtttca gaagacatgt agccccattt
1621 gaggcacgag gccaggcaga tcccacttgc agcctcccca ggtgtctgcc ccgcgtttcc
1681 tgcctgcgga ccagatgaat gtagcagatc cccagcctct ggcctcctgt tcgcctcctc
1741 tacaatttgc cattgtttct cctgggttag gccccggctt cactggttga gtgttgctct
1801 ctagtttcca gaggcttaat cacaccgtcc tccacgccat ttccttttcc ttcaagccta
1861 gcccttctct cattatttct ctctgaccct ctccccactg ctcatttgga tcccagggga
1921 gtgttcaggg ccagccctgg ctggcatgga gggtgaggct gggtgtctgg aagcatggag
1981 catgggactg ttcttttaca agacaggacc ctgggaccac agagggcagg aacttgcaca
2041 aaatcacaca gccaagccag tcaaggatgg atgcagatcc agaggtttct ggcagccagt
2101 acctcctgcc ccatgctgcc cgcttctcac cctatgtggg tgggaccaca gactcacatc
```

```
2161 ctgaccttgc acaaacagcc cctctggaca cagccccatg tacacggcct caagggatgt
2221 ctcacatcct ctgtctattt gagacttaga aaaatcctac aaggctggca gtgacagaac
2281 taagatgatc atctccagtt tatagaccag aaccagagct cagagaggct agatgattga
2341 ttaccaagtg ccggactagc aagtgctgga gtcgggacta acccaggtcc cttgtcccaa
2401 gttccactgc tgcctcttga atgcagggac aaatgccaca cggctctcac cagtggctag
2461 tggtgggtac tcaatgtgta cttttgggtt cacagaagca cagcacccat gggaagggtc
2521 catctcagag aatttacgag cagggatgaa ggcctccctg tctaaaatcc ctccttcatc
2581 ccccgctggt ggcagaatct gttaccagag gacaaagcct ttggctcttc taatcagagc
2641 gcaagctggg agcacaggca ctgcaggaga gaatgcccag tgaccagtca ctgaccctgt
2701 gcagaacctc ctggaagcga gctttgctgg gagagggggt agctagcctg agagggaacc
2761 ctctaaggga cctcaaaggt gattgtgcca ggctctgcgc ctgccccaca ccctccctta
2821 ccctcctcca gaccattcag gacacaggga aatcagggtt acaaatcttc ttgatccact
2881 tctctcagga tcccctctct tcctaccctt cctcaccact tccctcagtc ccaactcctt
2941 ttccctattt ccttctcctc ctgtctttaa agcctgcctc ttccaggaag acccccctat
3001 tgctgctggg gctccccatt tgcttacttt gcatttgtgc ccactctcca cccctgctcc
3061 cctgagctga aataaaaata caataaactt actataaaga tgc
```

(SEQ ID NO:17)

GenBank ACCESSION NM_000616

VERSION NM_000616.3 GI:91992151

An amino acid sequence of human CD4 is:

```
 1 mnrgvpfrhl llvlqlallp aatqgkkvvl gkkgdtvelt ctasqkksiq fhwknsnqik
61 ilgnqgsflt kgpsklndra dsrrslwdqg nfpliiknlk iedsdtyice vedqkeevql
121 lvfgltansd thllqgqslt ltlesppgss psvqcrsprg kniqggktls vsqlelqdsg
181 twtctvlqnq kkvefkidiv vlafqkassi vykkegeqve fsfplaftve kltgsgelww
241 qaerasssks witfdlknke vsvkrvtqdp klqmgkklpl hltlpqalpq yagsgnltla
301 leaktgklhq evnlvvmrat qlqknltcev wgptspklml slklenkeak vskrekavwv
361 lnpeagmwqc llsdsgqvll esnikvlptw stpvqpmali vlggvaglll figlgiffcv
421 rcrhrrrqae rmsqikrlls ekktcqcphr fqktcspi
```

(SEQ ID NO:18)

GenBank ACCESSION P01730

VERSION P01730.1 GI:116013

Levels of CD4 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CD4 levels are known

and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), Miltenyi Biotec (Bergisch Gladbach, DE), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

CD25

CD25 (cluster of differentiation 25; also referred to as ISG20 (Interferon-stimulated gene 20 kDa protein)) is the alpha chain of the IL-2 receptor. It is a type I transmembrane protein present on activated T cells, activated B cells, some thymocytes, myeloid precursors, and oligodendrocytes that associates with CD122 to form a heterodimer that can act as a high-affinity receptor for IL-2.

CD25 is expressed in most B-cell neoplasms, some acute nonlymphocytic leukemias, neuroblastomas, and tumor infiltrating lymphocytes. Its soluble form, called sIL-2R, may be elevated in these diseases and is occasionally used to track disease progression.

A nucleotide (cDNA) sequence of human CD25 is:

```
  1 cctgacatgg agcctgccag ctccgtcagc cctgactcgg cccggagctg agctccccac
 61 ctgccggtag cccaggagat ggagcagccc agcccacgtg cccggccttc cgcccctgac
121 ttcacttgat aacaaactag aaactgaaac agggtcggga tgccgatgcc ggcttggagt
181 tagagatgag tcaccgctga gagcagctgc agtagctgag cagtggcagc agagaggcag
241 acgtgagctg agggcgcaga ggcaggcagc atctctgagg gtccccaagg agcatggctg
301 ggagccgtga ggtggtggcc atggactgcg agatggtggg gctggggccc caccgggaga
361 gtggcctggc tcgttgcagc ctcgtgaacg tccacggtgc tgtgctgtac gacaagttca
421 tccggcctga gggagagatc accgattaca gaacccgggt cagcggggtc acccctcagc
481 acatggtggg ggccacacca tttgccgtgg ccaggctaga gatcctgcag ctcctgaaag
541 gcaagctggt ggtgggtcat gacctgaagc acgacttcca ggcactgaaa gaggacatga
601 gcggctacac aatctacgac acgtccactg acaggctgtt gtggcgtgag gccaagctgg
661 accactgcag gcgtgtctcc ctgcgggtgc tgagtgagcg cctcctgcac aagagcatcc
721 agaacagcct gcttggacac agctcggtgg aagatgcgag ggcaacgatg gagctctatc
781 aaatctccca gagaatccga gcccgccgag ggctgccccg cctggctgtg tcagactgaa
841 gccccatcca gcccgttccg cagggactag aggctttcgg ctttttggga cagcaactac
901 cttgcttttg gaaaatacat ttttaatagt aaagtggctc tatattttct ctacgcaaaa
961 aaaaaaaaaa aaaa
```

(SEQ ID NO:19)

GenBank ACCESSION NM_002201
VERSION NM_002201.4 GI:34147571

An amino acid sequence of human CD25 is:

```
 1 magsrevvam dcemvglgph resglarcsl vnvhgavlyd kfirpegeit dyrtrvsgvt
61 pqhmvgatpf avarleilql lkgklvvghd lkhdfqalke dmsgytiydt stdrllwrea
121 kldhcrrvsl rvlserllhk siqnsllghs svedaratme lyqisqrira rrglprlavs
181 d
```

(SEQ ID NO:20)

GenBank ACCESSION Q96AZ6
VERSION Q96AZ6.2 GI:57012967

Levels of CD25 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CD25 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

CD161

CD161 (cluster of differentiation 161) is also known as killer cell lectin-like receptor subfamily B, member 1, KLRB1, NKR-P1A.

Natural killer (NK) cells are lymphocytes that mediate cytotoxicity and secrete cytokines after immune stimulation. Several genes of the C-type lectin superfamily, including the rodent NKRP1 family of glycoproteins, are expressed by NK cells and may be involved in the regulation of NK cell function. The CD161 protein contains an extracellular domain with several motifs characteristic of C-type lectins, a transmembrane domain, and a cytoplasmic domain. CD161 is classified as a type II membrane protein. CD161 recognizes Lectin Like Transcript-1 (LLT1) as a functional ligand.

A nucleotide (cDNA) sequence of human CD161 is:

```
 1 gcctcacaga attgagagtt tgttcttaca cacaagttta atgccacctt cctctgtctg
61 ccatggacca acaagcaata tatgctgagt taaacttacc cacagactca ggcccagaaa
121 gttcttcacc ttcatctctt cctcgggatg tctgtcaggg ttcaccttgg catcaatttg
181 ccctgaaact tagctgtgct gggattattc tccttgtctt ggttgttact gggttgagtg
241 tttcagtgac atccttaata cagaaatcat caatagaaaa atgcagtgtg gacattcaac
301 agagcaggaa taaaacaaca gagagaccgg gtctcttaaa ctgcccaata tattggcagc
361 aactccgaga gaaatgcttg ttattttctc acactgtcaa cccttggaat aacagtctag
421 ctgattgttc caccaaagaa tccagcctgc tgcttattcg agataaggat gaattgatac
481 acacacagaa cctgatacgt gacaaagcaa ttctgttttg gattggatta aatttttcat
541 tatcagaaaa gaactggaag tggataaacg gctcttttt aaattctaat gacttagaaa
601 ttagaggtga tgctaaagaa aacagctgta tttccatctc acagacatct gtgtattctg
661 agtactgtag tacagaaatc agatggatct gccaaaaaga actaacacct gtgagaaata
721 aagtgtatcc tgactcttga
```

(SEQ ID NO:21)

GenBank ACCESSION NM_002258

VERSION NM_002258.2 GI:58761538

An amino acid sequence of human CD161 is:

```
 1 mdqqaiyael nlptdsgpes sspsslprdv cqgspwhqfa lklscagiil lvlvvtglsv
61 svtsliqkss iekcsvdiqq srnktterpg llncpiywqq lrekclllfsh tvnpwnnsla
121 dcstkessll lirdkdelih tqnlirdkai lfwiglnfsl seknwkwing sflnsndlei
181 rgdakensci sisqtsvyse ycsteirwic qkeltpvrnk vypds
```

(SEQ ID NO:22)

GenBank ACCESSION Q12918

VERSION Q12918.1 GI:74722301

Levels of CD161 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CD161 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

CCR7

CCR7 (C-C chemokine receptor type 7; also named CD197) is a member of the G protein coupled receptor family. This receptor was identified as a gene induced by the Epstein Barr virus (EBV), and is thought to be a mediator of EBV effects on B lymphocytes. CCR7 has been reported to be expressed in blood, bone marrow, lymph node, and intestine. It is particularly expressed in lymphoid tissues and in activated B and T lymphocytes and has been shown to control the migration of memory T cells to inflamed tissues, as well as stimulate dendritic cell maturation. The chemokine (C-C motif) ligand 19 (CCL19/ECL) has been reported to be a specific ligand of this receptor.

A nucleotide (cDNA) sequence of human CCR7 is:

```
   1 cacttcctcc ccagacaggg gtagtgcgag gccgggcaca gccttcctgt gtggttttac
  61 cgcccagaga gcgtcatgga cctggggaaa ccaatgaaaa gcgtgctggt ggtggctctc
 121 cttgtcattt tccaggtatg cctgtgtcaa gatgaggtca cggacgatta catcggagac
 181 aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga cgtgcggaac
 241 tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg cctactgggc
 301 aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat gaccgatacc
 361 tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc cttctgggcc
 421 tacagcgcgg ccaagtcctg ggtcttcggt gtccactttt gcaagctcat ctttgccatc
 481 tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat tgaccgctac
 541 gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct tctcatcagc
 601 aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc agagctcctg
 661 tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct catcacagag
 721 catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt tctggtcccc
 781 ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca ggcacgcaac
 841 tttgagcgca acaaggccat caaggtgatc atcgctgtgg tcgtggtctt catagtcttc
 901 cagctgccct acaatggggt ggtcctggcc cagacggtgg ccaacttcaa catcaccagt
 961 agcacctgtg agctcagtaa gcaactcaac atcgcctacg acgtcaccta cagcctggcc
1021 tgcgtccgct gctgcgtcaa ccctttcttg tacgccttca tcggcgtcaa gttccgcaac
1081 gatctcttca agctcttcaa ggacctgggc tgcctcagcc aggagcagct ccggcagtgg
1141 tcttcctgtc ggcacatccg gcgctcctcc atgagtgtgg aggccgagac caccaccacc
1201 ttctccccat aggcgactct tctgcctgga ctagagggac ctctcccagg gtccctgggg
1261 tggggatagg gagcagatgc aatgactcag gacatccccc cgccaaaagc tgctcaggga
1321 aaagcagctc tcccctcaga gtgcaagccc ctgctccaga agatagcttc accccaatcc
1381 cagctacctc aaccaatgcc aaaaaaagac agggctgata agctaacacc agacagacaa
1441 cactgggaaa cagaggctat tgtcccctaa accaaaaact gaaagtgaaa gtccagaaac
1501 tgttcccacc tgctggagtg aaggggccaa ggagggtgag tgcaaggggc gtgggagtgg
1561 cctgaagagt cctctgaatg aaccttctgg cctcccacag actcaaatgc tcagaccagc
```

```
1621 tcttccgaaa accaggcctt atctccaaga ccagagatag tggggagact tcttggcttg
1681 gtgaggaaaa gcggacatca gctggtcaaa caaactctct gaacccctcc ctccatcgtt
1741 ttcttcactg tcctccaagc cagcgggaat ggcagctgcc acgccgccct aaaagcacac
1801 tcatcccctc acttgccgcg tcgccctccc aggctctcaa caggggagag tgtggtgttt
1861 cctgcaggcc aggccagctg cctccgcgtg atcaaagcca cactctgggc tccagagtgg
1921 ggatgacatg cactcagctc ttggctccac tgggatggga ggagaggaca agggaaatgt
1981 caggggcggg gagggtgaca gtggccgccc aaggcccacg agcttgttct ttgttctttg
2041 tcacagggac tgaaaacctc tcctcatgtt ctgctttcga ttcgttaaga gagcaacatt
2101 ttacccacac acagataaag ttttcccttg aggaaacaac agctttaaaa gaaaaagaaa
2161 aaaaaagtct ttggtaaatg gcaaaaaaaa aaaaaaaaaa aaaaaaa
```

(SEQ ID NO:23)

GenBank ACCESSION NM_001838

VERSION NM_001838.3 GI:299473754

An amino acid sequence of human CCR7 is:

```
 1 mdlgkpmksv lvvallvifq vclcqdevtd dyigdnttvd ytlfeslcsk kdvrnfkawf
61 lpimysiicf vgllgnglvv ltyiyfkrlk tmtdtyllnl avadilfllt lpfwaysaak
121 swvfgvhfck lifaiykmsf fsgmllllci sidryvaivq avsahrhrar vllisklscv
181 giwilatvls ipellysdlq rssseqamrc slitehveaf itiqvaqmvi gflvpllams
241 fcylviirtl lqarnfernk aikviiavvv vfivfqlpyn gvvlaqtvan fnitsstcel
301 skqlniaydv tyslacvrcc vnpflyafig vkfrndlfkl fkdlgclsqe qlrqwsscrh
361 irrssmsvea ettttfsp
```

(SEQ ID NO:24)

GenBank ACCESSION P32248

VERSION P32248.2 GI:1352335

Levels of CCR7 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring CCR7 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

FOXP3

FOXP3 (forkhead box P3) is a gene involved in immune system responses. A member of the forkhead, winged helix (FOX) family of transcription factors, FOXP3 functions as a central regulator in the development and function of T regulatory (Treg) cells. FOX proteins are presumed to exert control via similar DNA binding interactions during transcription.

FOXP3 is a specific marker of natural T regulatory cells (nTregs,) and adaptive/induced T regulatory (a/iTregs) cells. In human disease, alterations in numbers of Treg cells that express FOXP3 are found in a number of disease states. Patients with an autoimmune disease such as systemic lupus erythematosus (SLE) have a relative dysfunction of FOXP3 positive cells. The FOXP3 gene is also mutated in the X-linked IPEX syndrome (Immunodysregulation, Polyendocrinopathy, and Enteropathy, X-linked), a primary immunodeficiency characterized by systemic T cell-mediated inflammation and excessive effector T cell cytokine activation and cytokine production. *FOXP3* is x-linked, meaning that only males are affected. IPEX is generally fatal within the first 1-2 years of life.

In animal studies, Tregs that express FOXP3 are critical in the transfer of immune tolerance. The induction or administration of FOXP3 positive T cells has, in animal studies, led to marked reductions in (autoimmune) disease severity in models of diabetes, multiple sclerosis, asthma, inflammatory bowel disease, thyroiditis and renal disease. In mice, a FOXP3 mutation (a frameshift mutation that result in protein lacking the forkhead domain) is responsible for 'Scurfy', an X-linked recessive mouse mutant that results in lethality in hemizygous males 16 to 25 days after birth. Similar to IPEX patients, scurfy mice display f CD4+ T-lymphocyte hyperactivation, extensive multiorgan infiltration, and elevation of numerous cytokines. This phenotype is that of mice with germline deletions of CTLA-4, TGF-β. The pathology observed in both scurfy mice and IPEX patients seems to arise from dysregulation of CD4+ T-cell activity. In mice overexpressing the FOXP3 gene, fewer T cells are observed. The remaining T cells have poor proliferative and cytolytic responses and poor IL-2 production, although thymic development appears normal. Histologic analysis indicates that peripheral lymphoid organs, particularly lymph nodes, lack typical cell numbers.

In mouse and human cells, FOXP3 can also be induced in mature peripheral naïve CD4+ T cells (i.e., non Treg cells) upon activation in the presence of TGFβ and IL-2. This process of iTreg differentiation is enhanced by all-trans retinoic acid (RA). In this instance, the regulation of FOXP3 expression can be reciprocally regulated with IL-17A expression. In human poised Th17 cells, however, FOXP3 expression can be induced concomitantly with IL-17, suggesting that these genes are regulated in a coordinated fashion in these poised cells.

A nucleotide (cDNA) sequence of human FOXP3 is:

```
   1 gcacacactc atcgaaaaaa atttggatta ttagaagaga gaggtctgcg gcttccacac
  61 cgtacagcgt ggtttttctt ctcggtataa aagcaaagtt gtttttgata cgtgacagtt
 121 tcccacaagc caggctgatc cttttctgtc agtccacttc accaagcctg cccttggaca
 181 aggacccgat gcccaacccc aggcctggca agccctcggc cccttccttg gcccttggcc
 241 catccccagg agcctcgccc agctggaggg ctgcacccaa agcctcagac ctgctggggg
 301 cccggggccc agggggaacc ttccagggcc gagatcttcg aggcggggcc catgcctcct
 361 cttcttcctt gaaccccatg ccaccatcgc agctgcagct gcccacactg cccctagtca
 421 tggtggcacc ctccggggca cggctgggcc ccttgcccca cttacaggca ctcctccagg
 481 acaggccaca tttcatgcac cagctctcaa cggtggatgc ccacgcccgg acccctgtgc
 541 tgcaggtgca ccccctggag agcccagcca tgatcagcct cacaccaccc accaccgcca
 601 ctggggtctt ctccctcaag gcccggcctg gcctcccacc tgggatcaac gtggccagcc
 661 tggaatgggt gtccagggag ccggcactgc tctgcacctt cccaaatccc agtgcaccca
 721 ggaaggacag cacccctttcg gctgtgcccc agagctccta cccactgctg gcaaatggtg
 781 tctgcaagtg gcccggatgt gagaaggtct tcgaagagcc agaggacttc ctcaagcact
 841 gccaggcgga ccatcttctg gatgagaagg gcagggcaca atgtctcctc cagagagaga
 901 tggtacagtc tctggagcag cagctggtgc tggagaagga gaagctgagt gccatgcagg
 961 cccacctggc tgggaaaatg gcactgacca aggcttcatc tgtggcatca tccgacaagg
1021 gctcctgctg catcgtagct gctggcagcc aaggccctgt cgtcccagcc tggtctggcc
1081 cccgggaggc ccctgacagc ctgtttgctg tccggaggca cctgtggggt agccatggaa
1141 acagcacatt cccagagttc ctccacaaca tggactactt caagttccac aacatgcgac
1201 ccccttttcac ctacgccacg ctcatccgct gggccatcct ggaggctcca gagaagcagc
1261 ggacactcaa tgagatctac cactggttca cacgcatgtt tgccttcttc agaaaccatc
1321 ctgccacctg gaagaacgcc atccgccaca acctgagtct gcacaagtgc tttgtgcggg
1381 tggagagcga gaaggggggct gtgtggaccg tggatgagct ggagttccgc aagaaacgga
1441 gccagaggcc cagcaggtgt tccaacccta cacctggccc ctgacctcaa gatcaaggaa
1501 aggaggatgg acgaacaggg gccaaactgg tgggaggcag aggtggtggg ggcagggatg
1561 ataggccctg gatgtgccca cagggaccaa gaagtgaggt ttccactgtc ttgcctgcca
1621 gggcccctgt tcccccgctg gcagccaccc cctcccccat catatccttt gccccaaggc
1681 tgctcagagg ggccccggtc ctggccccag cccccacctc cgccccagac acacccccca
```

```
1741 gtcgagccct gcagccaaac agagccttca caaccagcca cacagagcct gcctcagctg
1801 ctcgcacaga ttacttcagg gctggaaaag tcacacagac acacaaaatg tcacaatcct
1861 gtccctcact caacacaaac cccaaaacac agagagcctg cctcagtaca ctcaaacaac
1921 ctcaaagctg catcatcaca caatcacaca caagcacagc cctgacaacc cacacacccc
1981 aaggcacgca cccacagcca gcctcagggc ccacaggggc actgtcaaca caggggtgtg
2041 cccagaggcc tacacagaag cagcgtcagt accctcagga tctgaggtcc caacacgtgc
2101 tcgctcacac acacggcctg ttagaattca cctgtgtatc tcacgcatat gcacacgcac
2161 agccccccag tgggtctctt gagtcccgtg cagacacaca cagccacaca cactgccttg
2221 ccaaaaatac cccgtgtctc ccctgccact cacctcactc ccattccctg agccctgatc
2281 catgcctcag cttagactgc agaggaacta ctcatttatt tgggatccaa ggcccccaac
2341 ccacagtacc gtccccaata aactgcagcc gagctcccca caaaaaaaaa aaaaaaa
```

(SEQ ID NO:25)

GenBank ACCESSION NM_014009

VERSION NM_014009.3 GI:167466188

An amino acid sequence of human FOXP3 is:

```
 1 mpnprpgkps apslalgpsp gaspswraap kasdllgarg pggtfqgrdl rggahassss
61 lnpmppsqlq lptlplvmva psgarlgplp hlqallqdrp hfmhqlstvd ahartpvlqv
121 hplespamis ltppttatgv fslkarpglp pginvaslew vsrepallct fpnpsaprkd
181 stlsavpqss ypllangvck wpgcekvfee pedflkhcqa dhlldekgra qcllqremvq
241 sleqqlvlek eklsamqahl agkmaltkas svassdkgsc civaagsqgp vvpawsgpre
301 apdslfavrr hlwgshgnst fpeflhnmdy fkfhnmrppf tyatlirwai leapekqrtl
361 neiyhwftrm faffrnhpat wknairhnls lhkcfvrves ekgavwtvde lefrkkrsqr
421 psrcsnptpg p
```

(SEQ ID NO:26)

GenBank ACCESSION Q9BZS1

VERSION Q9BZS1.1 GI:14548061

Levels of FOXP3 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring FOXP3 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), Abnova (Taipei, Taiwan), BD Biosciences (San Jose, CA, USA),

eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

IL-22

Interleukin-22 (IL-22) is a member of the IL-10 superfamily (which includes IL-19, IL-20, IL-24, and IL-26), a class of potent mediators of cellular inflammatory responses. IL-22 is produced by activated dendritic cells and T cells and initiates innate immune responses against bacterial pathogens especially in epithelial cells. IL-22 can be produced by effector Th17 cells and likely plays a role in the coordinated response of both adaptive and innate immune systems.

IL-22 can contribute to immune disease through the stimulation of inflammatory responses, S100 proteins, and defensins. In some contexts, the pro-inflammatory versus tissue-protective functions of IL-22 are regulated by the often co-expressed cytokine IL-17A.

A nucleotide (cDNA) sequence of human IL-22 is:

```
 1 cgaccaggtt ctccttcccc agtcaccagt tgctcgagtt agaattgtct gcaatggccg
61 ccctgcagaa atctgtgagc tctttcctta tggggaccct ggccaccagc tgcctccttc
121 tcttggccct cttggtacag ggaggagcag ctgcgcccat cagctcccac tgcaggcttg
181 acaagtccaa cttccagcag ccctatatca ccaaccgcac cttcatgctg gctaaggagg
241 ctagcttggc tgataacaac acagacgttc gtctcattgg ggagaaactg ttccacggag
301 tcagtatgag tgagcgctgc tatctgatga agcaggtgct gaacttcacc cttgaagaag
361 tgctgttccc tcaatctgat aggttccagc cttatatgca ggaggtggtg cccttcctgg
421 ccaggctcag caacaggcta agcacatgtc atattgaagg tgatgacctg catatccaga
481 ggaatgtgca aaagctgaag gacacagtga aaaagcttgg agagagtgga gagatcaaag
541 caattggaga actggatttg ctgtttatgt ctctgagaaa tgcctgcatt tgaccagagc
601 aaagctgaaa aatgaataac taacccccct tccctgctag aaataacaat tagatgcccc
661 aaagcgattt tttttaacca aaaggaagat gggaagccaa actccatcat gatgggtgga
721 ttccaaatga acccctgcgt tagttacaaa ggaaaccaat gccacttttg tttataagac
781 cagaaggtag actttctaag catagatatt tattgataac atttcattgt aactggtgtt
841 ctatacacag aaaacaattt atttttaaa taattgtctt tttccataaa aaagattact
901 ttccattcct ttaggggaaa aaacccctaa atagcttcat gtttccataa tcagtacttt
961 atatttataa atgtatttat tattattata agactgcatt ttatttatat cattttatta
1021 atatggattt atttatagaa acatcattcg atattgctac ttgagtgtaa ggctaatatt
1081 gatatttatg acaataatta tagagctata acatgtttat ttgacctcaa taaacacttg
1141 gatatcc
```

(SEQ ID NO:27)

GenBank ACCESSION NM_020525
VERSION NM_020525.4 GI:41393566

An amino acid sequence of human IL-22 is:

```
 1 maalqksvss flmgtlatsc llllallvqg gaaapisshc rldksnfqqp yitnrtfmla
61 keasladnnt dvrligeklf hgvsmsercy lmkqvlnftl eevlfpqsdr fqpymqevvp
121 flarlsnrls tchiegddlh iqrnvqklkd tvkklgesge ikaigeldll fmslrnaci
```

(SEQ ID NO:28)

GenBank ACCESSION Q9GZX6
VERSION Q9GZX6.1 GI:17366814

Levels of IL-22 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (e.g., on cellular extracts or cell culture media), flow cytometry (e.g., measuring intracellular or cell surface levels), and other methods known in the art. Reagents for measuring IL-22 levels are known and available in the art, e.g., antibodies (e.g., flouresecently labeled antibodies) (e.g., for cell surface staining, e.g., to measure cell surface expression) are available from Abcam (Cambridge, UK), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Detection and Isolation of Cells

Cells that express a marker (e.g., protein) of interest (for example, on their cell surface) can be identified and/or separated from a mixture of cells (e.g., that contains more than one cell type), for example, by use of an agent (e.g., antibody or ligand) that binds to the marker.

Detection and separation techniques include, e.g., flow cytometry, fluorescence activated cell sorting (FACS), immunomagnetic selection in a positive or negative (i.e., depletion) direction using paramagnetic or superparamagnetic beads or particles, or positive or negative immunoaffinity selection on bead or fiber matrix columns.

For flow cytometry and FACS, these techniques can be done using the appropriate primary antibodies labeled directly or indirectly with any of a number of available fluorochromes with desired spectral properties. Indirect labeling can be achieved by interposing a fluorochrome labeled secondary, tertiary or higher order antibody specific for

the immunoglobulin species, class or subclass of the primary or preceding antibody, or to a hapten-like tag on the primary or preceding antibody. Examples of flourochromes include DNP, digoxin, fluorescein (FITC), PerCP/Cy5, PerCP/Cy7, Phycoerythrin (PE), RPE, RPE-Cy5, APC, Cascade Yellow, PerCP, Alexa FLUOR® 405, Alexa FLUOR® 430, Alexa FLUOR® 488, Alexa FLUOR® 647, Alexa FLUOR® 700, RPE-Alexa FLUOR® 750, Alexa FLUOR®780, eFLUOR® 660, APC-eFLUOR® 780, eFLUOR® 605NC , eFLUOR® 625NC, eFLUOR® 650NC, eFLUOR® 450, Red 613, Hydroxycoumarin, Aminocoumarin, Methoxycoumarin, Cascade Blue, PACIFIC BLUE®, PACIFIC ORANGE®, Lucifer yellow, NBD, TruRed, PerCP-Cy5.5, FluorX, BODIPY-FL, TRITC, X-Rhodamine, Lissamine Rhodamine B, Texas Red, propidium iodide, 7-AAD, and biotin, among others known in the art. Alternatively, immunoglobulin-binding proteins such as protein A, G or L, or ligand-binding molecules such as avidin or streptavidin with affinity to biotin or like molecules can be employed in place of any secondary or higher order antibody. FACS instruments, primary and indirect secondary antibodies and related reagents for these purposes, and cell labeling and sorting protocols are well-known to those skilled in the art. See, e.g., Becton-Dickinson Immunocytometry Systemx (San Jose, CA, USA), Pharmingen (San Diego, CA, USA), and R&D Systems (Minneapolis, MN, USA) and Southern Biotech (Birmingham, AL, USA).

Similar labeling strategies can be employed using the primary antibody or antibodies directly or indirectly linked to magnetic particles or other matrix materials. Magnetic particles in a variety of configurations and modifications, along with antibodies and/or other accessory reagents, magnetic separators and matrix materials, and both specific and generic selection protocols that can be adapted for these purposes by those skilled in the art are available from numerous suppliers, such as MACS Microbeads from Miltenyi Biotec (Auburn, CA, USA), DynaBeads from Invitrogen (Carlsbad, CA, USA), MagCellect from R&D Systems (Minneapolis, MN, USA), and RosetteSep from StemCell Technologies (Vancouver, BC, Canada). Cell surface antigens can be employed in other direct or indirect labeling techniques to enrich cell types from a mixture of cells by negatively selecting or depleting undesired cells using, without limitation, complement-mediated cell lysis. The cells to be depleted might be distinguished, for example, by one or more antigens associated with certain lineages or stage(s) of differentiation. Undesired cells can be bound by antibody that is bound (directly or indirectly) to beads and thereby removed from solution,

leaving behind the desired cells. In another technique, the undesired cells in the cell mixture are labeled directly or indirectly with antibodies that are able to activate or fix complement, and then incubated briefly (usually an hour or less) with a source of active complement at or near physiological temperature (e.g., 37°C) during which time these cells undergo lysis. A commonly used source of such complement, among others known to those in the art, is non-heat-inactivated newborn rabbit serum, available for example from Invitrogen (Carlsbad, CA, USA).

As one example, to isolate poised Th17 cells, peripheral blood mononuclear cells (PBMCs) are isolated from a buffy coat, e.g., by Ficoll gradient centrifugation. CD4+ memory (CD45RO+) T cells are isolated from the PBMCs, e.g., by magnetic separation. CD25- CRTH2- cells are isolated from the CD4+ CD45RO+ cells, e.g., by antibody staining and fluorescence activated cell sorting. The resulting cells can be stained with anti-CCR6 and anti-CXCR3 antibodies and sorted for CCR6+ CXCR3- and CCR6+ CXCR3+ populations. The cells can additionally or alternatively be stained and sorted for IFN-g- and IFN-g+ populations, e.g., CCR6+ IFN-g- and CCR6+ IFN-g+ populations can be isolated by sorting. The cells are stimulated with anti-CD3/CD28 (e.g., T cell receptor (TCR) stimulation). As a final step in the isolation, IL-17- cells are isolated by antibody staining and sorting for IL-17- cells (for example, by negative selection of IL-17 secreting (IL-17+) cells). These cells can be cultured in IL-2-containing media (e.g., for 6 days) to promote conversion to Th17 effector cells.

Test Compounds

As used herein, the term "test compound" refers to an agent that is being tested for its ability to affect the conversion of a poised Th17 cell into an effector Th17 cell. The test compound can be any agent including, but not restricted to, a peptide, a peptoid, a polypeptide (such as an antibody), a lipid, a metal, a nucleotide, a nucleotide analog, a nucleoside, a nucleic acid, an organic compound, an inorganic compound, a small organic or inorganic molecule, a chemical compound (e.g., a low molecular compound, or a high molecular compound), a pharmacological agent, an element, a saccharide, an isotope, a carbohydrate, an imaging agent, a lipoprotein, a glycoprotein, an enzyme, an analytical probe, a polyamine, and combinations and derivatives thereof. A test compound can be naturally occurring or synthetically prepared. It can be isolated from a microorganism, an

animal, or a plant, and can be produced recombinantly, or synthesized, e.g., by a chemical method. Examples of nucleic acid compounds that may be used include RNAi, such as shRNA, siRNA, esiRNA, miRNA, oligo DNA, oligo RNA, a ribozyme, and antisense nucleic acid, such as antisense RNA. The test compound can have a formula weight of less than about 10,000 grams per mole, less than 5,000 grams per mole, less than 1,000 grams per mole, or less than about 500 grams per mole. The test compound of the disclosure may comprise a mixture of substances (e.g., purified or partially purified), for example, the test compound can be an extract (e.g., an extract derived from a marine organism, plant, animal, soil, or a phage display library), or the product of mixed combinatorial syntheses. The mixture can be tested, e.g., in a method described herein, and the component that affects the conversion of a poised Th17 cell into an effector Th17 cell can be purified from the mixture in a subsequent step.

A test compound can be obtained, for example, using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries (such as libraries of peptides or polypeptides (e.g., antibodies), e.g., phage display or eukaryotic cell (e.g., yeast) display libraries) or small molecule libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection.

Test compounds can be derived or selected from libraries of synthetic or natural compounds. For instance, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), ChemBridge Corporation (San Diego, CA, USA), ICCB Known Bioactives library (Enzo Life Sciences, Plymouth Meeting, PA, USA), Aldrich (Milwaukee, WI, USA) ComGenex (Princeton, N.J., USA), Asinex (Moscow, RU), Tripos, Inc. (St. Louis, MO, USA), Otava (Kyiv, UA), Light Biologicals (Shirley, NY, USA), and ChemStar, Ltd. (Moscow, RU). A natural compound library is, for example, available from TimTec LLC (Newark, DE, USA). Libraries of natural compounds in the form of bacterial, fungal, plant and animal cell and tissue extracts can be used. Test compounds can be synthetically produced using combinatorial chemistry either as individual compounds or as mixtures. A collection of compounds made using combinatorial chemistry is a combinatorial library. RNAi (e.g., miRNA, siRNA, esiRNA, or shRNA) libraries can also be prepared, and are available from, for example, Sigma-Aldrich (St. Louis, MO,

USA), System Biosciences (Mountain View, CA, USA), Open Biosystems (Huntsville, AL, USA), and Applied Biosystems/Ambion (Austin, TX, USA).

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Pat. No. 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al., Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (e.g., PCT Publication No. WO 91/19735), encoded peptides (e.g., PCT Publication No. WO 93/20242), random bio-oligomers (e.g., PCT Publication No. WO 92/00091), benzodiazepines (e.g., U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., Proc. Nat. Acad. Sci. USA 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara et al., J. Amer. Chem. Soc. 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., J Amer. Chem. Soc. 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen et al., J. Amer. Chem. Soc. 116:2661 (1994)), oligocarbamates (Cho et al., Science 261:1303 (1993)), and/or peptidyl phosphonates (Campbell et al., J. Org. Chem. 59:658 (1994)), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra), peptide nucleic acid libraries (see, e.g., U.S. Pat. No. 5,539,083), antibody libraries (see, e.g., Vaughn et al., Nature Biotechnology, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al., Science, 274:1520-1522 (1996) and U.S. Pat. No. 5,593,853), small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN, January 18, page 33 (1993); isoprenoids, U.S. Pat. No. 5,569,588; thiazolidinones and metathiazanones, U.S. Pat. No. 5,549,974; pyrrolidines, U.S. Pat. Nos. 5,525,735 and 5,519,134; morpholino compounds, U.S. Pat. No. 5,506,337; benzodiazepines, U.S. Pat. No. 5,288,514, and the like). Additional examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909; Erb et al. (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckemmann et al. (1994). J. Med. Chem. 37:2678; Cho et al. (1993) Science 261:1303; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al. (1994) J. Med. Chem. 37:1233.

The test compounds of the present disclosure can also be obtained from: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but

with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckermann, R. N. et al. (1994) J. Med. Chem. 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the `one-bead one-compound` library method; and synthetic library methods using affinity chromatography selection. The biological libraries include libraries of nucleic acids and libraries of proteins. Some nucleic acid libraries encode a diverse set of proteins (e.g., natural and artificial proteins; others provide, for example, functional RNA and DNA molecules such as nucleic acid aptamers or ribozymes. A peptoid library can be made to include structures similar to a peptide library. (See also Lam (1997) Anticancer Drug Des. 12:145). A library of proteins may be produced by an expression library or a display library (e.g., a phage display library).

Libraries of compounds may be presented in solution (e.g., Houghten (1992) Biotechniques 13:412-421), or on beads (Lam (1991) Nature 354:82-84), chips (Fodor (1993) Nature 364:555-556), bacteria (Ladner, U.S. Pat. No. 5,223,409), spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull et al. (1992) Proc Natl Acad Sci USA 89:1865-1869) or on phage (Scott and Smith (1990) Science 249:386-390; Devlin (1990) Science 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. 87:6378-6382; Felici (1991) J. Mol. Biol. 222:301-310; Ladner, U.S. Pat. No. 5,223,409).

A test compound may optionally comprise a detectable label. Such labels include, but are not limited to, enzymatic labels, radioisotope or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds, and bioluminescent compounds.

High throughput screening methods can be used to evaluate test compounds. High throughput screening methods involve providing a library (e.g., a combinatorial chemical, iRNA (e.g., miRNA, siRNA, esiRNA, or shRNA), or peptide library) containing a large number of test compounds (library members). Such libraries are then screened in one or more assays, as described herein, to identify those library members (e.g., particular chemical species or subclasses) that display a desired characteristic activity.

Test compounds and/or libraries thereof can be screened in one or more assays, as described herein, to identify those test compounds and/or library members (particular chemical species or subclasses) that display a desired characteristic activity, e.g., have an effect on poised Th17 cell conversion. The compounds thus identified can serve as

conventional "lead compounds,"e.g., which are varied (e.g., by derivatization), or can themselves be used as modulators of poised Th17 cell conversion.

Assays

An assay for identifying and/or selecting test compounds which affect poised Th17 cell conversion can include contacting a poised cell with a test compound and determining the ability of the test compound to modulate (e.g., increase or decrease (e.g., inhibit)) poised Th17 cell conversion to an effector Th17 cell (e.g., under conditions that favor conversion). Determining the ability of the test compound to modulate poised Th17 cell conversion can be accomplished, for example, by determining the effects of the test compound on IL-17 (i.e., IL-17A) expression, e.g., by detecting IL-17 nucleic acid (e.g., mRNA or cDNA) or protein in the cell. Levels of IL-17 can be detected, e.g., by measuring mRNA levels (e.g., by RT-PCR), and/or by measuring protein levels, e.g., by Western blot analysis, ELISA (enzyme-linked immunosorbent assay) (e.g., on cells, cellular extracts, or cell culture media), or flow cytometry (e.g., measuring intracellular levels or secretion (e.g., with a secretion detection kit)).

In evaluating a population of poised Th17 cells, the number or percentage of cells that express or secrete IL-17 after exposure to the test compound, under conditions that favor conversion, can be measured. Cell-based systems can be used to identify compounds that decrease IL-17 expression and/or poised Th17 cell conversion, and vice versa (compounds that increase IL-17 expression and/or conversion). Cells can be exposed to a test compound, under conditions that favor conversion, and after exposure, the cells are assayed, for example, for expression (e.g., intracellular and/or secreted of protein) of IL-17. The poised cells can be preincubated with a test compound (e.g., for about 5, about 10, about 20, about 30, about 40, about 50, about 60, about 90, or about 120 minutes) and then exposed to conditions that favor conversion. The poised cells can be exposed to (e.g., contacted with) a test compound and to conditions that favor conversion concomitantly or at approximately the same time. The poised cells can be preincubated in conditions that favor conversion (e.g., for about 5, about 10, about 20, about 30, about 40, about 50, about 60, about 90, or about 120 minutes) and then exposed to (e.g., contacted with) a test compound.

In addition to evaluating the cells for IL-17 expression (and/or secretion), the cells can also be evaluated, e.g., for FOXP3, IFN-g, and/or IL-22 expression and for effects on

proliferation. For example, effects on proliferation can be assessed by counting cell numbers and/or by CFSE (carboxyfluorescein succinimidyl ester) labeling of the cells.

Controls (e.g., a negative control and/or a positive control) can be included in the assay. For example, the number of poised Th17 cells that are IL-17+ (express IL-17) after exposure to a test compound, under conditions that favor conversion, can be compared to the number of poised Th17 cells that express IL-17 in the absence of the test compound under identical assay conditions (negative control). For example, poised Th17 cells can be cultured in media that contains IL-2 (e.g., 10 IU/ml), IL-7 (e.g., 20 ng/mL), or IL-15 (e.g., 20 ng/mL) in the presence and absence of a test compound. After a period of culturing, the cells can be evaluated to determine the number or percentage of cells that express IL-17, e.g., the number or percentage of poised Th17 cells that have converted to effector Th17 cells. If the number or percentage of cells that express IL-17 in the culture containing the test compound after the period of culturing differs from the number or percentage of cells in the culture that does not contain the test compound, the test compound modulates IL-17 expression and/or conversion to effector Th17 cells. If a greater number or percentage of cells in the culture containing the test compound express IL-17 after the period of culturing as compared to the number or percentage of cells in the culture that does not contain the test compound, the test compound promotes IL-17 expression and/or conversion to effector Th17 cells. If a smaller number or percentage of cells in the culture containing the test compound express IL-17 after the period of culturing as compared to the number or percentage of cells in the culture that does not contain the test compound, the test compound decreases (e.g., inhibits) IL-17 expression and/or conversion to effector Th17 cells. The difference in number or percentage can be a statistically significant difference (e.g., statistical significance by a T test (e.g., student's T test) (e.g., with a probability (p) value of $p<0.05$ or <0.01)), or can be a difference of about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90%, or greater (e.g., 1.5-, 2-, 3-, 4-, 5-fold or greater). As another control, it is possible to compare assay results to a reference, e.g., a reference value, e.g., obtained from the literature, a prior-run assay, and so forth. Appropriate correlations and art known statistical methods can be used to evaluate an assay result(s). Similar controls and evaluations can be included in assays measuring FOXP3, IFN-g, and/or IL-22 expression.

In the assays described herein, more than one concentration (e.g., serial dilutions) of a test compound can be tested.

Rapamycin can block the conversion of poised Th17 cells to effector Th17 cells (under conditions that favor conversion). As shown herein, rapamycin decreases IL-17 expression and increases FOXP3 expression in treated poised Th17 cells. Rapamycin, e.g., at a concentration of 10 nM-300 nM (e.g., 10 nM-30 nM), can be used as a positive control in one or more assays. Use of rapamycin at these concentrations gives approximately 50-60% inhibition of poised cell conversion. For example, the amount by which a test compound decreases conversion (under conditions that favor conversion) can be compared to the amount of inhibition by rapamycin.

Particular populations of poised Th17 cells can be used in the methods described herein, e.g., CXCR3+ or CXCR3-, and/or IFN-g+ or IFN-g-, and/or CD161+ or CD161-, and/or CRTH2+ or CRTH2- subsets of poised Th17 cells can be used to evaluate the effects of a test compound on the conversion of poised Th17 cells to effector Th17 cells and/or IL-17 expression and/or FOXP3 expression in those particular subsets of poised cells (under conditions that favor conversion).

Cell Culture

Human poised Th17 cells can be cultured in media suitable for culturing hematopoietic cells. For example, the cells can be grown in X-vivo media (e.g., X-vivo 10, X-vivo 15, or X-vivo 20 media) (BioWhittaker; available from Lonza, Walkerville, MD, USA). Other suitable media are known in the art and include, for example, AIM V® or OPTMIZER™ CTS™ T-Cell Expansion medium (Invitrogen, Carlsbad, CA, USA); STEMLINE II® medium (Sigma-Aldrich, St. Louis, MO, USA); Yssels' medium (Gemini Bio-Products. West Sacramento, CA, USA); and so forth.

The media can be supplemented, e.g., with an antibiotic (e.g., penicillin and streptomycin (PenStrep), e.g., 1% Pen Strep)); HEPES (e.g., 1% HEPES); L-Glutamine (e.g., 1% L-Glutamine); and/or sodium pyruvate (e.g., 1% sodium pyruvate), as known in the art.

The cells can be cultured, e.g., at about 37°C with 5-10% CO_2 (e.g., 5% CO_2), as known in the art.

Examples of conditions that favor poised Th17 cell conversion are: an X-vivo media supplemented with 1% Pen Strep, 1% HEPES, 1% L-Glutamine, 1% sodium pyruvate, and a gc-cytokine, for example, 10 IU/ml (or 20 IU/ml) of IL-2, IL-7 (20 ng/ml), or IL-15 (20 ng/ml).

EXAMPLES

Example 1: Isolation of Human Poised Th17 Cells

The example provides a procedure that was performed to isolate peripheral blood mononuclear cells (PBMC) from buffy coat and isolation of CD4+ memory cells from PBMC by magnetic separation and further isolation of poised Th17 cells from the CD4+ memory cells by high speed sorting. The example also provides procedures for culturing the aforementioned sorted poised Th17 cells and for intracellular staining on day 6 to measure conversion of these poised cells into IL-17 producing cells.

IL-2 is added to freshly isolated memory T cell subsets prior to stimulation with anti-CD3/anti-CD28-coated beads to maintain IL-17 expression. Following FACS sorting of IL-17- or IL-17+ memory T cell subsets, these cells are cultured in IL-2-supplemented medium again. In this context, expansion of IL-17- or IL-17+ memory T cells is required to induce *de novo* IL-17 expression.

- **EQUIPMENT**
  - Beckman GS-6R centrifuge, or equivalent
  - Pipet–aid, Drummond, or equivalent
  - Pipettor, single channel, 10-200 ul calibrated, Rainin, or equivalent

- **MATERIALS**
  - Disposable pipette tips
  - Centrifuge tubes, 15- and 50 ml (Corning)
  - Hemacytometer and cover slip
  - Corning filter systems (500 ml filter unit; VWR Cat. No. 28199-803)
  - T75cm^2 tissue culture flasks (Costar, Cat. No. 430720)
  - Ficoll Paque (GE Amersham, Cat. No.)
  - Trypan Blue (GIBCO, Cat. No. 15250061)

- 6-Well Plate (Costar, Cat. No. 3156)
- 24-Well Plate (Costar, Cat. No. 3526)
- 48-well Plate (Costar, Cat. No. 3548)
- 96-well Round bottom Plate (Costar, Cat. No. 3599)
- 96-well V-bottom Plate (Costar, Cat. No. 3799)

- **ISOLATION OF PBMC FROM BUFFY COAT**
- **All centrifugation for PBMC isolation is performed at 20^0C**
  - Working in a bio-safety hood, transfer the contents from the buffy coat bag to a 50.0mL centrifuge tube (Approximately 30.0mL of buffy coat is received per bag).
  - Distribute 30.0mL of buffy coat into 6X 50.0mL conical.
  - Bring up the volume in each of the 6X50.0mL conical to 35.0mL with 1X DPBS.
  - Slowly underlay 12.0ml of Ficoll into each 50.0mL conical containing buffy coat diluted with 1X DPBS.
  - Centrifuge the buffy coat at 1800 rpm for 25 minutes (min.) without brakes.
  - Carefully remove the lymphocyte layer and transfer to another 50.0mL centrifuge tube.
  - Bring up the volume to 50.0mL with 1X DPBS.
  - Centrifuge at 1500 rpm for 10 min.
  - Aspirate the supernatant and resuspend the cell pellet in 50.0mL 1XDPBS.
  - Determine the viable cell yield by taking a small aliquot and diluting in trypan blue and using hemacytometer and cover slip.

- **ISOLATION OF CD4+ MEMORY T-CELLS FROM PBMC BY MAGNETIC SEPARATION**
- **All centrifugation during magnetic separation is performed at 4^0C**
  - **MATERIALS**
    - Phosphate Buffered Saline (Sigma, Cat. No. 14190)
    - Bovine Serum Albumin (Sigma, Cat. No. A7906)
    - EDTA (Sigma, Cat. No. 059K8706)

- RPMI 1640 Medium containing L-Glutamine and 25mM HEPES (Invitrogen, Cat. No. 22400105)
- Fetal Bovine Serum (Sigma, Cat. No. F2442)
- Pen Strep (Invitrogen, Cat. No 15140-122)
- Sodium Pyruvate (Invitrogen, Cat. No. 11360070)
- CD4+ Memory T-cell Isolation Kit (Miltenyi, Cat. No.130-091-893)
- LS Separation Columns (Miltenyi, Cat. No. 130-042-401)
- MACS Separator (Miltenyi, Cat. No. 130-090-076)

- **REAGENTS**
  - MACS Buffer: Prepare a buffer containing Phosphate buffered saline (pH 7.0), 0.5% Bovine Serum Albumin and 2mM EDTA. Sterile filter and store at 2-8°C.
  - T-cell Medium: Sterile filter 475.0mL of RPMI 1640 medium containing L-Glutamine and 25mM HEPES medium, 25.0mL fetal bovine serum, 5.0mL Pen Strep and 5.0mL Sodium Pyruvate. Store at 2-8^{0}C.

- **MAGNETIC LABELING AND ISOLATION CD4+ MEMORY T-CELLS**
- Determine cell number and aliquot 2X10^8 PBMC per 15.0mL centrifuge tube.
- Centrifuge at 1500 rpm for 10 min. and aspirate supernatant.
- Resuspend cell pellet in 40ul of MACS buffer per 10^7 cells.
- Add 10ul of memory CD4+ T-cell Biotin-Antibody cocktail per 10^7 cells.
- Mix well and incubate in the refrigerator at 2-8^{0}C for 10 minutes.
- Add 30ul of buffer per 10^7 cells.
- Add 20ul of Anti-biotin microbeads per 10^7 cells.
- Mix well and incubate in the refrigerator at 2-8^{0}C for 15 minutes.
- During this incubation, prepare LS columns by placing the column in the magnetic field and rinsing with 3.0mL of MACS buffer.
- Wash cells with 10.0mL of MACS buffer.
- Aspirate supernatant and resuspend cell pellet in 1.0mL MACS buffer (500ul per 10^8 cells).
- Apply the cell suspension the LS column.

- Collect the cells that pass through the column and wash the column 3X with 3.0mL MACS buffer.
- The total effluent is the enriched CD4+ Memory T-cells.
- Spin the cells at 1500 rpm for 10 min. and aspirate supernatant.
- Resuspend in T-cell medium and determine the viable cell number by trypan blue stain and counting by hemacytometer.
- Resuspend cells at 3 million cells per mL (A buffy coat yields approximately 60-100 million CD4+ memory T-cells).
- Transfer cells to T75cm^2 tissue culture flask and rest cells overnight at 37^{0}C, 5% CO_2.

- **ISOLATION AND CULTURE OF POISED TH17 CELLS**
  - **REAGENTS**
  - Anti human CCR6-PercpCy5.5 (Biolegend, Cat. No 335505)
  - Anti human CRTH2-APC (B D Pharmingen, Cat. No. 558042)
  - Anti human CXCR3-A488 (Biolegend, Cat. No. 334901)
  - Anti human CD25-A700 (Biolegend, Cat. No. 302622)
  - IL-17 Secretion Assay-Detection Kit-PE (Miltenyi, Cat. No. 130-094-537)
  - Recombinant human IL-2 (B D Bioscience, Cat. No 354043)
  - Anti CD3/CD28 beads (Invitrogen, Cat. No 111-31D)
  - FACS Buffer**:** Sterile filter 490.0mL of 1xDPBS and 10.0mL of fetal bovine serum. Store at 2-8^{0}C.
  - Sorting Buffer: RPMI 1640 medium containing 1% PenStrep, 2-8°C
  - Th17 Culture Medium: Sterile filter X-vivo15 media containing 1% Pen Strep, 1% HEPES, 1% L-Glutamine, 1% Sodium Pyruvate. Store at 2-8°C.

  - **EQUIPMENT**
  - BD FACS Aria II

  - **STAINING AND HIGH SPEED SORTING OF POISED TH17 CELLS**
  - Remove CD4+ memory T-cells from the incubator and spin cells down.

- Aspirate supernatant.
- Resuspend cells at 20 million cells per mL in FACS buffer.
- Add Anti-human CCR6-PrecpCy5.5, CRTH2-APC, CXCR3-A488 and CD25-A700 at 1:30.
- Incubate at room temperature for 20 minutes.
- Wash 1X with FACS buffer and aspirate supernatant.
- Resuspend cells at 30 million cells per mL in sorting buffer.
- Sort cells on FACS Aria II, by gating on CD25-Negative CRTH2-Negative cells and sorting the following cell populations:
    - CCR6-
    - CCR6+CXCR3-
    - CCR6+CXCR3+
- Collect the sorted cells in 12X75mm BD Polystyrene tubes containing 1.0mL T-cell medium.
- Resuspend sorted cell populations at 2million cells per ml in T-cell medium containing 10 IU recombinant human IL-2.
- Place cells overnight in the incubator at 37°C, 5% CO_2.

- **IL-17 SECRETION AND CULTURE**
- Remove cells from the incubator and stimulate the sorted CD4+memory cell populations for a period of 18 hours at 37°C, 5% CO_2 with anti CD3/CD28 beads at bead to cell ratio of 3:1.
- Remove cells and wash cells in ice cold MACS buffer at 1500rpm for 10 minutes.
- Aspirate supernatant.
- Resuspend cells in 80ul of cold medium per 10^7 cells.
- Add 20ul of IL-17 Catch Reagent per 10^7 cells and incubate cells on ice for 5 minutes.
- Add 10.0mL of warm T-cell media per 10^7 cells.
- Incubate cells at 37°C, 5% CO_2 for 45 minutes on a shaker.
- Wash cells with ice cold MACS buffer and aspirate supernatant.
- Resuspend cells in 80ul of cold MACS buffer per 10^7 cells.

- Add 20ul of IL-17 PE detection antibody per 10^7 cells.
- Incubate cells on ice for 10 minutes.
- Wash cells with ice cold MACS buffer and aspirate supernatant.
- Resuspend cells at 20 million cells per mL in sorting buffer.
- Sort IL-17 negative cells from each of the following cell population using FACS Aria II cell sorter:
    - CCR6-
    - CCR6+CXCR3-
    - CCR6+CXCR3+
- Collect the sorted cells in 12X75mm BD Polystyrene tubes containing 1.0mL T-cell medium.
- Resuspend sorted cell populations at 2million cells per ml in Th17culture medium containing 10 IU/ml recombinant human IL-2.
- Place cells in the incubator at 37°C, 5% CO_2.
- Split and replenish cells with 10 IU/ml recombinant human IL-2 in Th17 culture media for 5 days.
- Harvest cells on day 6.

- **DAY 6 INTRACELLULAR STAIN ON POISED TH17 CELLS**
    - **REAGENTS**
        - PMA-Phorbol 12-myristate 13-acetate (Sigma, Cat. No. P8139). Prepare a stock solution of 1.62mM by dissolving in ethanol. Store small aliquots at -80°C.
        - Ionomycin (Sigma, Cat. No.I0634) Prepare a stock solution of 13.4mM by dissolving in ethanol. Store small aliquots at -80°C.
        - BrefeldinA (Sigma, Cat. No.B7651). Prepare a stock solution of 5mg/mL by dissolving in ethanol. Store small aliquots at -80°C.
        - Anti human IFN-g-FITC (Biolegend, Cat. No. 502506)
        - Anti Human IL-17 Pacific Blue (Biolegend, Cat. No. 512312)
        - Fixation Buffer:1X PBS+4%Paraformaldhyde
        - Permebilization Buffer: 1XPBS+1%BSA+0.5% Saponin

- **EQUIPMENT**
  - BD LSR II

- **INTRACELLULAR STAINING**
  - Remove the cells from the incubator and transfer 300 ul of the cells to a 96-well V-bottomed plate.
  - Wash 1X with T-cell media.
  - Stimulate cells with PMA and Ionomycin in the presence of brefeldin A for 3 hours at 37°C, 5% CO2.
  - After 3 hours, spin cells and discard supernatant.
  - Add 75ul of fixation buffer and incubate at room temperature for 20 minutes.
  - Add 150ul of 1XPBS and spin cells at 2000 rpm for 1 minute.
  - Aspirate the supernatant.
  - Add 50ul of anti-human IFN-g FITC and anti-human IL-17A Pacific Blue antibodies in permeabilization buffer at a dilution of 1:50.
  - Incubate at 4°C for 20 minutes.
  - Wash the cells 1X with PBS.
  - Resuspend the cells in 150ml of PBS+1% Paraformaldehyde and acquire the samples using BD LSRII flow cytometer.

Example 2: Screening Compounds for Effect on Poised Th17 Cell to Effector Th17 Cell Conversion

The example provides a procedure that was performed to evaluate test compounds for their effects on poised Th17 cell conversion to effector Th17 cells.

- **METHOD TO SCREEN COMPOUNDS THAT INHIBIT THE PRODUCTION OF IL-17 IN POISED TH17 CELLS**
  - Make a series of five, 10-fold serial dilution of the test compounds, in X-vivo 15 medium (BioWhittaker) starting at 2X concentration of 10um.
  - X-vivo 15 media containing the same volume of DMSO as the highest concentration of the test compound serves as a control.

- Add $5x10^4$ poised memory cells from the sort (CCR6-; CCR6+CXCR3-; and CCR6+CXCR3+ populations) in 100 ul X-vivo 15 medium containing recombinant human IL-2 at 2X concentration of 20 IU/mL to a round bottomed 96-well plate.
- Add 100 ul of the test compound from the serial dilutions (10uM, 1uM, 0.1uM, 0.01uM, 0.001uM) made as mentioned above in duplicate.
- Incubate at 37°C, 5%CO_2.
- Split the cells every other day by adding compound and recombinant human IL-2 in X-vivo 15 medium.
- Analyze cells on day 6 by intracellular staining for IL-17A and IFN-g.

Example 3: Human CCR6+ memory T cells are poised to express IL-17

Human CD4+ naïve T cells (T_N) (CCR7hi CD45RO-) were FACS sorted and activated using anti-CD3/anti-CD28 coated beads (Dynal) (2 beads/cell) in the presence of TGFbeta (30ng/mL), IL-6 (30ng/mL), anti-IL-4 (0.5ug/mL), and anti-IFNγ (1ug/mL) (all from R&D Systems) for 2 days. Cells were then expanded in IL-1B (20ng/mL), IL-2 (10U/mL), and IL-23 (20ng/mL) for 5-7 more days to induce Th17 differentiation. The anti-CCR7 antibody was obtained from BioLegend. Cytokine production was determined by intracellular cytokine staining following a 4-hour restimulation with PMA (20nM) and ionomycin (1uM) in the presence of brefeldin A (BFA) (20ug/mL). The results are shown in FIG. 1. These data demonstrate that only a small minority (typically 1-5%) of naïve human T cells can differentiate into Th17 cells *in vitro*.

Human memory T cells (CD4+ CD45RO+) (T_{MEM}) were stimulated through the T Cell Receptor (TCR) using antibody-coated beads as in FIG. 1 for 18-24 hours to induce cytokine expression. These activated cells were harvested and stained for CCR6 expression and active IL-17A secretion using a commercially-available kit (Miltenyi). Stained cells were FACS sorted to obtain: (1) CCR6- IL-17A-, (2) CCR6+ IL-17A-, and (3) CCR6+ IL-17A+ cells. Each population was subsequently cultured in medium supplemented with 10U/mL interleukin (IL)-2. After 6 days in culture, cytokine production was determined as in FIG. 1. The results are shown in FIG. 2. These data demonstrate that a sizeable portion of CCR6+ IL-17A- human memory T cells (typically ~30%) can be induced to express IL-17A following *in vitro* expansion in IL-2-containing medium. Importantly, this capacity to

express IL-17A is restricted to CCR6+ memory T cell compartment as CCR6- IL-17A- T cells produce very low amounts of IL-17A.

Naïve T cells differentiated to Th17 as in FIG. 1 were stained as in FIG. 2 for expression of CCR6 and IL-17A following restimulation on day 7 for 18-24 hours with antibody-coated dynabeads (2 beads/cell). These cells were sorted as in FIG. 2 and cultured for an additional 7 days in IL-2-supplemented medium (10U/mL). Cytokine production was determined as above following restimulation with PMA (20nM) and ionomycin (1uM). The results are shown in FIG. 3. These data show that CCR6+ IL-17A- T cells derived differentiated *in vitro* from naïve precursors are fundamentally different from CCR6+ IL-17A- memory T cells (poised Th17 cells) isolated *ex vivo*, in that they cannot be induced to express IL-17A. Unlike poised Th17 cells which are CD45RO+, T cells derived differentiated *in vitro* from naïve precursors are CD45RO-.

Memory CCR6- IL-17A- and CCR6+ IL-17A- T cells were sorted and cultured in IL-2-supplemented medium as in FIG. 2. Cells were harvested at the indicated time points and restimulated with PMA (20nM) and ionomycin (1uM) to determine IL-17 production. See FIG. 4. Data are presented as the mean percentage of IL-17A+ T cells $\pm$ SD from triplicate samples. These experiments show the rapid kinetics of IL-17A induction in CCR6+ but not CCR6- IL-17A- human memory T cells.

Memory CCR6- IL-17A- or CCR6+ IL-17A- T cells were sorted as described above and cultured for 6 days in the presence of the indicated recombinant cytokines and neutralizing Abs. IL-17A production was determined after restimulation with PMA and ionomycin. The results are shown in FIG. 5. Data are presented as the mean percentage of IL-17A+ T cells $\pm$ SD from triplicate samples. These experiments demonstrate that IL-2 signaling is the principle driver of IL-17A expression in CCR6+ IL-17A- human memory T cells. Whereas IL-7 or IL-15 can substitute for IL-2 in this regard, IL-1beta, IL-6, IL-21, or IL-23, which collectively drive IL-17A production in human naïve T cells, have little impact on IL-17A expression in CCR6+ IL-17A- human memory T cells.

CCR6- IL-17A- or CCR6+ IL-17A- T cells were sorted as described above and cultured for 6 days in IL-2 alone (10U/mL) or with IL-2 (10U/mL) and IL-23 (20ng/mL). On day 6, cells were harvested, restimulated for 18-24 hours using antibody-coated dynabeads (2 beads/cell) and IL-17A-secreting T cells were FACS sorted following staining with IL-17A secretion kit (Miltenyi). These IL-17A+ T cells were then cultured for an

additional 6 days in IL-2 alone or IL-2 plus IL-23 as above. Cells were restimulated with PMA (20nM) and ionomycin (1uM) to determine IL-17 production via intracellular cytokine staining. The results are shown in FIG. 6. Bar graphs showing the percentage of IL-17A+ T cells at the end of the two-week cultures are presented from single samples. All data are representative of at least 3 independent experiments. These data demonstrate that IL-2 alone is sufficient to maintain stable IL-17A expression within CCR6+ human memory T cells.

Example 4: Expression of CXCR3 defines at least 2 distinct poised human Th17 cell populations

Human CD4+ memory T cells were stained for expression of CXCR3 prior to TCR-activation, then stimulated with PMA (20nM) and Ionomycin (1uM) in the presence of brefeldin A (20ug/mL). Following 4 hours of stimulation, the cells were further stained for CCR6, CD161, IL-17A and IFN-g. The anti-CD161-PE antibody was from BD Biosciences; the remaining antibodies were as described above. Percent positive of CD161 and IFN-g within each of CCR6+ CXCR3+ and CCR6+ CXCR3- is shown. The results are shown in FIG. 7. These experiments demonstrate that CCR6+ CXCR3- IL-17A-, and CCR6+ CXCR3+ IL-17A- subsets both express the Th17-associated cell surface receptor, CD161, but differ in their expression of the Th1-associated cytokine IFNg (i.e., these subsets are phenotypically distinct).

Human memory T cells (CD4+ CD45RO+) were FACS sorted into: (1) CCR6- IFNg- IL-17A-, (2) CCR6- IFNg+ IL-17A-, (3) CCR6+ IFNg- IL-17A-, (4) CCR6+ IFNg+ IL-17A-, or (5) CCR6+ IL-17A+ cells. Gene expression of Th1-, Th2-, or Th17-associated transcripts was measured by quantitative nuclease protection assays (High Throughput Genomics, Inc.) in sorted T cell subsets. The results are shown in FIG. 8. Data are presented as mean relative transcript abundance $\pm$ SD normalized to the endogenous control genes *Rpl19* and *Ywhaz*. These experiments show that CCR6+ IFNg+ IL-17A- T cells express several Th1-associated genes, but also express the Th17-associated transcription factor RORgt (human RORgt is also referred to as RORC2), similar to other CCR6+ T cell subsets. These data suggest that expression or lack of expression of CXCR3 or IFNg define distinct T cell subsets that are both poised to express IL-17A.

Human CCR6- IL-17A-, CCR6+ CXCR3- IL-17A-, CCR6+ CXCR3+ IL-17A-, or CCR6+ IL-17A+ T cells were FACS sorted and cultured for 6 days in IL-2 supplemented medium (10IU/mL). Cells were restimulated with PMA (20nM) and ionomycin (1uM) in the presence of brefeldin A (20ug/mL) for 4 hours on day 6 and cytokine production was determined via intracellular staining. The results are shown in FIG. 9, and suggest that both CCCR6+ CXCR3- IL-17A- and CCR6+ CXCR3+ IL-17A- T cells are similar in their ability to express IL-17A, but differ in that CXCR3+ T cells also stably express the Th1-associated cytokine IFNg. Therefore, CCR6+ CXCR3- and CCR6+ CXCR3+ T cells are likely functionally distinct pro-inflammatory T cell subsets *in vivo*.

Human CCR6- IL-17A-, CCR6+ CXCR3- IL-17A-, or CCR6+ CXCR3+ IL-17A- T cells were FACS sorted from 5 individual healthy adult donors and cultured in IL-2-containing medium for 6 days as in FIG. 9. The percentage of IL-17 positive cells in each expanded population was determined following restimulation with PMA (20nM) and ionomycin (1uM) as described above. The results are shown in FIG. 10. These data show that CCR6+ CXCR3- IL-17A- T cells differentiate into IL-17A producing T cells with a slightly higher frequency than do CCR6+ CXCR3+ IL-17A- T cells, although both subsets differentiate into IL-17 secreting cells at a much higher frequency compared to CCR6- T cells.

Example 5: Induction of IL-17A and FOXP3 expression in poised Th17 cells

Human poised Th17 (CCR6+ CXCR3- IL-17A-) cells were FACS sorted and cultured with recombinant human IL-2 (10U/mL), anti-human IL-2 (1ug/mL), or recombinant human IL-2 (10U/mL) plus 30ng/mL rapamycin for 6 days. IL-17A and FOXP3 expression were determined via intracellular staining following restimulation with PMA and ionomycin as above. The results are shown in FIG. 11. These results show that IL-17A and FOXP3 expression are induced in poised Th17 cells in an IL-2-dependent manner and that rapamycin, in the presence of IL-2, inhibits the induction of IL-17A, whilst enhancing FOXP3, expression.

Human naïve T cells (CD45RA+), or memory T cell subsets CCR6- IL-17A-, CCR6+ CXCR3- IL-17A-, or CCR6+ CXCR3+ IL-17A- were FACS sorted and either left unstimulated (grey, shaded peaks), or cultured in 10U/mL IL-2-containing medium for 24 hours (black outline peaks). Cells were harvested and Stat3 and Stat5 tyrosine

phosphorylation was analyzed via flow cytometry following intracellular staining using phospho-specific monoclonal antibodies (BD Biosciences). The results are shown in FIG. 12. The results demonstrate that exposure of all T cell subsets, most notably all 3 memory T cell subsets (bottom three rows), to IL-2 induces tyrosine phosphorylation of both Stat5 and Stat3. These results are representative of 4 independent experiments.

Example 6: Identification of poised Th17 cells in mice

Spleen and lymph nodes harvested from wild-type C57B6/J mice purchased from the Jackson laboratory were pooled. CD4+ T cells were enriched via magnetic sorting using the mouse CD4+ T cell isolation kit II as per manufacturer's instructions (Miletnyi Biotech, USA). These cells were stimulated using plate-bound anti-CD3/anti-CD28. Briefly, tissue culture wells were coated with 0.3 mg/mL goat-anti-hamster IgG (MP Biomedicals, Inc.) for 1 hour at 37°C. Coated wells were washed twice with sterile PBS to remove unbound antibody, and cells were immediately added along with 0.3ug/mL anti-CD3 (145-2C11; eBioscience), and 0.5ug/mL anti-CD28 (BD Bioscience). Cells were stimulated for 14-18 hours at 37°C to induce cytokine secretion. Activated cells were then stained with: CD4-Pacific Orange (Invitrogen), CD62L-Pacific Blue (eBioscience), CCR6-PE (Biolegend), IL-17A-biotin (Miltenyi Biotech), and streptavidin-APC (eBioscience) in accordance with the cytokine capture assay product manual (Miltenyi Biotech, Inc.). Cells were sorted on a FACSAria II (BD Biosciences) into the following subsets (shown in FIG. 13A): $CD62L^{lo}$ CCR6- IL17A-, $CD62L^{lo}$ CCR6+ IL17A-, and $CD62L^{lo}$ CCR6+ IL17A+. CD62L is expressed on naïve, but not memory T cells. Therefore, $CD62L^{lo}$ cells are used so as to obtain pure populations of either CCR6+ or CCR6- memory T cells. Because CCR6 is exclusively expressed on memory T cells, failure to include the CD62L marker would result in a CCR6- population of cells that contained both naïve and memory T cells, and therefore would not be a fair comparison with the exclusively memory CCR6+ cell fraction. "Lo", in reference to expression of CD62L in memory T cell subsets (e.g., $CD62L^{lo}$), refers to no or minimal levels of staining, as compared to a "hi" (high) level of staining for the same marker in a given population of cells. For example, in panel (ii) of FIG. 13A, levels of CD62L staining are measured on the x-axis. In this panel, at least two populations of cells, with two different levels of CD62L staining are seen. The two left boxed populations (containing 2.7 and 67.1 percent of the total cells) have lo levels of CD62L staining as

compared to the right boxed population; the right boxed population (containing 18.4 percent of the total cells) has hi levels of CD62L staining. "Lo" expression of CD62L can be operationally defined by the expression of CD62L within the CCR6+ subset (e.g., because CCR6+ T cells are exclusively memory T cells); a corresponding gate is thus set to isolate CCR6- CD62Llo memory T cells.

2×10^4 cells of each phenotype were resuspended in 100 uL mouse T cell medium (see recipe below) supplemented with 10U/mL recombinant human IL-2 (Invitrogen) and 10^4 mouse T cell expander beads (1:2 beads:cells ratio) (Invitrogen). Cells were cultured for 6 days in round-bottom 96-well plates and fresh IL-2-supplemented mouse T cell medium was added on day 2 and day 4.

To prepare mouse T cell medium, standard DMEM + 4.5g/L glucose (MediaTech, Inc.) was supplemented with 10% Fetal bovine serum (Thermo Fisher), and 1% of each of the following: MEM Essential Vitamin solution (Gibco), Non Essential Amino Acids (Gibco), Sodium Pyruvate (Gibco), 1M HEPES (Gibco), Penicillin/ Streptomycin (Gibco), L-glutamine (Gibco) plus 2-mercaptoethanol (Sigma-Aldrich), where a 14.3M stock solution of 2-mercaptoethanol is diluted to 5 mM in sterile water containing 100x (400 mM) L-glutamine, and L-Arginine/ L-Asparagine/ Folic acid mix (see recipe/ protocol below).

100X Arg/Asp/Folic Acid:

L-arginine-HCl-11.6gms

L-asparagine- 3.6gms

Folic acid- 0.6gms

1M NaOH- 27.2mL approx

1. Dissolve arginine, asparagine, folic acid in ~800mL ddH_2O
2. Add 1M NaOH very slowly until folic acid dissolves (less than 27.2mL may be needed)
3. Bring volume up to 1L with ddH_2O
4. Filter with 0.22um filter and store in 50.0mL aliquots in -20^0C

On day 6, cells were restimulated with PMA (10nM), Ionomycin (1uM), and Brefeldin A (10ug/mL) for 3 hours, as described for human T cell stimulation, and these cells were stained with 1 ug/mL anti-IFNg-Percp-Cy5.5 (eBioscience), and 1 ug/mL anti-IL17A-APC (Biolegend). Data were acquired on LSRII Fortessa flow cytometer (BD Biosciences) and analyzed via FloJo software (Treestar, Inc.). The results are shown in FIG. 13B. The data show that, similar to human memory T cells, CCR6+ IL17A-, but not CCR6-

IL17A- mouse memory T cells induce *de novo* expression of IL-17A in response to IL-2 signaling. These data indicate that poised Th17 cells are present in mice and the same cytokine signals (i.e., IL-2, IL-7, or IL-15) drive conversion of poised Th17 (IL17A-) cells into IL-17A-secreting cells.

As poised Th17 cells are present in mice as well as in humans, mouse poised Th17 cells can be isolated and used in the same types of assays as human poised Th17 cells, e.g., as described herein. For example, the effects of test compounds on the conversion of poised Th17 cells into effector Th17 cells can be evaluated in the mouse cells.

Example 7: Variation on the human poised Th17 cell isolation protocol and its use for screening of small molecule (or RNAi) libraries

We have shown that IL-2, IL-7, or IL-15 induces de novo IL-17 expression in ex vivo-isolated CCR6+ IL-17- (poised Th17) cells. The protocol used to isolate pure CCR6+ IL-17- memory T cell populations yields typically fewer than 10^6 cells per 500 mL of human peripheral whole blood. In order to develop a protocol more amenable to larger scale screens (e.g., small molecule or RNAi screens), we have developed methods wherein resting (i.e., not stimulated through TCR) CCR6+ memory T cells (CD4+ CD45RO+ CCR6+ CD25-), or total memory T cells (CD4+ CD45RO+) are purified and stimulated with IL-7 or IL-15 to induce IL-17 expression. This protocol allows for more rapid and less expensive isolation of larger subsets of cells that can range between 0.2-1 x 10^8 cells per 500 mL of human peripheral whole blood.

Briefly, total CD4+ CD45RO+ memory T cells are isolated from whole blood via immunomagnetic depletion columns (StemSep). CCR6+ CD25- memory T cells can be FACS sorted from the total memory cell preparation using the antibodies described above. Resting total memory or CCR6+ CD25- memory T cells are then cultured in serum-free Ex vivo-15 T cell medium as described above supplemented with either 20 ng/mL recombinant IL-7 or IL-15 (both from R&D systems), or cultured in the absence of exogenous cytokines for 6 days. Small molecule compounds are added at day 0 and again at day 3. Cells are then stimulated with PMA and ionomycin on day 6 to induce activation of the cells; supernatants are harvested to measure cytokine secretion by cytokine-specific (e.g., IL-17A) ELISA, and a CellTiter-Glo luminescent assay (Promega) is performed on the cells to quantify the number of viable T cells within each well. The effects of compounds on IL-7 or IL-15-

induced IL-17 secretion within CCR6+ or total memory T cells is normalized to cell numbers as determined by CellTiter-Glo. An exemplary data set showing the utility of the screening protocol is shown in FIGS. 14A and 14B. IL-7 or IL-15 is used for screening purposes because resting CCR6+ or total memory T cells are used and the receptors for these cytokines, unlike the high affinity IL-2 receptor that is only expressed on recently activated T cells, are constitutively expressed on resting memory T cells (human and mouse).

Example 8: CCR6+ IL-17- memory T cells isolated from rheumatoid arthritis patients upregulate IL-17 expression in response to γc-cytokine stimulation

The fact that *ex vivo*-isolated human CCR6+ IL-17- memory T (TM) cells can induce *de novo* IL-17 expression upon γc-cytokine stimulation indicates that current methods used for *ex vivo* analyses of IL-17 expression underestimates the frequency of human memory T cells that can express IL-17 in inflammatory settings, and would therefore be considered human Th17 cells. Because a number of studies investigate changes in Th17 frequencies within autoimmune patient cohorts, we asked whether CCR6+ IL-17- memory T cells isolated from the peripheral blood of patients with rheumatoid arthritis (RA) could be similarly induced to express IL-17 by IL-2 stimulation. The frequency of IL-17-producing cells within CCR6- or CCR6+ subsets was determined from the peripheral blood of 4 RA donors in the following two manners: (1) directly *ex vivo* following 4 hour PMA and ionomycin stimulation, or (2) following the expansion of FACS-sorted CCR6- IL-17- or CCR6+ IL-17- T cell subsets in IL-2 followed by 4 hour PMA and ionomycin stimulation. Indeed, we observed that CCR6+, but not CCR6-, IL-17- memory T cells from RA patients upregulated IL-17 after culture with IL-2 to similar levels observed in healthy adult donors (FIG. 15). Moreover, the frequency of IL-17-producing T cells within expanded CCR6+ IL-17- T cells was substantially higher than those observed for *ex vivo*-stimulated CCR6+ memory T cells. Thus, the frequency of IL-17+ T cells following *ex vivo* stimulation within autoimmune patient blood samples does not capture the full repertoire of memory T cells that can express IL-17 in response to inflammatory cytokine stimulation (e.g., IL-2, IL-7, or IL-15).

Example 9: Heterogeneous CCR6+ memory T cells are poised to upregulate IL-17 expression in response to gc-cytokine stimulation

Human Th17 cells (i.e., IL-17+ T cells) are reportedly enriched within subsets of CCR6+ memory T cells that are CXCR3- or CD161+ compared to CCR6+ CXCR3+ or CCR6+ CD161- cells. Indeed, a significant proportion of human CCR6+ memory T cells co-expressed either CD161 or CXCR3; CD161 expression is enriched within CCR6+ cells, whereas expression of CXCR3 is enriched within the CCR6- compartment (FIG. 16A). Consistent with previous studies, CCR6+ CD161+ T cells are highly enriched for IL-17-producing cells upon *ex vivo* stimulation compared to CCR6+ CD161- cells, whereas CCR6+ CXCR3+ cells produce less IL-17 but more IFNγ than CCR6+ CXCR3- cells (FIG. 16A). As shown in FIG. 16A, *ex vivo* (day 0) isolated CD4+ memory T cells (TM cells) were co-stained with CCR6, CD161, and CXCR3. The frequency of CCR6+ cells positive or negative for either CD161 (two left panels), or CXCR3 (two right panels) are shown. Ex vivo cytokine production was determined following PMA and ionomycin stimulation within each gated CCR6+ TM cell subset as indicated. FACS plots show the intracellular expression of IL-17, IL-22, and IFNγ.

To address the possibility that IL-17 upregulation by CCR6+ IL-17- cells is restricted to pre-defined CCR6+ sub-populations, we fractionated CCR6+IL-17- cells based on either CD161 or CXCR3 expression and expanded these subsets in the presence of IL-2. After expansion and restimulation, we found that all CCR6+ IL-17- subsets were capable of high-level IL-17 production in contrast to CCR6- memory T cells (FIGS. 16B and 16C). Consistent with the *ex vivo* analyses of these subsets, however, the frequency of IL-17+ cells was somewhat higher (approximately 2-fold) in the CD161+ or CXCR3- subsets compared to CD161- or CXCR3+ counterparts, respectively (FIGS. 16B and 16C). Moreover, CCR6+ CXCR3+ IL-17- cells maintained their propensity to express IFNγ as compared to CCR6+ CXCR3- memory cells (FIGS. 16B and 16C). Therefore, heterogeneous sub-populations of CCR6+ T cells share a capacity to express IL-17 in response to γc-cytokine stimulation. FACS sorted CCR6- IL-17-, CCR6+ CD161+/- IL-17- (FIG. 16B) or CCR6+ CXCR3+/- IL-17- (FIG. 16C) cells were cultured in IL-2-supplemented medium for 7 days and restimulated with PMA and ionomycin. Cytokine production was determined by intracellular staining for IL-17, IL-22, and IFNγ. Each set of data represent 2-3 independent experiments using cells from different adult donors.

Example 10: *Ex vivo*-isolated CCR6+ IL-17+ and CCR6+ IL-17- memory T cells are indistinguishable following culture with gc-cytokines

A more comprehensive analyses of cytokine gene expression in *ex vivo*-isolated and TCR/ IL-2-stimulated human CCR6+ IL-17- and CCR6+ IL-17+ cells revealed that these two cell types were nearly indistinguishable following restimulation with PMA and ionomycin. Specifically, a number of pro-inflammatory cytokines canonically associated with the Th17 lineage (*Il17a, Il17f, Il22, Il26, Ccl20*) were comparably expressed in both CCR6+ IL-17- and CCR6+ IL-17+ cells, though both CCR6+ cell types expressed substantially higher levels of these cytokines as compared to CCR6- TM cells (FIG. 17). Importantly, differential expression of Th17-signature cytokines by both CCR6+ populations as compared to CCR6- T cells was specific, as all three populations expressed roughly equivalent levels of other cytokines not related to the Th17 lineage, namely *Tnf, Il4*, and *Ifng* (FIG. 17).

Cytokine gene expression was determined using quantitative nuclease protection assay (qNPA; see below) in CCR6- IL17-, CCR6+ IL-17-, or CCR6+ IL-17+ populations expanded for 6-7 days in IL-2-supplemented medium and restimulated with PMA and ionomycin. Cytokine mRNA expression was normalized to a housekeeper gene (Rpl19) and normalized values are presented as a fold-change on a scatter plot (where x axis = CCR6+ IL-17-/ CCR6- IL-17-, and y axis = CCR6+ IL-17+/ CCR6- IL-17-).

For quantitative nuclease protection assays, T cell cultures were collected after PMA and ionomycin activation. Cells were resuspended at 4x 10^6/mL in sample lysis buffer (High Throughput Genomics, Inc., Tuscon, AZ), heated to 95°C for 10 minutes and stored at -80°C. mRNA levels were quantified in sample lysates by quantitative nuclease protection assay (qNPA; High Throughput Genomics, Inc., Tuscon, AZ) using 4 probes per transcript (see below for sequences), and samples were normalized based on abundance of a control (housekeeper) gene (e.g., *Rpl19*). Assay quality was monitored by a negative control (Arabidopsis thaliana DNA binding / transcription factor (Ant). Human oligonucleotide probes were generated against: the housekeeper gene ribosomal protein L19 (*Rpl19*; NM_000981), Interleukin-17A (*Il17a*; NM_002190), and the negative control Arabidopsis thaliana DNA binding / transcription factor (Ant). Raw expression levels were normalized to a housekeeper gene (Rpl19). Probe sequences are listed below:

Rpl19
(1): 5′-AATGAAATCGCCAATGCCAACTCCCGTCAGCAGATCCGGAAGCTCATCAA-3′ (SEQ ID NO:29),
Rpl19
(2): 5′-TGATCATCCGCAAGCCTGTGACGGTCCATTCCCGGGCTCGATGCCGGAAA-3′ (SEQ ID NO:30),
Rpl19
(3):5′-CAGAGAAGGTCACATGGATGAGGAGAATGAGGATTTTGCGCCGGCTGCTC-3′ (SEQ ID NO:31),
Rpl19
(4): 5′-CAAGCGGATTCTCATGGAACACATCCACAAGCTGAAGGCAGACAAGGCCC-3′ (SEQ ID NO:32).

Ant
(1): 5′-CAGTCACTGAGCTTATCCATGAGCCCTGGGTCACAATCTAGCTGCATCAC-3′ (SEQ ID NO:33),
Ant
(2): 5′-CTCCAAAGGTGGAGGATTTCTTTGGGACCCATCACAACAACACAAGTCAC-3′ (SEQ ID NO:34),
Ant
(3): 5′-CGAAGGAACAACAACAGCATTGTCGTCAGGAATACTGAAGACCAAACCGC-3′ (SEQ ID NO:35),
Ant
(4): 5′-GGGAGGTTATGATATGGAGGAGAAAGCTGCTCGAGCATATGATCTTGCTG-3′ (SEQ ID NO:36).

Il17a
(1): 5′-CCCTCAGGAACCCTCATCCTTCAAAGACAGCCTCATTTCGGACTAAACTC-3′ (SEQ ID NO:37),
Il17a
(2): 5′-TAACACTTGGCCAAGATATGAGATCTGAATTACCTTTCCCTCTTTCCAAG-3′ (SEQ ID NO:38),
Il17a
(3): 5′-TGATGGTCAACCTGAACATCCATAACCGGAATACCAATACCAATCCCAAA-3′ (SEQ ID NO:39),
Il17a
(4): 5′-CCTGGTCCTGCGCAGGGAGCCTCCACACTGCCCCAACTCCTTCCGGCTGG-3′ (SEQ ID NO:40).

Il17f
(1): 5′-GTCATCCACCATGTGCAGTAAGAGGTGCATATCCACTCAGCTGAAGAAGC-3′ (SEQ ID NO:41),
Il17f
(2): 5′-ATGACAGTGAAGACCCTGCATGGCCCAGCCATGGTCAAGTACTTGCTGCT-3′ (SEQ ID NO:42),
Il17f
(3): 5′-TCAAGGAAAGGAAGACATCTCCATGAATTCCGTTCCCATCCAGCAAGAGA-3′ (SEQ ID NO:43),
Il17f
(4): 5′-GGACTCTTAATAAGACCTGCACGGATGGAAACAGAAAATATTCACAATGT-3′ (SEQ ID NO:44).

Il22
(1): 5′- CACTGCAGGCTTGACAAGTCCAACTTCCAGCAGCCCTATATCACCAACCG-3′ (SEQ ID NO:45),
Il22
(2): 5′- GCTAAGGAGGCTAGCTTGGCTGATAACAACACAGACGTTCGTCTCATTGG-3′ (SEQ ID NO:46),
Il22
(3): 5′- CTTCTCTTGGCCCTCTTGGTACAGGGAGGAGCAGCTGCGCCCATCAGCTC-3′ (SEQ ID NO:47),
Il22
(4): 5′- CTCGAGTTAGAATTGTCTGCAATGGCCGCCCTGCAGAAATCTGTGAGCTC-3′ (SEQ ID NO:48).

Il4
(1): 5′- GGCGGGCTTGAATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGG-3′ (SEQ ID NO:49),
Il4
(2): 5′-GCTGATAAGATTAATCTAAAGAGCAAATTATGGTGTAATTTCCTATGCTG-3′ (SEQ ID NO:50),
Il4
(3): 5′-TTTGTCAGCATTGCATCGTTAGCTTCTCCTGATAAACTAATTGCCTCACA-3′ (SEQ ID NO:51),
Il4
(1): 5′-TTTCCTATTGGTCTGATTTCACAGGAACATTTTACCTGTTTGTGAGGCAT-3′ (SEQ ID NO:52).

Il21
(1):5′-CTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACAGAAACACAG-3′ (SEQ ID NO:53),
Il21
(2): 5′-GAAGGCCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCA-3′ (SEQ ID NO:54),
Il21
(3): 5′-GGATCTAACTTGCAGTTGGACACTATGTTACATACTCTAATATAGTAGTG-3′ (SEQ ID NO:55),
Il21
(4): 5′-CCAGTCCTGGCAACATGGAGAGGATTGTCATCTGTCTGATGGTCATCTTC-3′ (SEQ ID NO:56).

Il26
(1): 5′-CCCAAGCTGTTGACGCTCTCTATATCAAAGCAGCATGGCTCAAAGCAACG-3′ (SEQ ID NO:57),
Il26
(2): 5′-TAGTCACTCTGTCTCTTGCCATTGCCAAGCACAAGCAATCTTCCTTCACC-3′ (SEQ ID NO:58),
Il26
(3): 5′-CGGCATGTTAGGTGATTCAGAATAGACAAGAAGGATTTAGTAAATTAACG-3′ (SEQ ID NO:59),
Il26
(4): 5′-AAGTACATTGTGTCAACTTAATTTAAAGTATGTAACCTGAATTAACTCGT-3′ (SEQ ID NO:60).

Ifng
(1): 5′-CCAGTGCTTTAATGGCATGTCAGACAGAACTTGAATGTGTCAGGTGACCC-3′ (SEQ ID NO:61),
Ifng
(2): 5′-CTGGTGCTTCCAAATATTGTTGACAACTGTGACTGTACCCAAATGGAAAG-3′ (SEQ ID NO:62),
Ifng
(3): 5′-AAGAACTACTGATTTCAACTTCTTTGGCTTAATTCTCTCGGAAACGATGA-3′ (SEQ ID NO:63),
Ifng
(4): 5′-AAATGAATATCTATTAATATATGTATTATTTATAATTCCTATATCCTGTG-3′ (SEQ ID NO:64).

Tnf (1): 5′-CAAGACCACCACTTCGAAACCTGGGATTCAGGAATGTGTGGCCTGCACAG-3′ (SEQ ID NO:65),

Tnf (2): 5′-GGACCTCTCTCTAATCAGCCCTCTGGCCCAGGCAGTCAGATCATCTTCTC-3′ (SEQ ID NO:66),

Tnf (3): 5′-GTGTCTGTAATCGCCCTACTATTCAGTGGCGAGAAATAAAGTTTGCTTAG-3′ (SEQ ID NO:67),

Tnf (4): 5′-TGTAGCCCATGTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGC-3′ (SEQ ID NO:68).

Ccl20 (1): 5′-CACTTGCACATCATGGAGGGTTTAGTGCTTATCTAATTTGTGCCTCACTG-3′ (SEQ ID NO:69),

Ccl20 (2): 5′-TTTGTTTAAGCATCACATTAAAGTTAAACTGTATTTTATGTTATTTATAG-3′ (SEQ ID NO:70),

Ccl20 (3): 5′-ATAAAATTATATTTGGGGGGGAATAAGATTATATGGACTTTCTTGCAAGC-3′ (SEQ ID NO:71),

Ccl20 (4): 5′-GCGAATCAGAAGCAGCAAGCAACTTTGACTGCTGTCTTGGATACACAGAC-3′ (SEQ ID NO:72).

Il2 (1): 5′-CACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGG-3′ (SEQ ID NO:73),

Il2 (2): 5′-AAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAG-3′ (SEQ ID NO:74),

Il2 (3): 5′-CTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAATCC-3′ (SEQ ID NO:75),

Il2 (4): 5′-GCTACCTATTGTAACTATTATTCTTAATCTTAAAACTATAAATATGGATC-3′ (SEQ ID NO:76).

Tgfb1 (1): 5′-CGAGCCCTGGACACCAACTATTGCTTCAGCTCCACGGAGAAGAACTGCTG-3′ (SEQ ID NO:77),

Tgfb1 (2): 5′-AACACATCAGAGCTCCGAGAAGCGGTACCTGAACCCGTGTTGCTCTCCCG-3′ (SEQ ID NO:78),

Tgfb1 (3): 5′-CCTACATTTGGAGCCTGGACACGCAGTACAGCAAGGTCCTGGCCCTGTAC-3′ (SEQ ID NO:79),

Tgfb1 (4): 5′-GTGACAGCAGGGATAACACACTGCAAGTGGACATCAACGGGTTCACTACC-3′ (SEQ ID NO:80).

Example 11: CCR6+IL-17+ and CCR6+IL-17- T cells proliferate similarly in response to IL-2 stimulation

FACS sorted anti-CD3/anti-CD28 activated CCR6+IL-17A+, or CCR6+IL-17A- cells were labeled with CFSE or eFluor 670 (i.e., e670), respectively, plated in mixed cultures (co-cultures) and stimulated with IL-2 to monitor proliferation. Dye dilution in CFSE-labeled (CCR6+IL-17A+) or eFluor 670-labelled (CCR6+IL-17A-) cells were analyzed on day0 (directly post-labeling), day 2 and day 4 by FACS analysis (data not shown). Fold expansion was calculated by normalizing the mean fluorescence intensities (MFI's) at day 2 or 4 to day 0. The results are shown in FIG. 18A and show that both populations of cells proliferate similarly in response to IL-2 stimulation. The co-cultures were stimulated with PMA and ionomycin on day 6. CCR6+IL-17A+ or CCR6+IL-17A- cells were gated based on CFSE or eFluor 670 fluorescence and IL-17 expression was determined by intracellular staining. The results are shown in FIG. 18B. Data shown are from one representative donor out of three donors and independent experiments. Isolated T cells were labeled with CFSE (Invitrogen) or eFluor670 (eBioscience) per manufacturers instructions.

Example 12: Example of high throughput assay used to screen compound library in 384-well format

For reagent preparation and PBMC isolation, refer to conversion protocol (Example 3).

Materials:

FACS Buffer: Sterile filtered Phosphate Buffered Saline with 2% fetal bovine serum

Freeze Media: 90% Fetal Bovine Serum +10% DMSO

Easysep Human Memory CD4+ T-cell enrichment Kit-Stem cell Technologies, Cat. No. 19157

Easy50 Easysep Magnet, Stem cell Technologies, Cat. No. 18002

CELL TITER-GLO®, Promega, Cat. No. G7571

Human IL-17A 384 tissue culture kit, Meso Scale Discovery, Cat. No. K251ATB-2

Isolation of Memory CD4+ T-cells

- For Labeling up to $2X10^9$ cells

- Prepare mononuclear cell suspension at a concentration of $5X10^7$ cells/mL in FACS buffer in a 50mL conical tube.
- Add 50ul/mL Easysep enrichment cocktail.
- Mix well and incubate at room temperature for 10 minutes.
- Vortex Easysep magnetic particles for 30 seconds vigorously and add 50ul/mL.
- Mix well and incubate at room temperature for 10 minutes.
- Bring the cell suspension to a volume of 50.0ml by adding FACS buffer.
- Mix the cells gently in the tube by pipetting up and down 2 or 3 times.
- Place the cells in the conical tube without the cap into the magnet for 10 minutes.
- Remove cells by carefully pipetting the cell suspension into a new 50.0mL conical.
- Spin cells and discard supernatant.
- Determine cell number.
- Immediately freeze cells in Freeze Media at $4x10^7$ cells/mL.
- Place cells at -80°C overnight and transfer to Liquid Nitrogen the next day.

Compound Dilution

- Transfer 2ul of each test compound to a well of a 96-well plate.
- On the day of the assay, add 8ul of DMSO to each well.
- Transfer 2ul of 1:5 diluted compound to 198ul Xvivo media in a 96-well.
- The final concentration of the compound in the assay is 1/10,000
- The biological replicates are set up in two identical plates.

HT Screen (HTS) set up

- On the day of assay, thaw sufficient vials of memory CD4+ T-cells in a water bath.
- Bring each vial to a volume of 12.0mL by adding 11.0mL of Xvivo media, bringing the final concentration of cells to $3.3X10^6$ cells/mL.
- Add 27ul of IL-15 at a final concentration of 20ng/mL to appropriate wells.
- To plate MIN control wells (16 wells total), add media alone without IL-15.
- To plate MAX control wells (16 wells total), add DMSO alone with IL-15 with no test compound.
- Transfer 3ul of each test compound at 1:500 dilutions in the 96-well plate, to appropriate wells of 384-well plates (e.g., compounds from four 96-well plates per 384-well plate).
- Add 30ul of cells to each well of the 384 well plates.
- Place the plates in the incubator at 37°C, 5% CO_2 for 6 days.
- Do not open the incubator or disturb the plates during this incubation period.

Day 6 HTS Read Out

Reagents

- PMA-Phorbol 12-myristate 13-acetate (Sigma, Cat. No.P8139). Prepare a stock solution of 1.62mM by dissolving in ethanol. Store small aliquots at -80°C. Use at 10nM.
- Ionomycin (Sigma, Cat. No.I0634). Prepare a stock solution of 13.4mM by dissolving in ethanol. Store small aliquots at -80°C. Use at 1uM.

Method

- Remove the plates from the incubator.
- Add 10ul of PMA/Ionomycin at 7X concentration.
- Shake the plates briefly on a shaker at 600 rpm.
- Place the plates in the incubator for 4 hours.
- After 4 hours, remove the plate and shake briefly on a shaker for few minutes.
- Spin the plate down at 2000 rpm for 2 minutes.

- Remove 30ul of supernatant and transfer to a 384-well plate for measuring IL-17 concentration.
- Measure cell viability as follows.

Cell Viability by CELL TITER-GLO®

- Thaw the CELL TITER-GLO® Buffer, and equilibrate to room temperature prior to use.
- Transfer the entire volume of CELL TITER-GLO® Buffer into the amber bottle containing CELL TITER-GLO® Substrate to reconstitute the lyophilized enzyme/substrate mixture. This forms the CELL TITER-GLO® Reagent.
- Add 30ul of CELL TITER-GLO® Reagent that is equal to the volume of cell culture medium present in each well.
- Mix contents for 2 minutes on an orbital shaker to induce cell lysis.
- Allow the plate to incubate at room temperature for 10 minutes to stabilize luminescent signal.
- Record luminescence using Fluostar Optima (BMG Labtech).

IL-17 ELISA by Meso Scale Discovery(MSD)

- Add 10ul of the supernatant to the ELISA plates pre-coated with IL-17A antibody.
- Seal the plate and incubate on a shaker at room temperature for 2 Hours.
- Add 10ul of 1.0ug/mL IL-17A detection antibody in kit component Diluent 100.
- Seal the plate and incubate on a shaker at room temperature for 2 Hours.
- Wash the plate 3times with PBS+0.05% Tween.
- Add 35ul of kit component 2X read buffer to each well and read the plate on Sector imager6000 (Meso Scale Discovery).

Example 13: Conversion of CCR6+ memory murine T cells into IL-17A-secreting effectors

CD4+ T cells from the spleen and peripheral lymph nodes of Il17a-IRES-GFP mice (Biocytogen, Inc.) were purified via magnetic cell sorting (STEMCELL Technologies, Inc.). Enriched T cell preparations were stained with anti-TCRbeta-PECy7 clone H57-597, anti-CD4-PerCP-Cy5.5 clone GK1.5, anti-CD25-APC clone 3C7, anti-CD62L-Pacific Blue

clone MEL-14, anti-CD44-Alexafluor 700 clone IM7, and anti-CCR6-PE clone 29-2L17 (1:200 dilutions except anti-CCR6 1:100; BioLegend, Inc.). CD4+ memory T cells (TCRbeta+ CD4+ CD25- CD62L- CD44hi) were FACS sorted into CCR6+ or CCR6- cells (FACS Aria II – BD Bioscience). These cells were then stimulated using anti-CD3/ anti-CD28 coated beads (2 beads: 1 cell ratio) for 18 hours and then harvested to sort out IL-17A (GFP)- or IL-17A (GFP)+ cells. Sorted CCR6- GFP-; CCR6+ GFP-; and CCR6+ GFP+ cells were then cultured in IMDM medium (Invitrogen Corporation - supplemented as described in Example 6 for DMEM: 4.5g/L glucose (MediaTech, Inc.), 10% Fetal bovine serum (Thermo Fisher), and 1% of each of the following: MEM Essential Vitamin solution (Gibco), Non Essential Amino Acids (Gibco), Sodium Pyruvate (Gibco), 1M HEPES (Gibco), Penicillin/ Streptomycin (Gibco), L-glutamine (Gibco) plus 2-mercaptoethanol (Sigma-Aldrich), where a 14.3M stock solution of 2-mercaptoethanol is diluted to 5 mM in sterile water containing 100x (400 mM) L-glutamine, and L-Arginine/ L-Asparagine/ Folic acid mix) for 6 days in the presence of anti-CD3/ anti-CD28 coated beads (2 beads: 1 cell ratio) and 10 IU/mL recombinant human IL-2 (BD Bioscience). Expanded cells were restimulated with PMA and ionomycin in the presence of Brefeldin A (20 ug/ml) for 4 hours and then fixed, permeabilized, and stained to detect intracellular expression of IL-17A (anti-IL-17A-Pacific Blue clone TC11-18H10.1, 1:200; BioLegend, Inc.) (FIG. 19). Data acquisition was performed on LSR II Fortessa (BD Bioscience) and analyzed using FloJo software (TreeStar, Inc.). The results show that, similar to human CCR6+ T cells, mouse CCR6+ memory T cells that lack IL-17A expression ex vivo can produce de novo IL-17A upon stimulation with gamma common-utilizing cytokines.

WHAT IS CLAIMED IS:

1. An isolated human poised Th17 cell, wherein the cell is CD4+ (expresses CD4), CD25- (does not express CD25), CD45RO+ (expresses CD45RO), CCR6+ (expresses CCR6 (Chemokine (C-C motif) receptor 6)) and IL-17- (does not express interleukin (IL)-17).

2. The cell of claim 1, wherein the cell is CXCR3+ (expresses CXCR3 (chemokine (C-X-C motif) receptor 3)).

3. The cell of claim 1, wherein the cell is CXCR3- (does not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

4. A method of isolating human poised Th17 cells, the method comprising:

isolating or having isolated CD4+ CD45RO+ CD25- CCR6+ and IL-17- cells from a mixture of cells;

thereby isolating human poised Th17 cells, wherein the poised Th17 cells are CD4+ CD45RO+ CD25- CCR6+ and IL-17-.

5. The method of claim 4, wherein the method further comprises isolating or having isolated CXCR3+ cells from the human poised Th17 cells.

6. The method of claim 4, wherein the method further comprises isolating or having isolated CXCR3- cells from the human poised Th17 cells.

7. A method of converting a human poised Th17 cell, wherein the poised Th17 cell is CD4+ CD25- CD45RO+ CCR6+ IL-17-, to a human effector Th17 cell, the method comprising:

culturing the human poised Th17 cell in a culture medium that comprises interleukin (IL)-2, IL-7, or IL-15, wherein the human effector Th17 cell is IL-17+.

8. The method of claim 7, further comprising stimulating the cell through the T cell receptor (TCR).

9. The method of claim 7, wherein the human poised Th17 cell is CXCR3+.

10. The method of claim 7, wherein the human poised Th17 cell is CXCR3-.

11. A method of evaluating a test compound for its ability to modulate conversion of human poised Th17 cells, wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17-, to human effector Th17 cells, wherein the human Th17 effector cells are IL-17+, the method comprising:

incubating the human poised Th17 cells with a test compound, wherein the incubating occurs in conditions that are favorable for conversion of the human poised Th17 cells to human effector Th17 cells in the absence of the test compound; and

evaluating whether the test compound modulates conversion of the human poised Th17 cells to human effector Th17 cells.

12. The method of claim 11, wherein the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

13. The method of claim 11, wherein the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

14. The method of claim 11, wherein the evaluating comprises determining the percentage of IL-17+ cells.

15. The method of claim 11, wherein the evaluating comprises determining the number of IL-17+ cells.

16. The method of claim 11, wherein the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

17. A method of evaluating a test compound for its ability to modulate IL-17 expression, the method comprising:

incubating human poised Th17 cells with a test compound, wherein the human poised Th17 cells are CD4+ CD25- CD45RO+ CCR6+ IL-17- and wherein the incubating occurs in conditions that promote IL-17 expression by the cells in the absence of the test compound; and

evaluating whether the test compound modulates IL-17 expression by the cells.

18. The method of claim 17, wherein the cells are CXCR3+ (express CXCR3 (chemokine (C-X-C motif) receptor 3)).

19. The method of claim 17, wherein the cells are CXCR3- (do not express CXCR3 (chemokine (C-X-C motif) receptor 3)).

20. The method of claim 17, wherein the evaluating comprises determining the percentage of IL-17 expressing cells.

21. The method of claim 17, wherein the evaluating comprises determining the number of IL-17 expressing cells.

22. The method of claim 17, wherein the evaluating comprises measuring the amount of IL-17 (e.g., secreted IL-17).

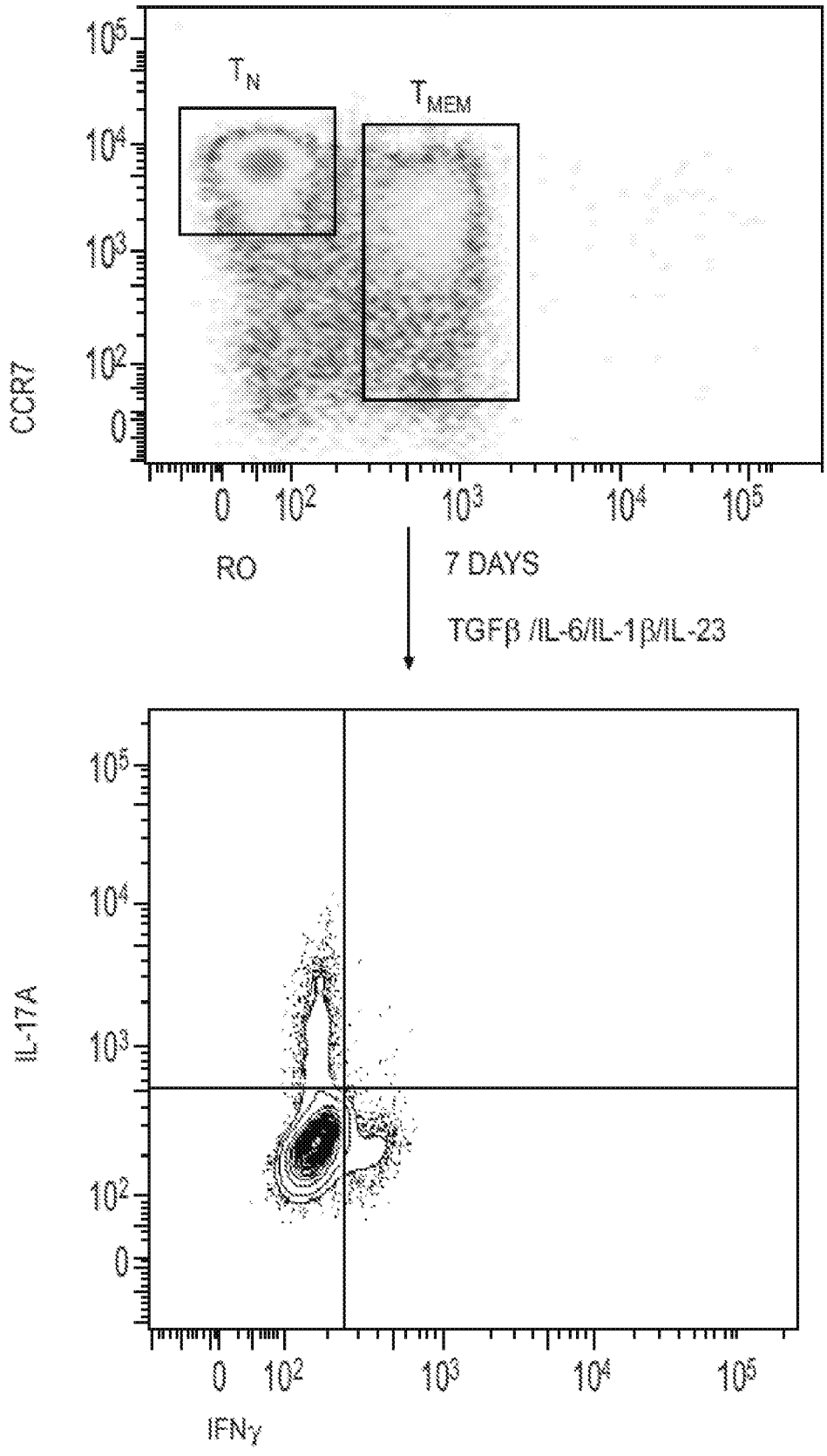

T_N
T_{MEM}
CCR7
10^5
10^4
10^3
10^2
0
0 10^2 10^3 10^4 10^5
RO
7 DAYS
TGFβ /IL-6/IL-1β/IL-23
IL-17A
IFNγ

FIG. 1

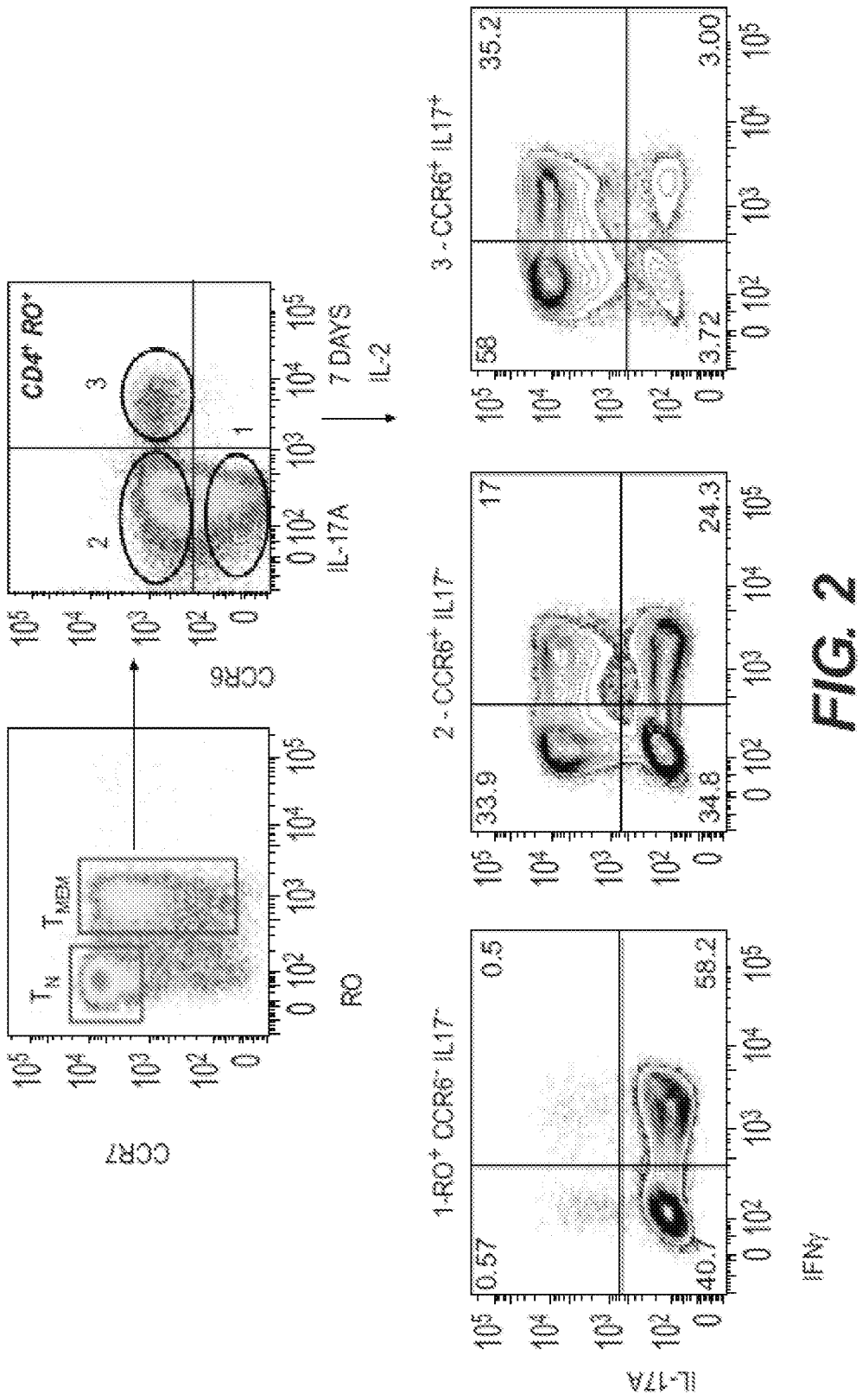

CCR7
RO
T_N
T_{MEM}
CCR6
CD4+ RO+
1
2
3
IL-17A
7 DAYS
IL-2
1-RO+ CCR6- IL17-
2 - CCR6+ IL17-
3 - CCR6+ IL17+
0.5
58.2
0.57
40.7
33.9
34.8
17
24.3
35.2
3.00
58
3.72
IL-17A
IFNγ

FIG. 2

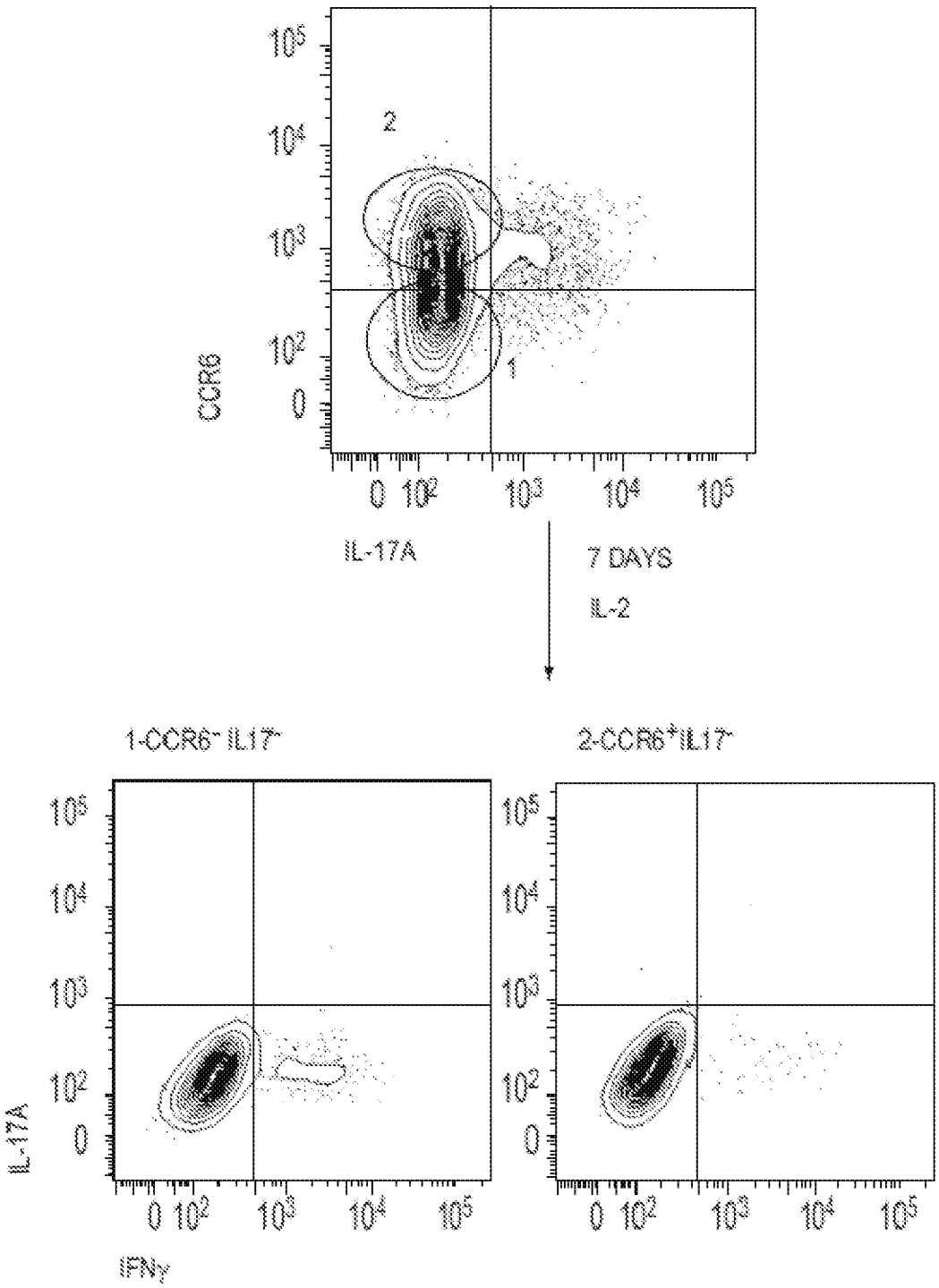


FIG. 3

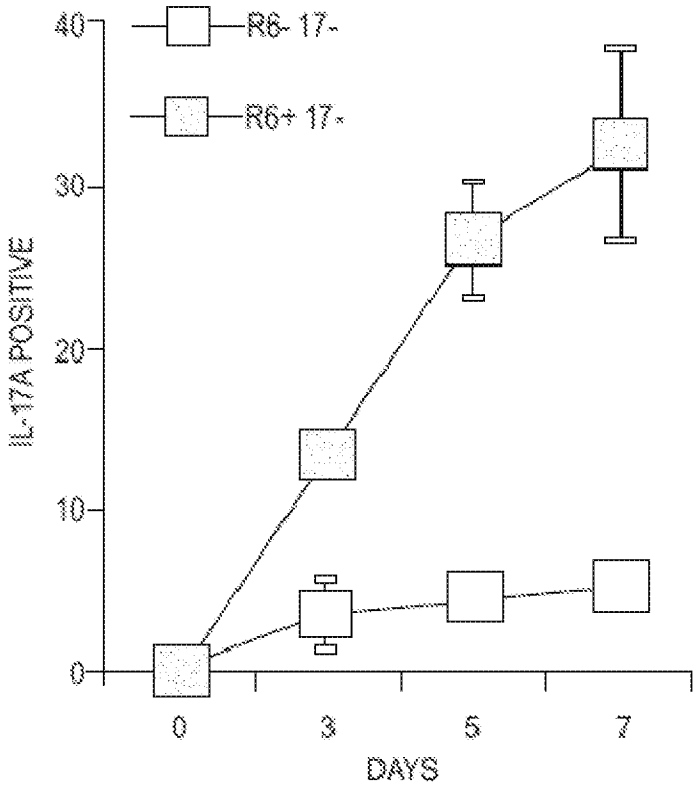


FIG. 4

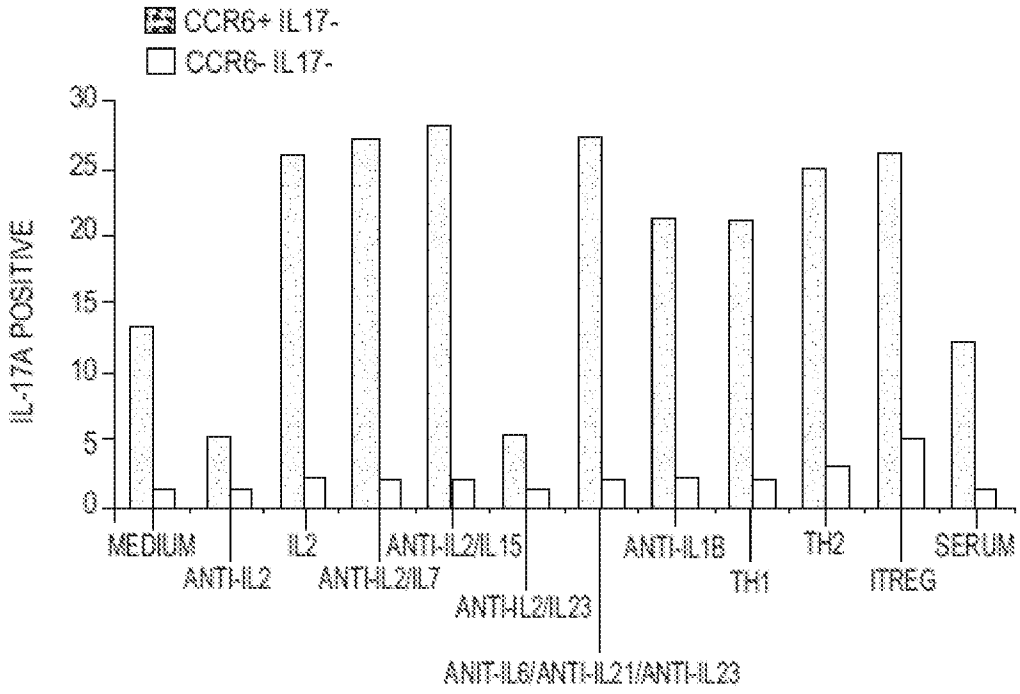

CCR6+ IL17-
CCR6- IL17-
IL-17A POSITIVE
30
25
20
15
10
5
0
MEDIUM
ANTI-IL2
IL2
ANTI-IL2/IL7
ANTI-IL2/IL15
ANTI-IL2/IL23
ANIT-IL6/ANTI-IL21/ANTI-IL23
ANTI-IL1B
TH1
TH2
ITREG
SERUM

FIG. 5

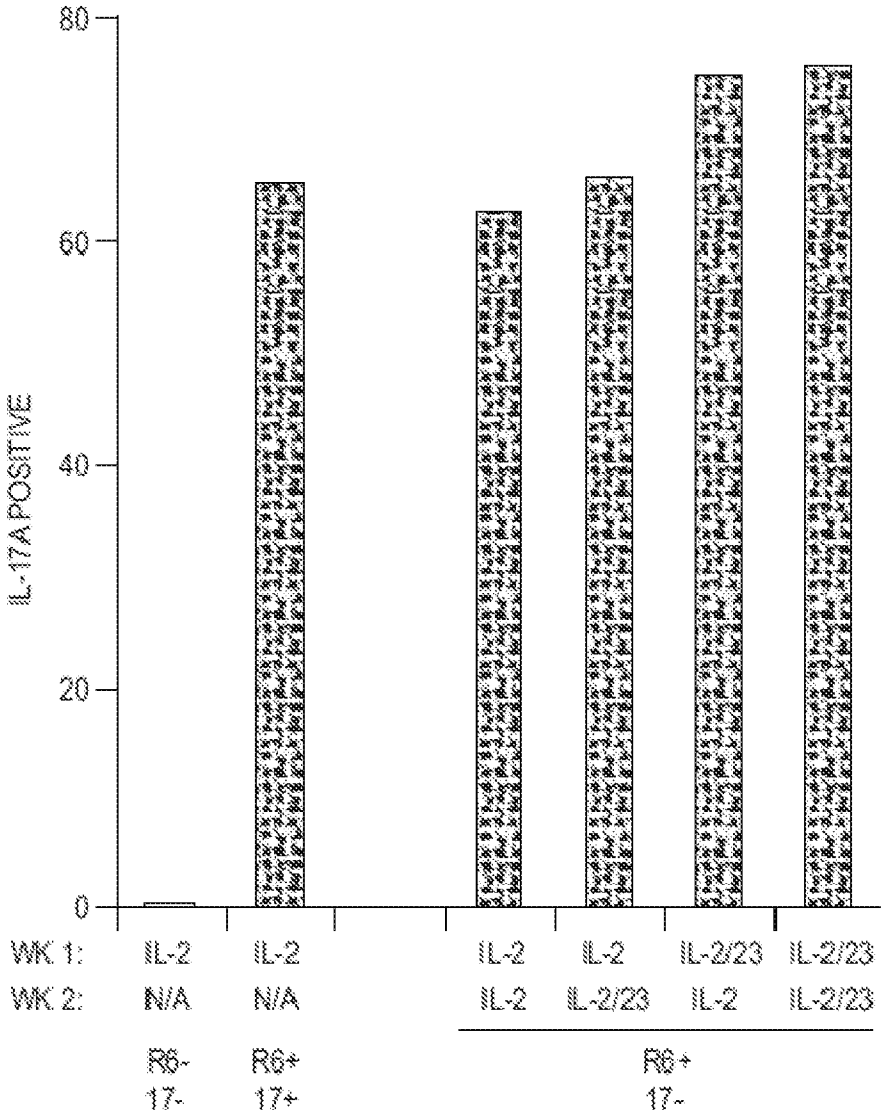

80
60
40
20
0
IL-17A POSITIVE
WK 1:
WK 2:
IL-2
N/A
IL-2
N/A
IL-2
IL-2
IL-2
IL-2/23
IL-2/23
IL-2
IL-2/23
IL-2/23
R6-
17-
R6+
17+
R6+
17-

FIG. 6

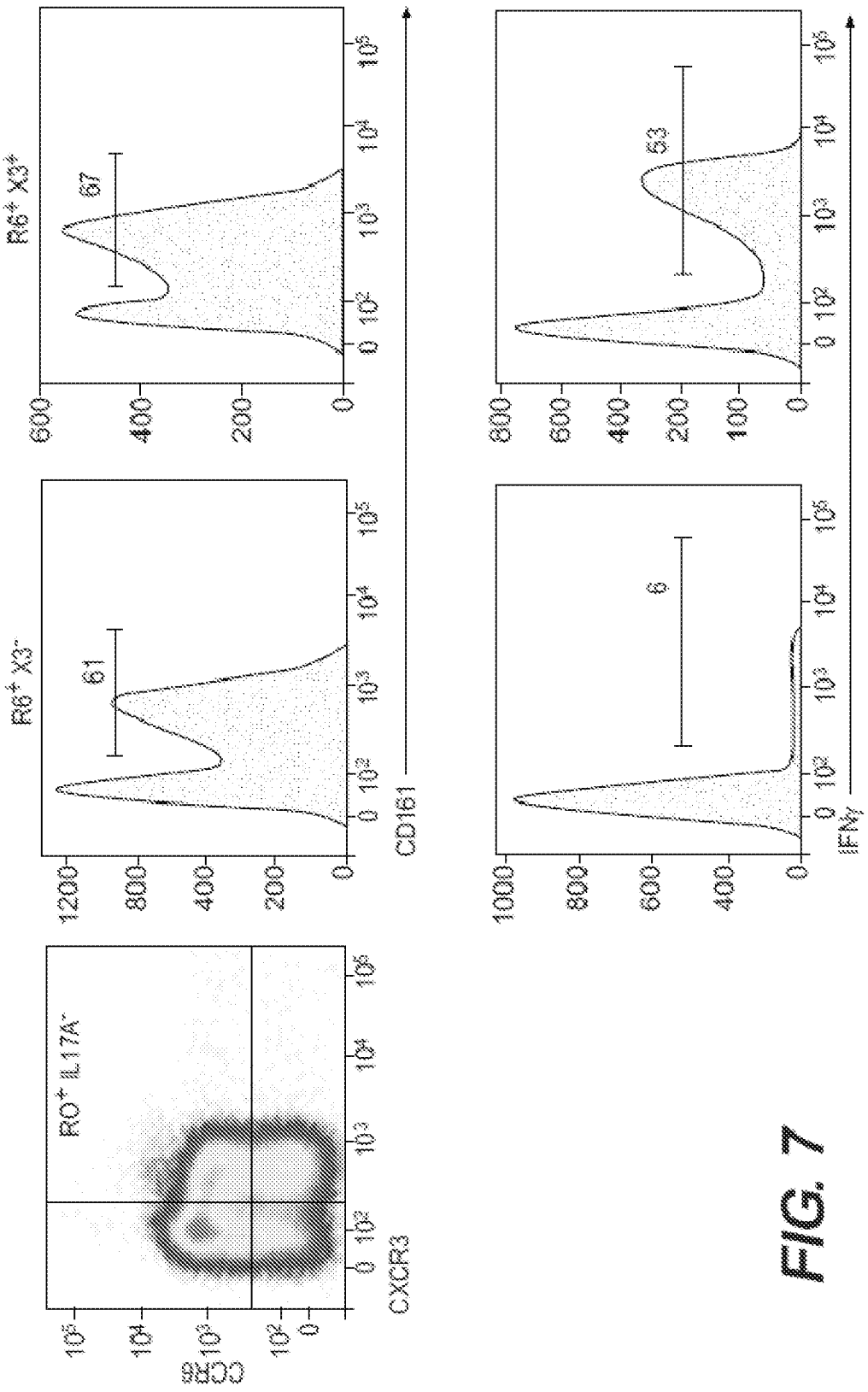


FIG. 7

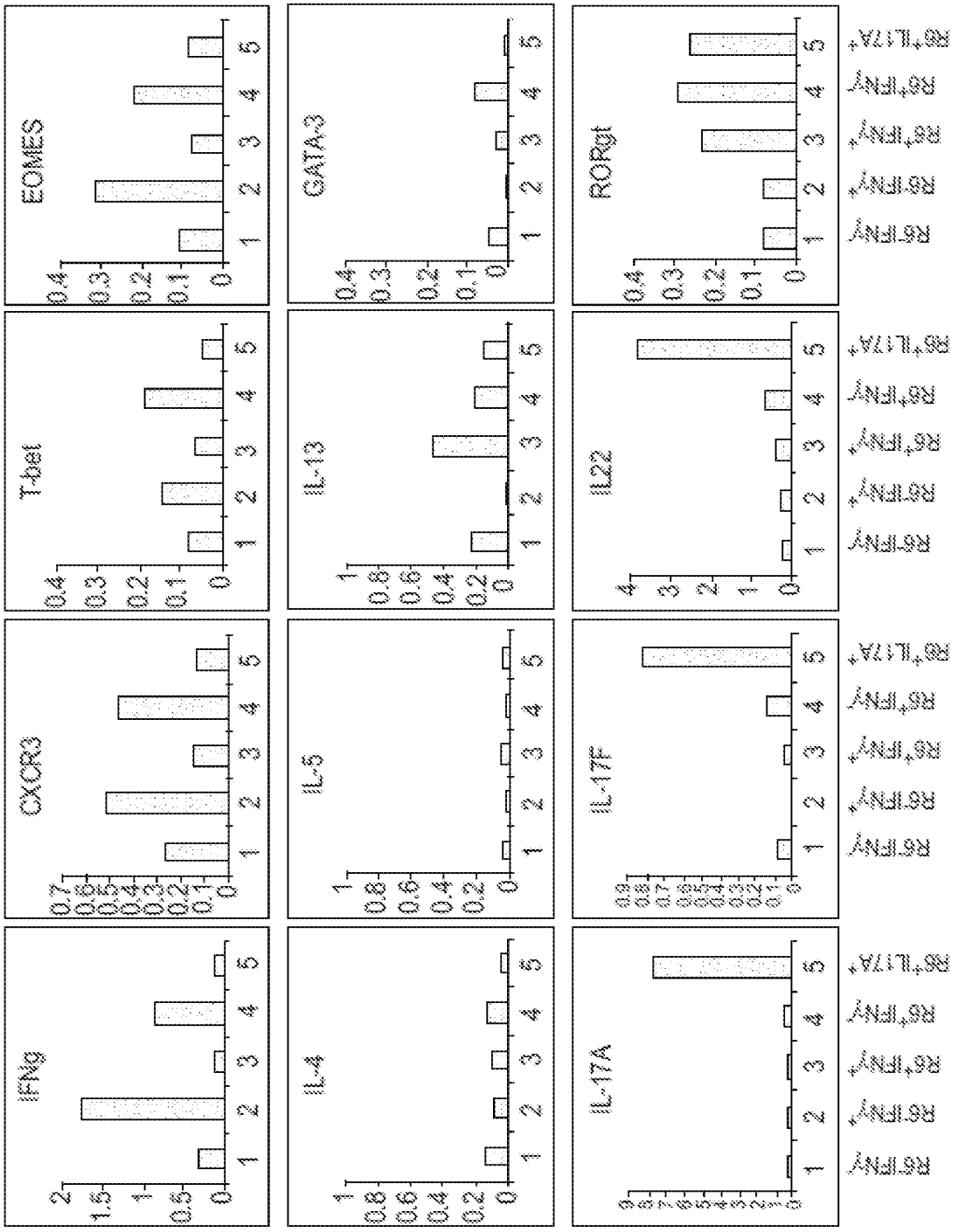

Th1
Th2
Th17
IFNg
IL-4
IL-17A
CXCR3
IL-5
IL-17F
T-bet
IL-13
IL22
EOMES
GATA-3
RORgt
R6-IFNγ-
R6+IFNγ+
R6+IFNγ+
R6+IFNγ+
R6+IL17A+

FIG. 8

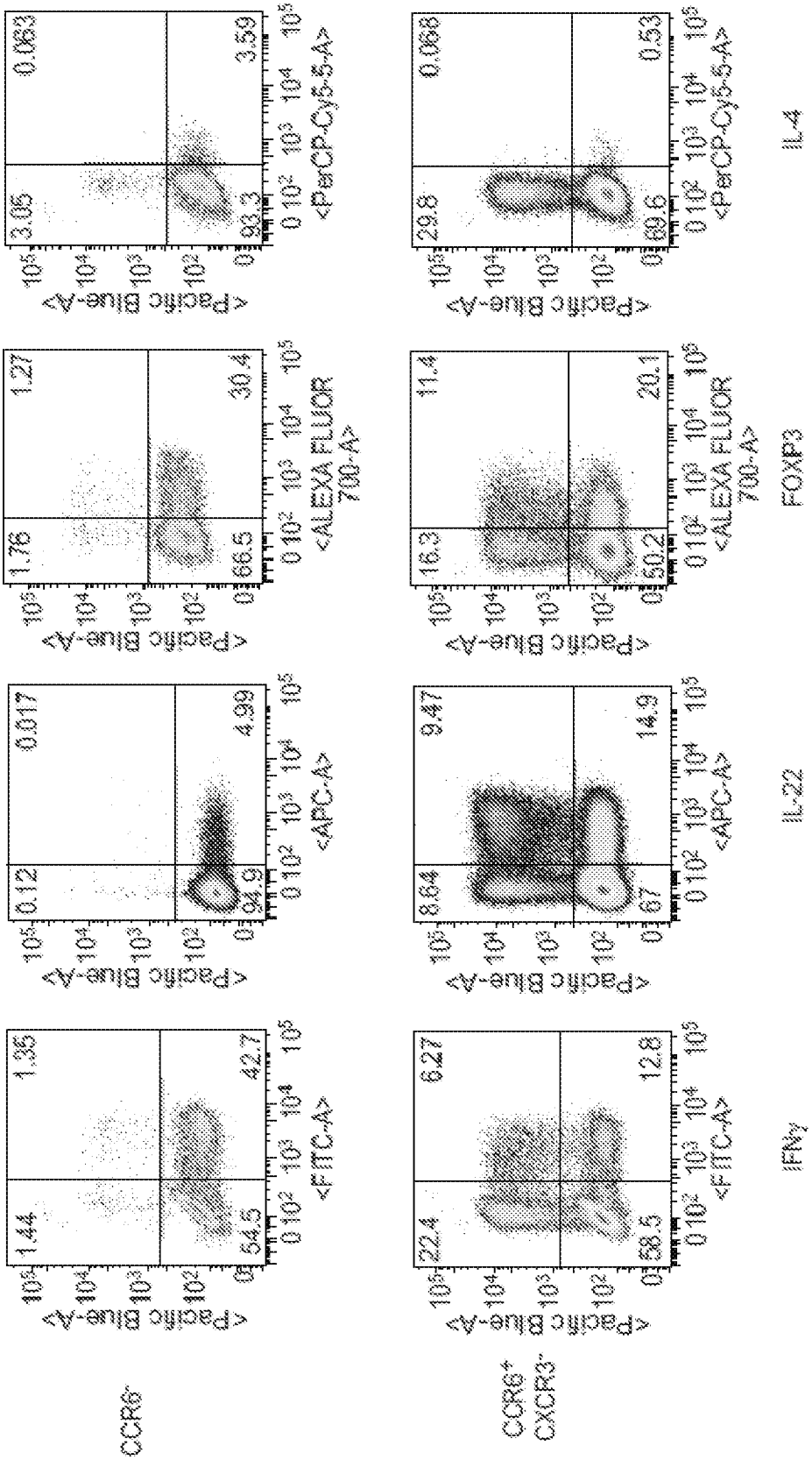

CCR6-
CCR6+ CXCR3-
IFNγ
IL-22
FOXP3
IL-4
<FITC-A>
<APC-A>
<ALEXA FLUOR 700-A>
<PerCP-Cy5-5-A>
<Pacific Blue-A>
1.44
1.35
54.5
42.7
0.12
0.017
94.9
4.99
1.76
1.27
66.5
30.4
3.05
0.083
93.3
3.59
22.4
6.27
58.5
12.8
8.64
9.47
67
14.9
16.3
11.4
50.2
20.1
29.8
0.066
69.6
0.53

FIG. 9A

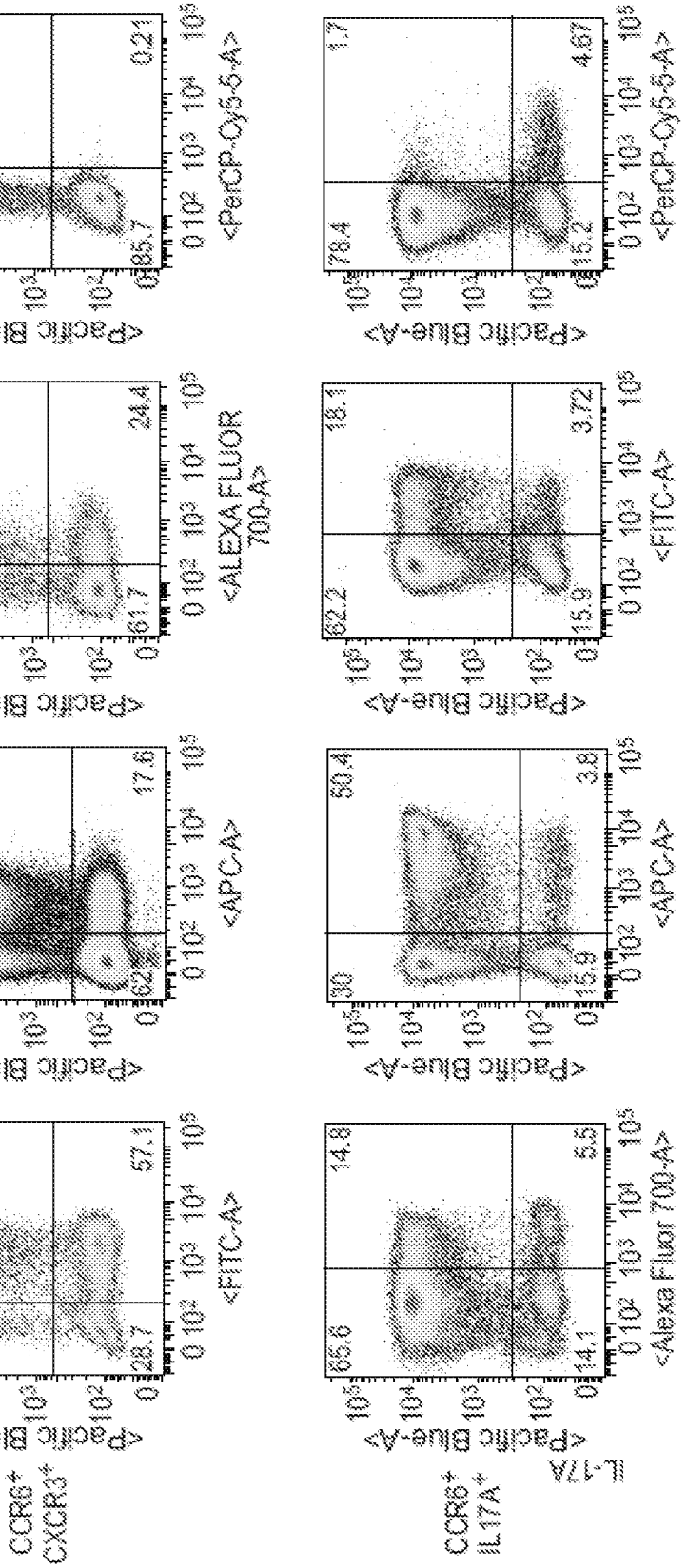

CCR6+ CXCR3+
CCR6+ IL17A+
IFNγ
IL-22
FOXP3
IL-4
IL-17A
<FITC-A>
<APC-A>
<ALEXA FLUOR 700-A>
<PerCP-Cy5-5-A>
<Alexa Fluor 700-A>
<Pacific Blue-A>
3.74
10.5
28.7
57.1
8.68
11.7
62.2
17.6
8.68
5.27
61.7
24.4
14.1
0.011
85.7
0.21
65.6
14.8
14.1
5.5
30
50.4
15.9
3.8
62.2
18.1
15.9
3.72
78.4
1.7
15.2
4.67

FIG.9B

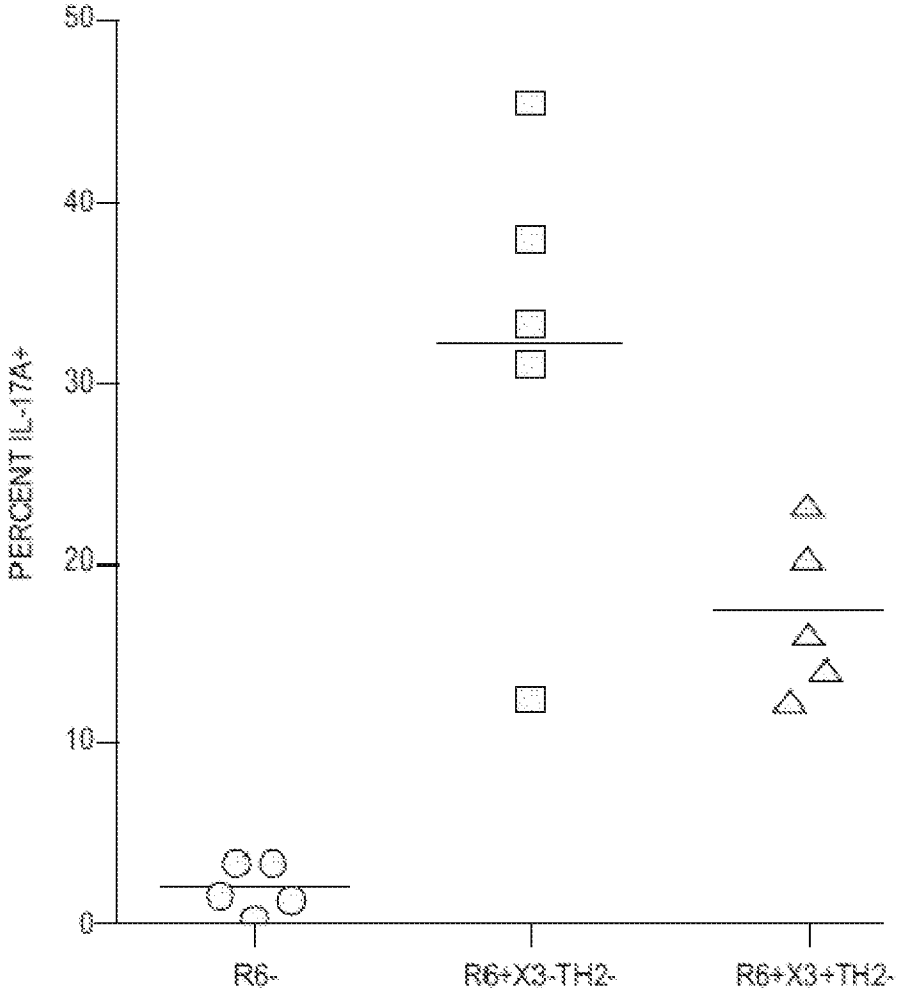


FIG. 10

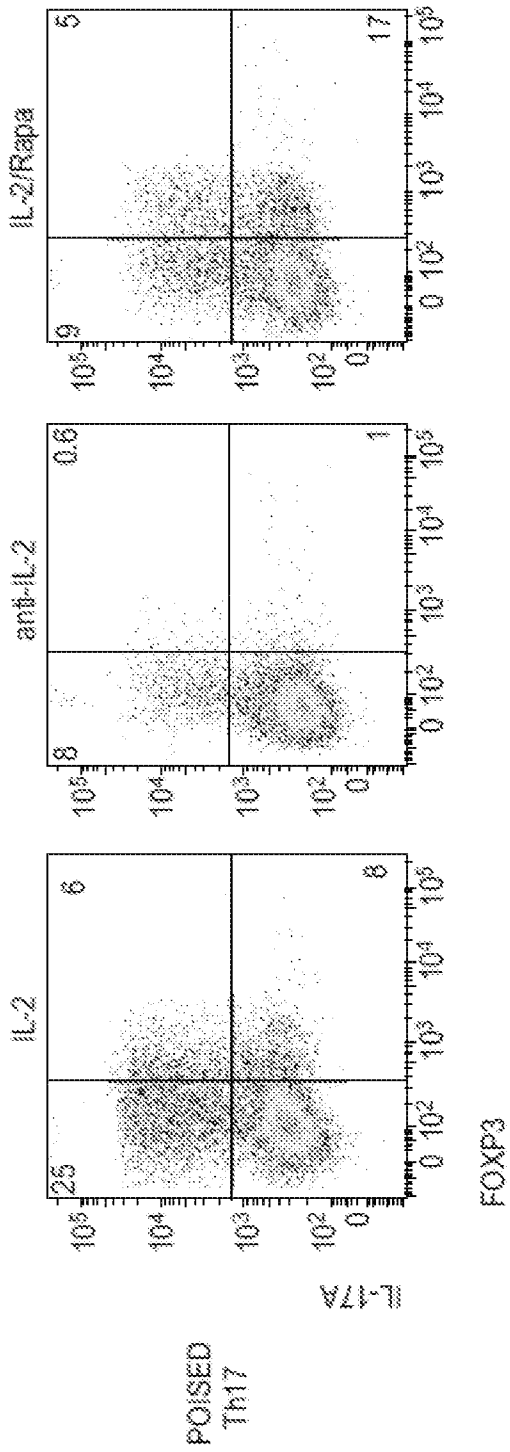

POISED Th17
IL-17A
FOXP3
IL-2
anti-IL-2
IL-2/Rapa
25
6
8
8
0.6
1
9
5
17
0
10^2
10^3
10^4
10^5
10^6

FIG.11

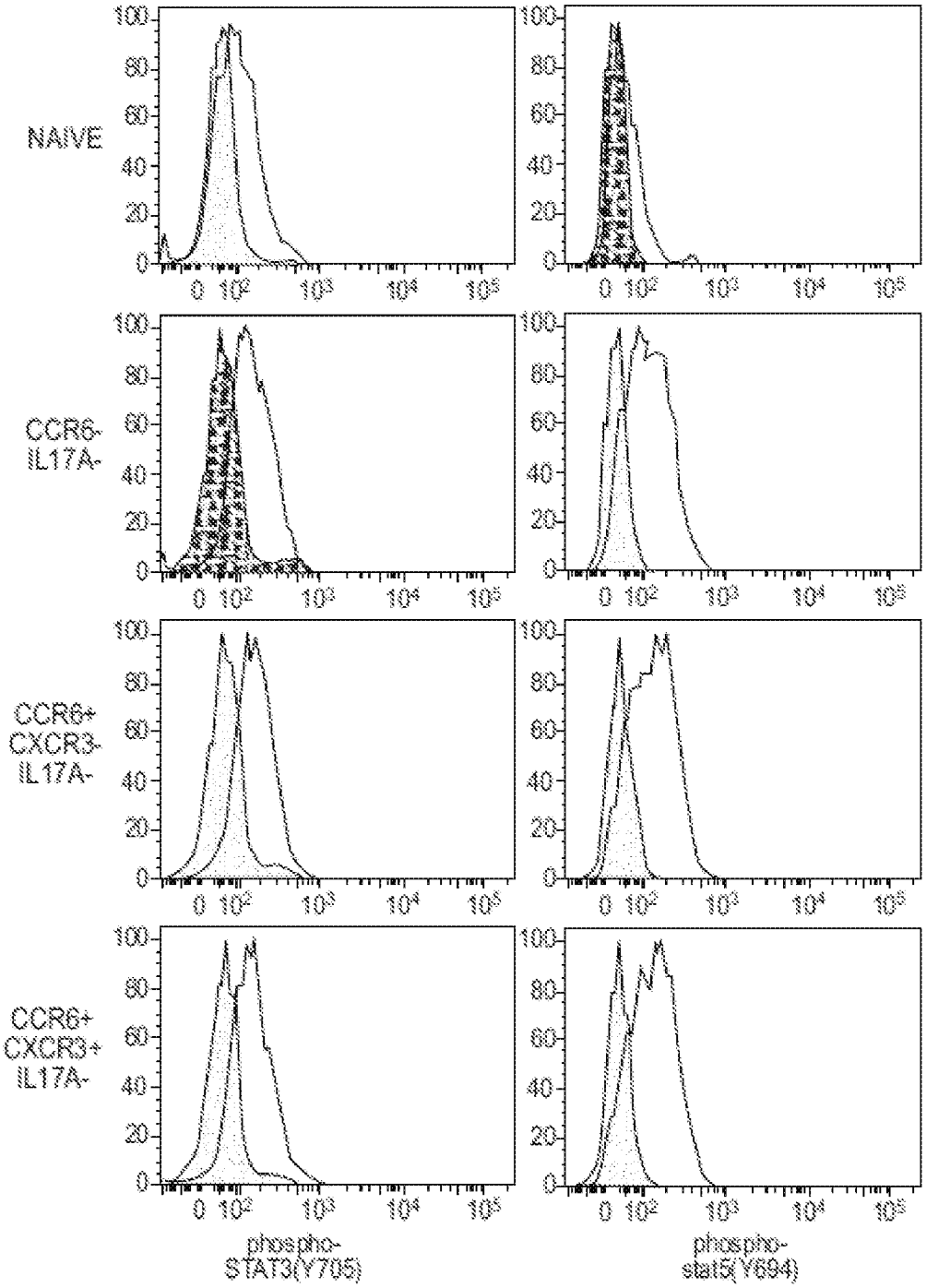

NAIVE
CCR6-
IL17A-
CCR6+
CXCR3-
IL17A-
CCR6+
CXCR3+
IL17A-
phospho-
STAT3(Y705)
phospho-
stat5(Y694)

FIG. 12

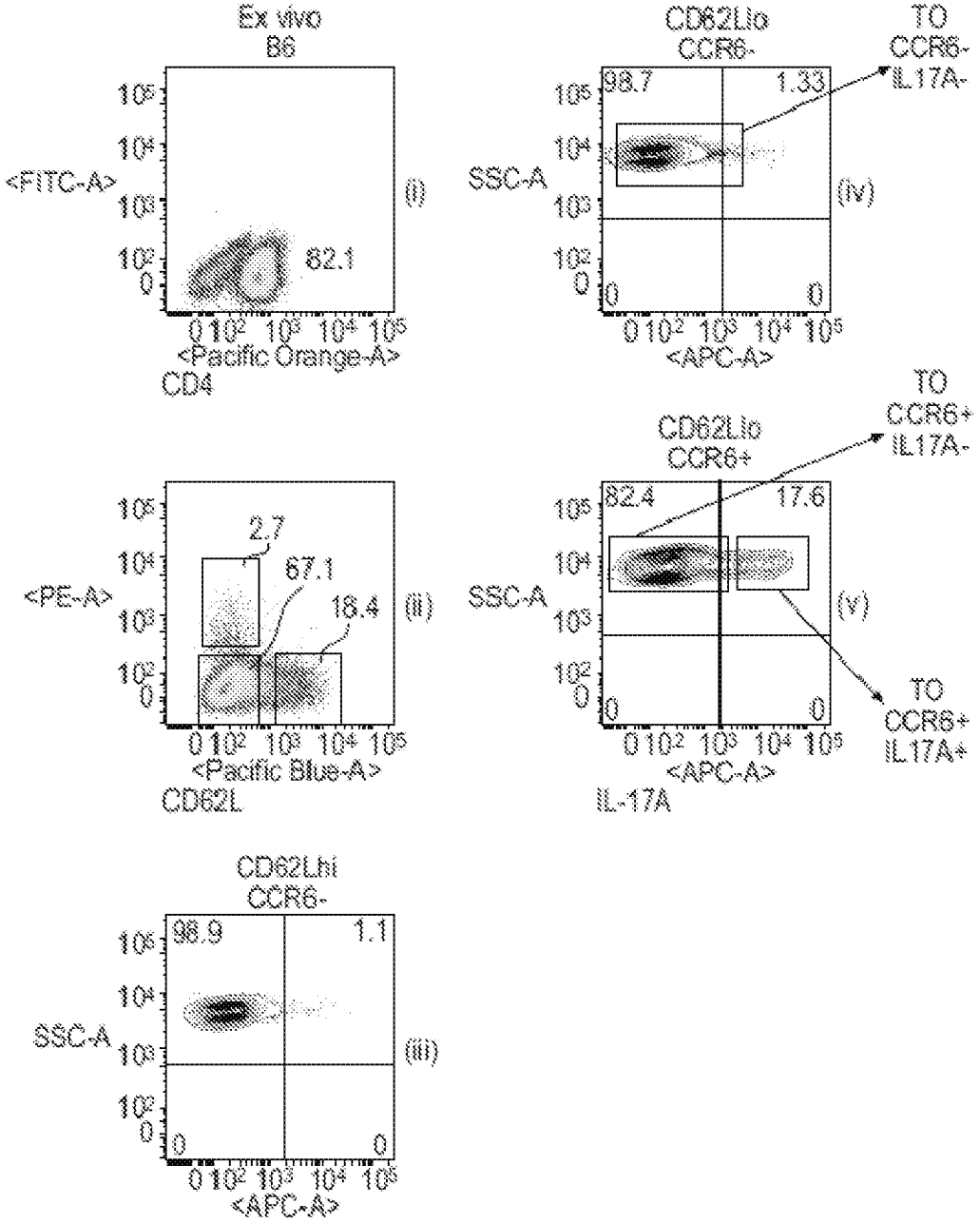

Ex vivo
B6
<FITC-A>
(i)
82.1
<Pacific Orange-A>
CD4
CD62Lio
CCR6-
98.7
1.33
TO
CCR6-
IL17A-
SSC-A
(iv)
<APC-A>
<PE-A>
2.7
67.1
18.4
(ii)
<Pacific Blue-A>
CD62L
CD62Lio
CCR6+
82.4
17.6
TO
CCR6+
IL17A-
(v)
TO
CCR6+
IL17A+
<APC-A>
IL-17A
CD62Lhi
CCR6-
98.9
1.1
SSC-A
(iii)
<APC-A>

FIG. 13A

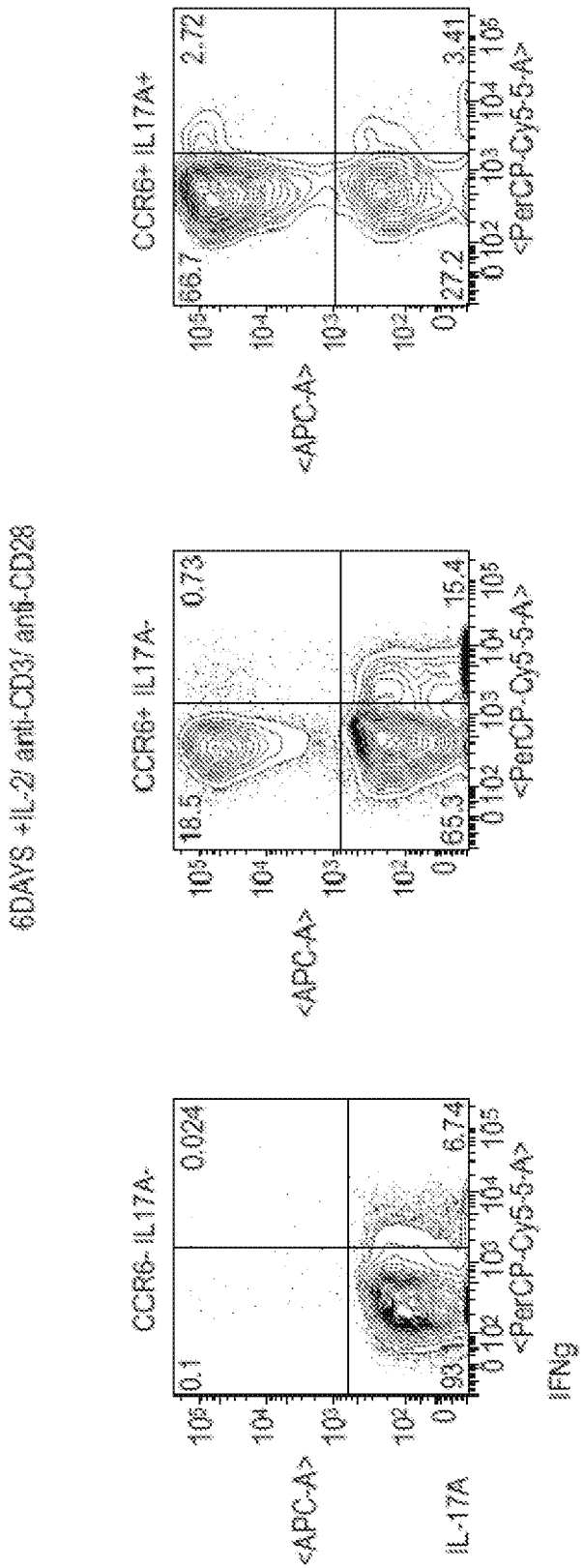

6DAYS +IL-2/ anti-CD3/ anti-CD28
CCR6- IL17A-
CCR6+ IL17A-
CCR6+ IL17A+
0.024
0.73
2.72
10.1
18.5
66.7
93.1
65.3
27.2
6.74
15.4
3.41
<APC-A>
<PerCP-Cy5-5-A>
IL-17A
IFNg

FIG. 13B

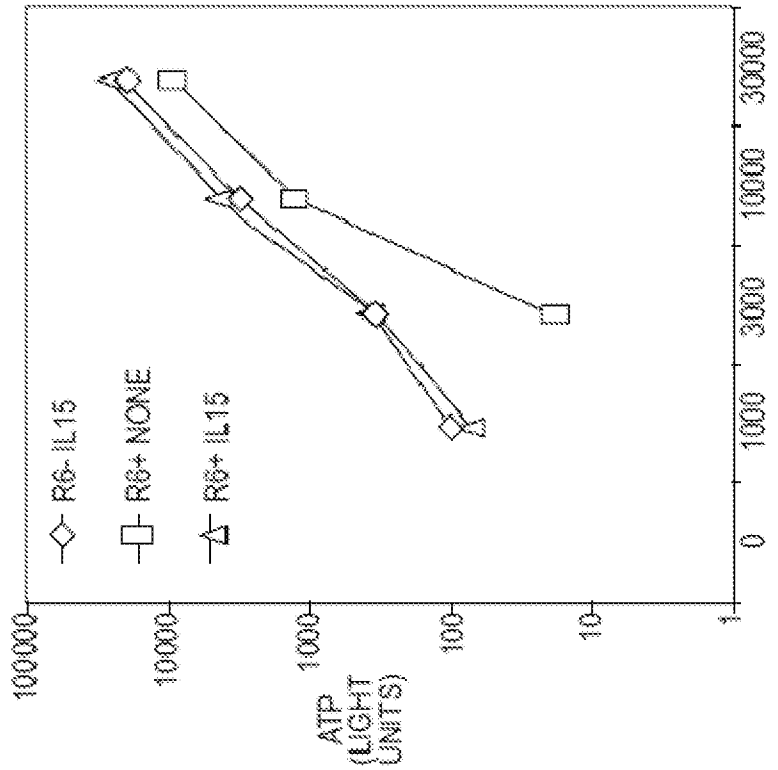


FIG. 14B

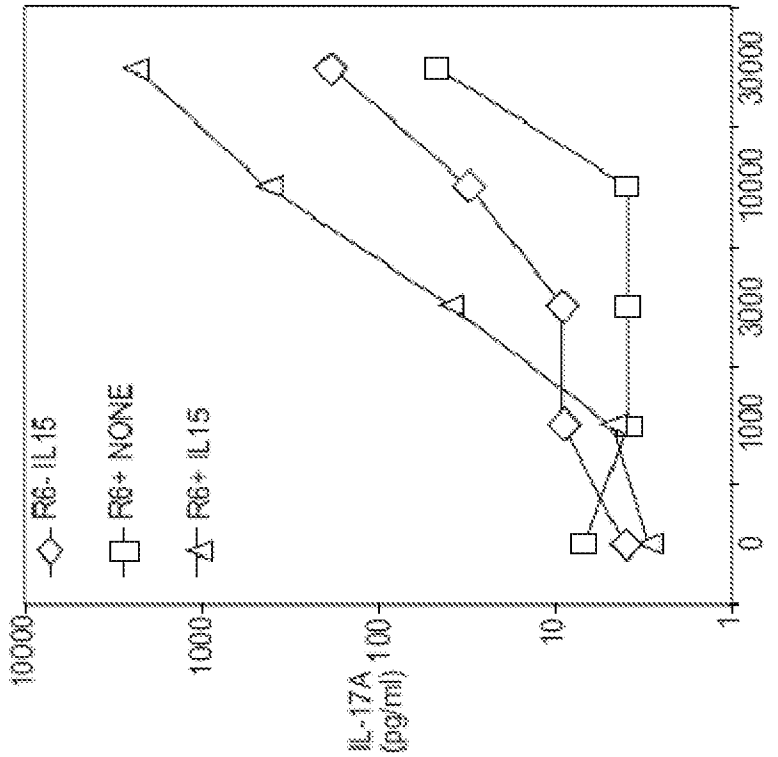


FIG. 14A

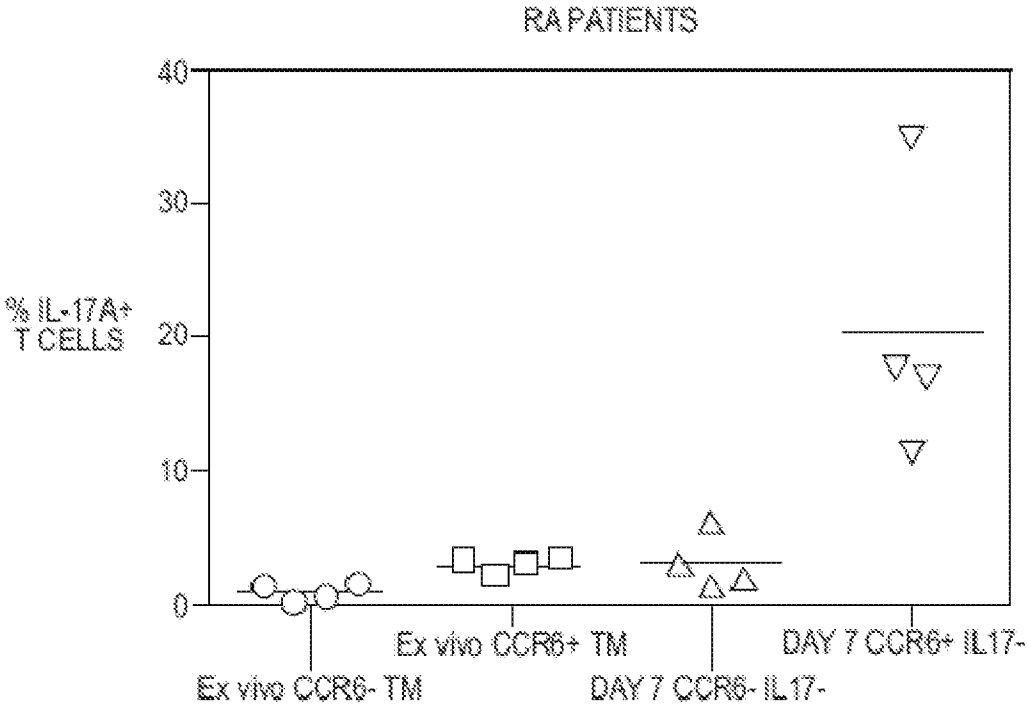
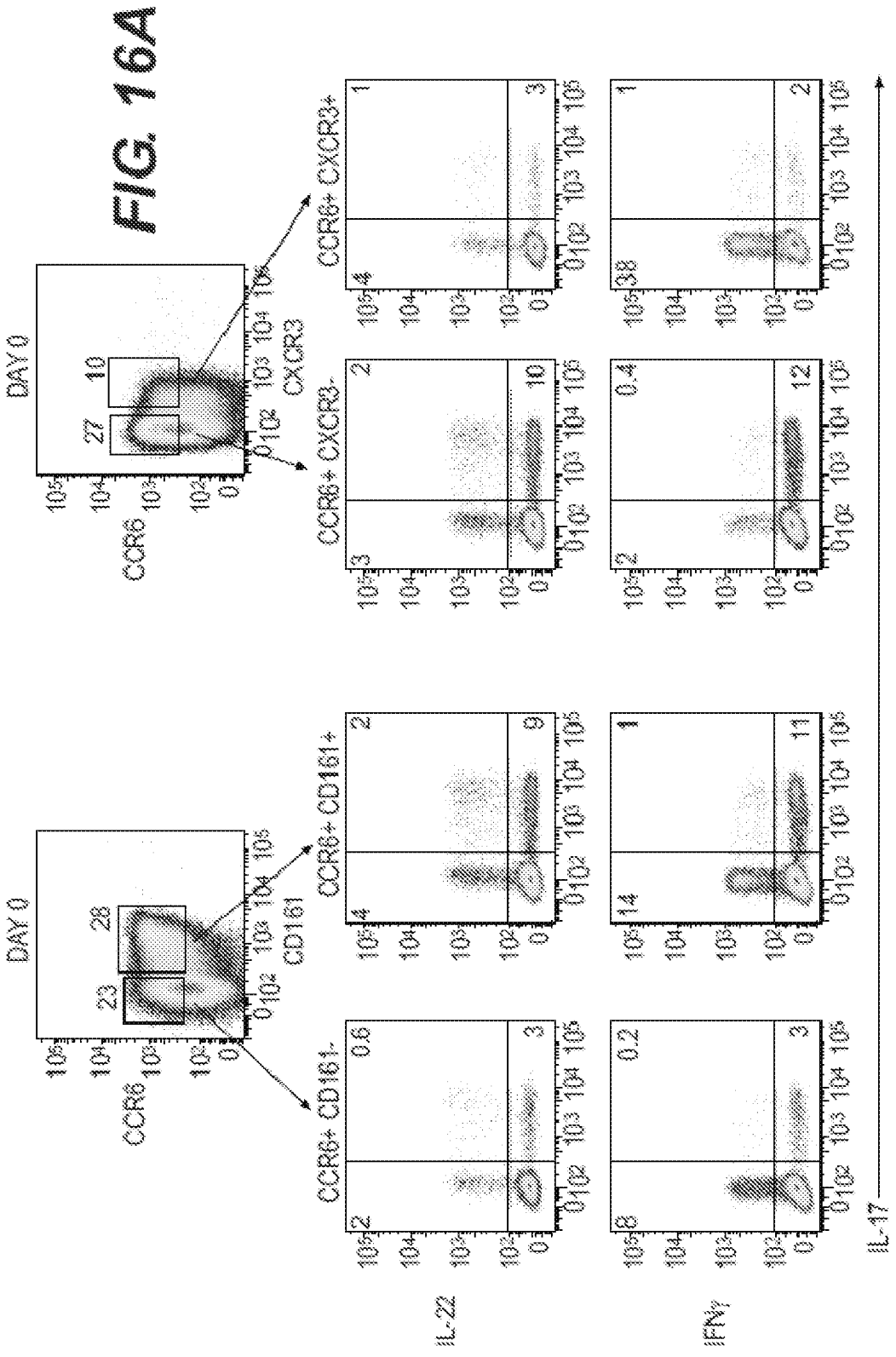

RA PATIENTS
% IL-17A+ T CELLS
40
30
20
10
0
Ex vivo CCR6- TM
Ex vivo CCR6+ TM
DAY 7 CCR6- IL17-
DAY 7 CCR6+ IL17-

FIG. 15

FIG. 16A


DAY 0
CCR6
CXCR3
CD161
CCR6+ CXCR3+
CCR6+ CXCR3-
CCR6+ CD161+
CCR6+ CD161-
IL-22
IFNγ
IL-17

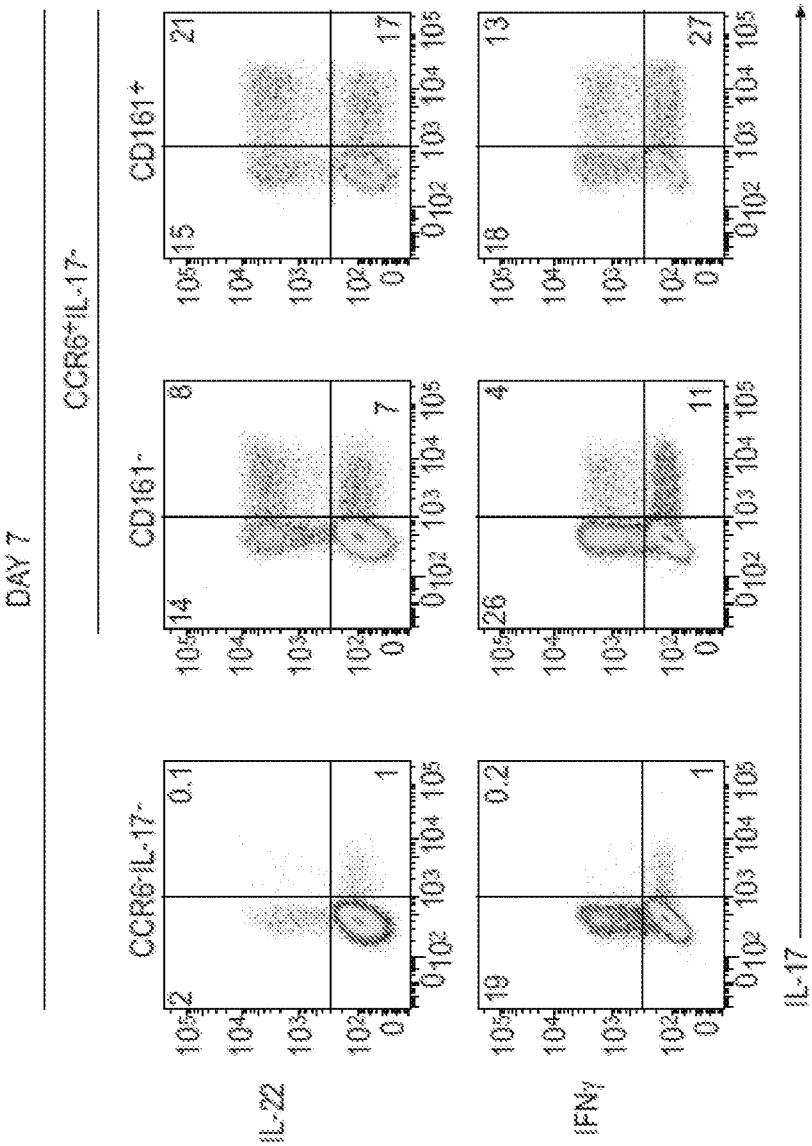

DAY 7
CCR6+IL-17-
CD161-
CD161+
CCR6-IL-17-
IL-22
IFNγ
IL-17
2
0.1
1
14
8
7
19
0.2
1
26
4
11
15
21
17
18
13
27

FIG. 16B

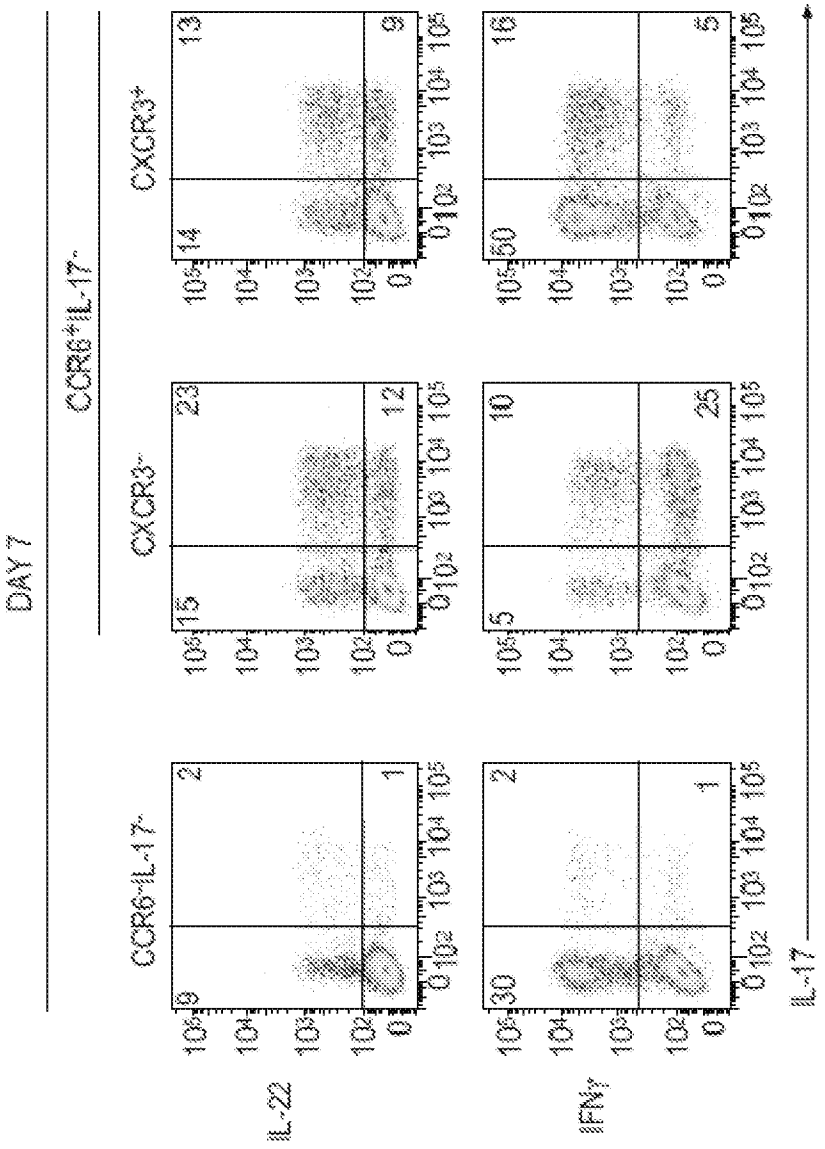

DAY 7
CCR6+IL-17+
CCR6+IL-17-
CXCR3-
CXCR3+
IL-22
IFNγ
IL-17
0
2
1
15
23
12
14
13
9
30
2
1
5
10
25
50
16
5

FIG. 16C

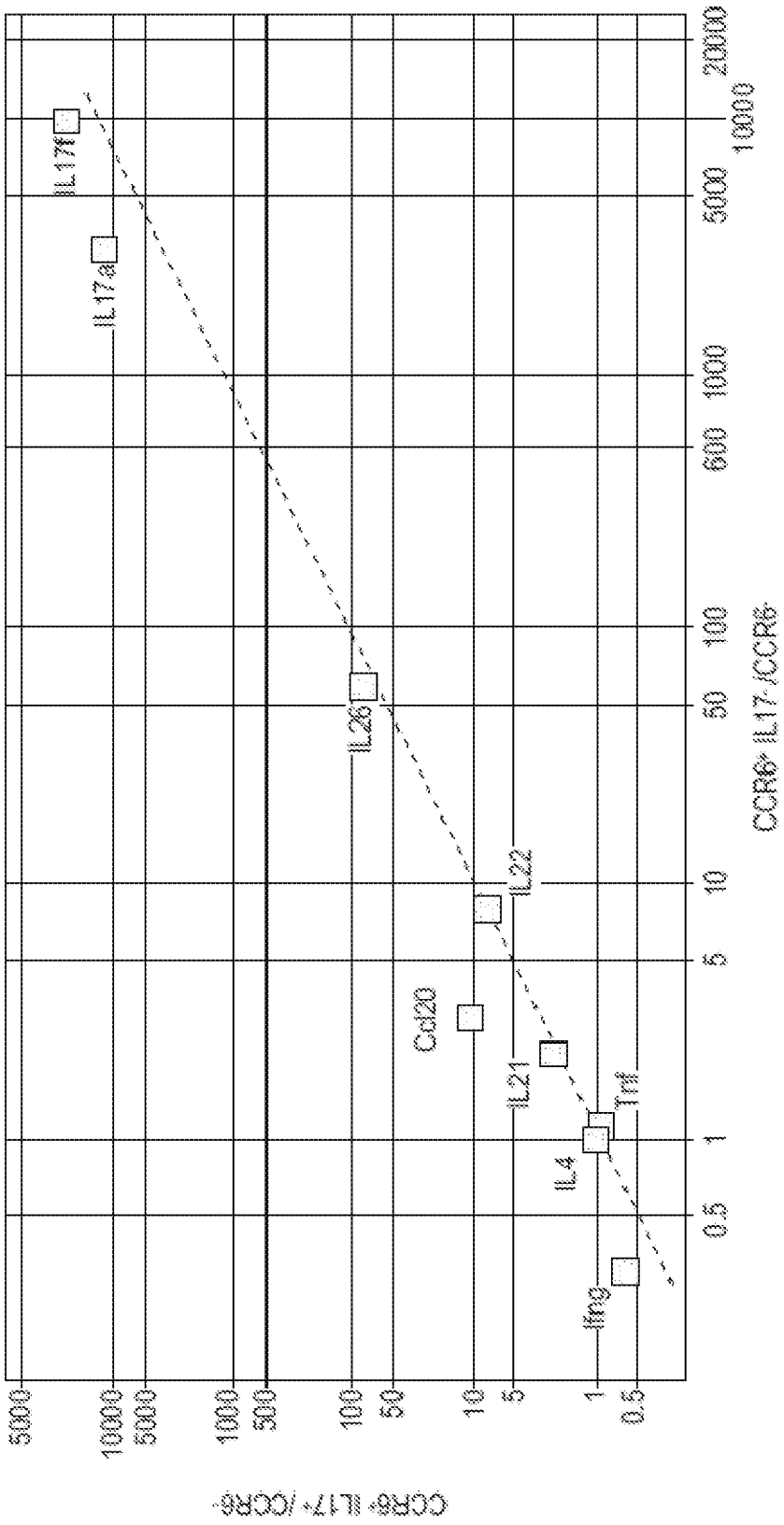

IL17f
IL17a
IL26
IL22
Cd20
IL21
IL4
Tnf
Ifng
5000
10000
5000
1000
500
100
50
10
5
1
0.5
0.5
1
5
10
50
100
600
1000
5000
10000
20000
CCR6+ IL17+/CCR6+
CCR6+ IL17-/CCR6+

FIG. 17

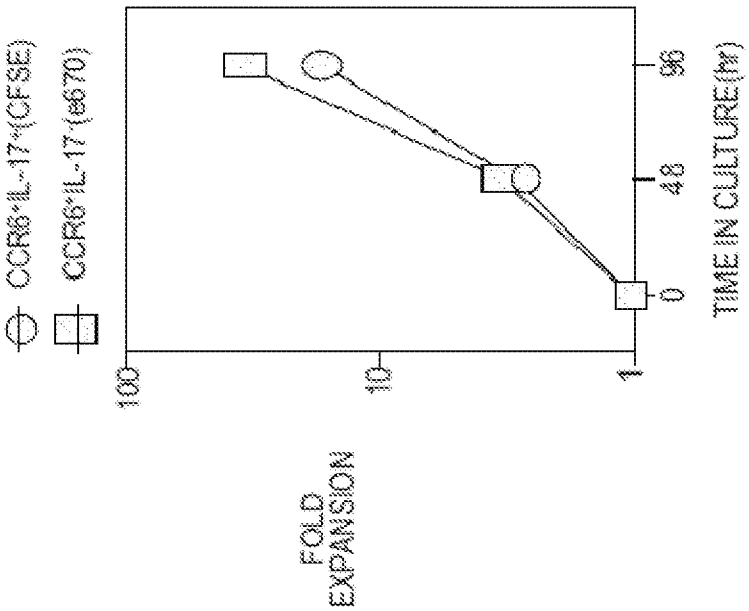


FIG. 18A

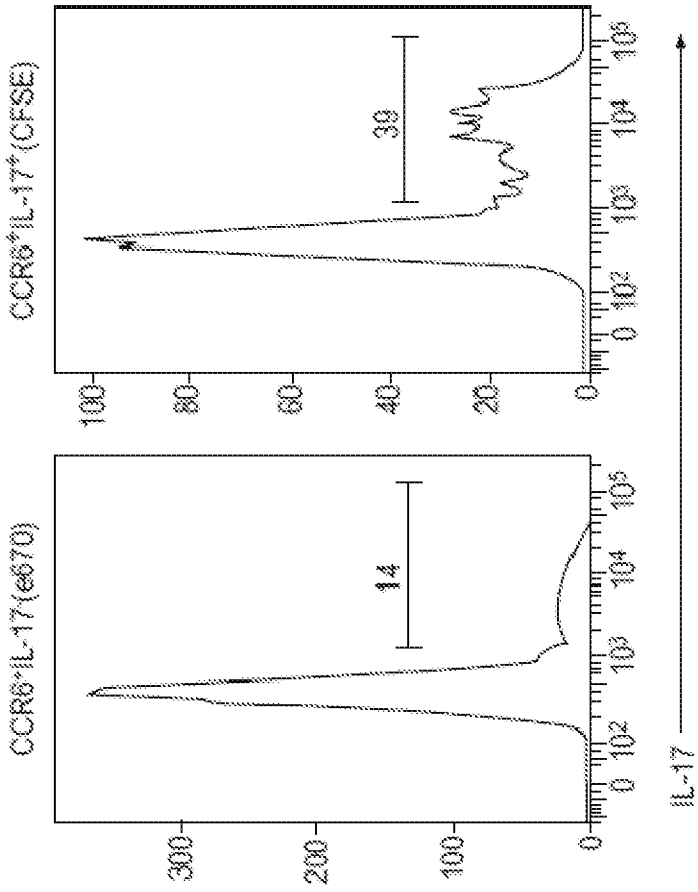


FIG. 18B

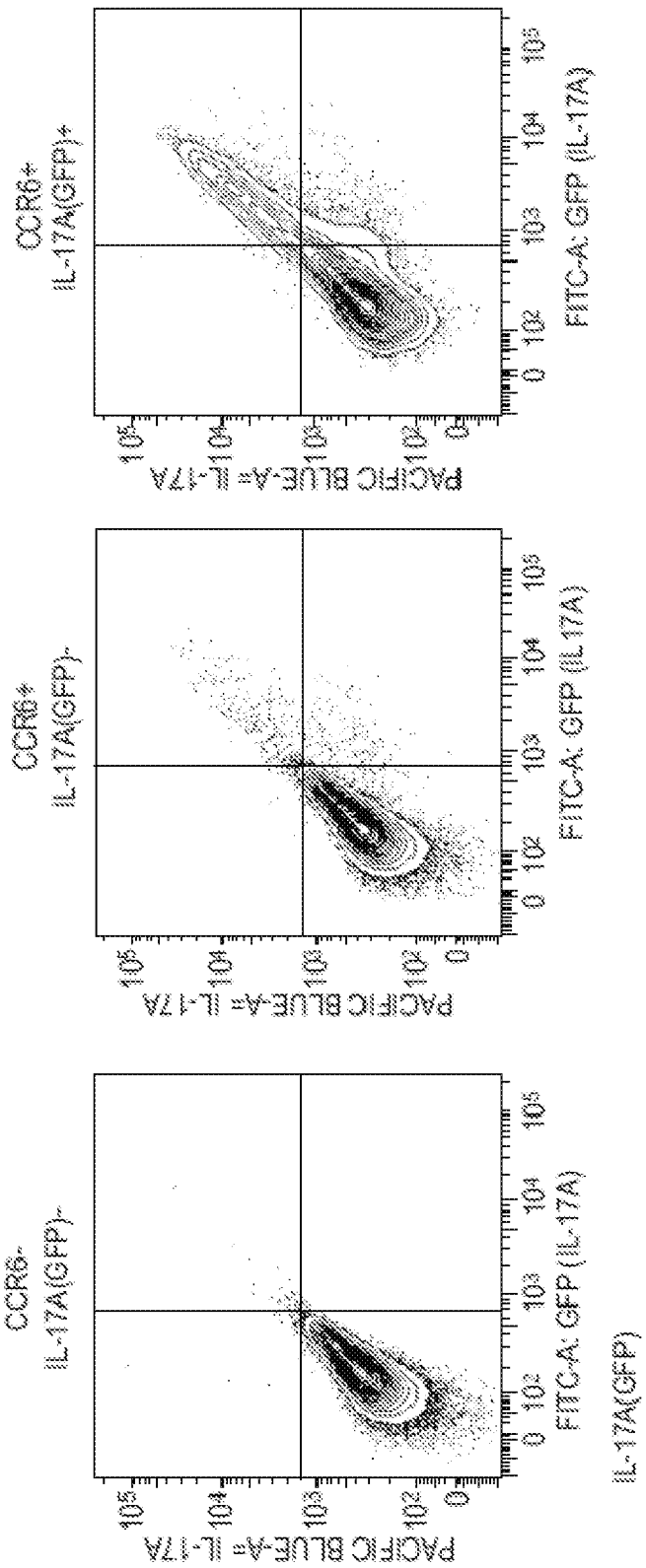

CCR6+
IL-17A(GFP)+
CCR6+
IL-17A(GFP)-
CCR6-
IL-17A(GFP)-
PACIFIC BLUE-A= IL-17A
FITC-A: GFP (IL-17A)
IL-17A(GFP)

FIG. 19