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(54) **Title:** ARIH2 REGULATES DENDRITIC CELL ACTIVATION AND AUTOIMMUNITY

a	Stage	+/+	+/-	-/-	Total	% +/-
	E14.5	10	12	7	29	24%
	PND1	6	14	7	27	26%
	PND2	2	6	3	11	27%
	PND3	5	12	2	20	10%
	PND6	5	6	2	20	10%
	PND8	9	10	1	20	5% *
	3 wk	137	242	13	392	3.3% **

(57) **Abstract:** Aih2 negatively regulates dendritic cell (DC) activation and inhibits NFκB signaling. Methods and compositions are provided for suppressing immune response, reducing chronic inflammation, treating autoimmune diseases, preventing diabetes and inhibiting dendritic cell activation by increasing the biological activity of Aih2. Also provided are methods and compositions for enhancing immune response, treating infections, treating cancer and enhancing DC activation by decreasing the biological activity of Aih2.

ARIH2 REGULATES DENDRITIC CELL ACTIVATION AND AUTOIMMUNITY

RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. Provisional Application No. 61/391010, filed on October 7, 2010, the entire content of which, including the specification and the drawings, is hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] The present technology relates generally to methods and compositions for treating inflammation, a bacterial infection and cancer.

BACKGROUND

[0003] Throughout the disclosure, various technical and patent literature are identified by a bibliographic citation or by reference to an Arabic numeral. The full bibliographical citations of these literatures, references, identified by an Arabic numeral can be found immediately preceding the claims. The contents of all technical and patent literature are incorporated into the present disclosure by reference in their entirety.

[0004] Ariadne 2 (Arih2) is a highly conserved E3 ligase with a unique organization of RING Fingers, categorized as an *all-trans* retinoic acid inducible RING Finger (TRIAD) domain⁶⁻¹⁰. Little is known about the functions of Arih2, even though studies in *Drosophila* and *Arabidopsis*^{7,11-13} and in vitro mammalian cells have implicated a role for Arih2 in haematopoiesis³⁻⁶.

SUMMARY OF THE INVENTION

[0005] It is discovered that Arih2 negatively regulates dendritic cell (DC) activation and inhibits NF κ B signaling. Accordingly, it is demonstrated that Arih2 inhibits

immune response and reduces inflammation and also inhibition of Arih2 leads to enhanced immune response and DC activation.

[0006] The present inventors discovered that in the mixed 129JOla x C57BL/6 background, loss of Arih2 led to a lymphoproliferative phenotype and early mortality. This was accentuated on the C57BL/6 background with Arih2 deficiency causing embryonic lethality at day E16.5. Lethality was partially rescued by MyD88 deficiency, indicating a role for Arih2 in innate immune function.

[0007] It is also found that Arih2^{-/-} dendritic cells (DC) expressed high levels of activation markers and were responsible for the induction of diverse autoimmune diseases including diabetes in a mouse model. This was in part due to increased and sustained nuclear p65/RelA and a hyperactive NFκB response. Adoptive transfer models confirmed that deficiency of Arih2 specifically in the haematopoietic compartment was able to recapitulate the lymphoproliferative phenotype.

[0008] Consistent with a break in tolerance, DC-T cell homeostasis was altered. Increased serum immunoglobulin levels, and dramatic increases in peripheral and organ infiltrating T cells, macrophages, granulocytes and DCs were observed. These results indicate Arih2 as a novel gene involved in immune regulation by antagonizing DC activation. This also establishes Arih2 as having a critical role in the maintenance of peripheral tolerance and the pathogenesis of autoimmunity and has implications for immunotherapies and dendritic cell-based vaccines.

[0009] Accordingly, it is discovered herein that increased biological activity of Arih2 can result in (a) suppression of immune response, (b) reduction of chronic inflammation, (c) treatment of autoimmune diseases, (d) prevention of diabetes that is caused by autoimmune reactions and (e) inhibition of DC activation.

[0010] In the same vein, decreased biological activity of Arih2 can give rise to (a) enhanced immune response, (b) treatment of infections, (c) treatment of cancer and (d) DC activation.

[0011] Accordingly, one embodiment of the present disclosure provides a method for inhibiting activation of a dendritic cell, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, contacting the dendritic cell with an effective amount of an agent that increases the biological activity of Aih2 in the dendritic cell, thereby inhibiting activation of the dendritic cell.

[0012] In one aspect, the dendritic cell underexpresses the Aih2. In another aspect, the method further comprises contacting the dendritic cell with an effective amount of an agent that decreases the biological activity of MyD88. Such contacting, in some aspects, can be in vitro, ex vivo or in vivo.

[0013] Another embodiment of the present disclosure provides a method for suppressing an immune response in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby suppressing the immune response. In some aspects, the patient is in need of immunosuppression for a graft versus host disease.

[0014] Also provided, in one embodiment, is a method for reducing inflammation in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby reducing inflammation. In one aspect, the inflammation is chronic inflammation.

[0015] Further provided, in yet another embodiment, is a method for treating an autoimmune disease or condition in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby treating the autoimmune disease.

[0016] Yet another embodiment of the present disclosure provides a method for preventing diabetes in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of

an agent that increases the biological activity of Arih2, thereby preventing diabetes. In one aspect, the patient is suffering from an autoimmune reaction or disorder.

[0017] In any of the above embodiments, Arih2 can be underexpressed in dendritic cells in the patient. In another aspect, the method of any of the embodiments can further comprise administering to the patient an agent that decreases the biological activity of MyD88.

[0018] In any of the above embodiments, the agent that increases the biological activity of Arih2 comprises an Arih2 protein, a transcription regulator of Arih2, a polynucleotide encoding the Arih2 protein, a vector comprising the polynucleotide, a small molecule Arih2 activator or an equivalent of each thereof or a stem cell comprising each thereof.

[0019] In one aspect, the vector is a viral vector. In another aspect, the viral vector further comprises a transcription regulator. In some aspects, the viral vector is selected from the group of an adenovirus, adeno-associated virus, lentivirus or retrovirus.

[0020] In one aspect, the Arih2 protein or polynucleotide of the above embodiment is encapsulated. In another aspect, the Arih2 protein further comprises a cell penetrating peptide (CPP) that can be chemically or recombinantly linked to the Arih2 protein.

[0021] In some aspects, a cell penetrating peptide can be a HIV-TAT peptide.

[0022] Further, one embodiment of the present disclosure provides a method for promoting activation of a dendritic cell, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, contacting the dendritic cell with an agent that decreases the biological activity of Arih2, thereby promoting activation of the dendritic cell.

[0023] In one aspect, the dendritic cell overexpresses Arih2. In another aspect, the method further comprises contacting the dendritic cell with an effective amount of

an agent that increases the biological activity of MyD88. In yet another aspect, the contacting is in vitro or in vivo.

[0024] Yet another embodiment of the present disclosure provides a method for enhancing an immune response in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that decreases the biological activity of Aih2, thereby enhancing the immune response in the patient.

[0025] One embodiment of the present disclosure provides a method for treating an infection in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that decreases the biological activity of Aih2, thereby treating the infection in the patient. In one aspect, the infection is a bacterial infection.

[0026] Another embodiment of the present disclosure provides a method for treating a cancer patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that decreases the biological activity of Aih2, thereby treating cancer in the patient.

[0027] In one aspect, the cancer patient suffers one or more cancer selected from an adenocarcinoma, a leukemia, a lymphoma, a melanoma, a myeloma, a sarcoma or a teratocarcinoma. In another aspect, the cancer patient suffers from a cancer in one or more of adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid or uterus.

[0028] In one aspect of these embodiments, the method further comprises administering to the patient a chemotherapy, a biological cancer therapy or a radiation therapy. In another aspect, Aih2 is overexpressed in the patient.

[0029] An agent that decreases the biological activity of Arih2 can be a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to Arih2 DNA or mRNA, or a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA, a vector comprising the polynucleotide, an antibody or an antibody fragment that specifically recognizes the Arih2 protein, a small molecule Arih2 inhibitor or an equivalent of each thereof or a stem cell comprising each thereof.

[0030] Another embodiment of the present invention is a method for decreasing nuclear expression of I κ B β in a patient in need thereof, comprising administering to the patient in need thereof an effective amount of an agent that increases the biological activity of Arih2, thereby decreasing nuclear expression of I κ B β .

[0031] Another embodiment of the present invention is a method for increasing nuclear expression of I κ B β in a patient in need thereof, comprising administering to the patient in need thereof an effective amount of an agent that decreases the biological activity of Arih2, thereby increasing nuclear expression of I κ B β .

[0032] In one aspect, the vector is a viral vector. In another aspect, the viral vector is selected from the group of an adenovirus, adeno-associated virus, lentivirus or retrovirus.

[0033] The present disclosure, in one embodiment, provides a cell comprising, or alternatively consisting essentially of, or yet alternatively consisting of, a recombinant Arih2 protein or polynucleotide. In one aspect, the Arih2 protein is fused to a cell penetrating peptide.

[0034] The present disclosure, in another embodiment, provides a cell comprising, or alternatively consisting essentially of, or yet alternatively consisting of, an agent that decreases the biological activity of Arih2. In one aspect, the cell is a stem cell. In another aspect, the cell is a stem cell. In yet another aspect, the stem cell is a monoblast stem cell.

[0035] Further, the present disclosure also provides a kit for use in inhibiting dendritic cell activation, reducing chronic inflammation or treating an autoimmune

disease, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, an effective amount of an agent that increases the biological activity of Arih2 and instructions to use.

[0036] In another embodiment, the present disclosure provides a kit for use in promoting dendritic cell activation, treating an infection or treating a cancer, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, an effective amount of an agent that decreases the biological activity of Arih2 and instructions to use.

[0037] Also provided is a method for determining whether a subject is likely to develop a chronic inflammation, an autoimmune disease or diabetes, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, determining in a sample isolated from the subject the expression level of Arih2, wherein an underexpression of Arih2 determines that the subject is likely to develop a chronic inflammation, an autoimmune disease or diabetes.

[0038] Yet also provided is a method for determining whether a patient is suitable for a treatment comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administration of an effective amount of an agent that increases the biological activity of Arih2, wherein the patient suffers from a chronic inflammation, an autoimmune disease or diabetes, comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an underexpression of Arih2 determines that the patient is suitable for the treatment.

[0039] Still another embodiment of the present disclosure provides a method for determining whether a subject is likely to develop an infection or cancer, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, determining in a sample isolated from the subject the expression level of Arih2, wherein an overexpression of Arih2 determines that the subject is likely to develop an infection or cancer.

[0040] Further provided is a method for determining whether a patient is suitable for a treatment comprising, or alternatively consisting essentially of, or yet

alternatively consisting of, administration of an effective amount of an agent that decreases the biological activity of Arih2, wherein the patient suffers from an infection or cancer comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an overexpression of Arih2 determines that the patient is suitable for the treatment.

[0041] Still further provided is a method for identifying an agent suitable for increasing or decreasing the biological activity of Arih2, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, contacting a dendritic cell with a candidate agent and determining the activation of the dendritic cell, wherein an increased activation indicates that the candidate agent is suitable for decreasing the biological activity of Arih2 or a decreased activation indicates that the candidate agent is suitable for increasing the biological activity of Arih2.

[0042] Still further provided is a method for enhancing potency of a vaccine in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of the vaccine and an effective amount of an agent that decreases the biological activity of Arih2, thereby enhancing vaccination of the vaccine in the patient.

[0043] The administration of the vaccine and the agent can be concurrent or sequential.

[0044] The agent that decreases the biological activity of Arih2 can comprise a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to Arih2 DNA or mRNA, or a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA, a vector comprising the polynucleotide, an antibody or an antibody fragment that specifically recognizes the Arih2 protein, a small molecule Arih2 inhibitor or an equivalent of each thereof or a stem cell comprising each thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0045] **FIG. 1** shows that Arih2 deficient mice have reduced survival and develop a lymphoproliferative phenotype. **a.** Number and ratio of offspring from 129JOla x

C57BL/6 Aih2^{+/-} intercrosses (*p <0.05, ** p<0.0001). **b.** Survival curve for gene targeted Aih2 mice on 129JOla x C57BL/6 background. **c.** Weight (g) of 129JOla x C57BL/6 Aih2^{-/-} and Aih2^{+/-} pups and adults, with mean $\pm$ standard deviation. *p<0.02 at all stages for comparisons between genotypes; PND1 p<0.0001, PND2 p=0.012, PND3 p=0.019, PND6 p=0.007, 5wk+ p=0.002. n=3-8 for PND analysis, n=9-13 for mice older than 5 weeks (5wk+). **d.** Haematoxylin and eosin (H+E) staining of liver, heart and lung showing cellular infiltrates. *a*=alveoli; *b*=bronchiole; *ec*=endocardium; *my*=myocardium; *hv*=hepatic venule. Black bar indicates 100 μ m. **e.** Number and ratio of offspring from C57BL/6 Aih2^{+/-} intercrosses. * p<0.05, ** p<0.0001. **f.** Embryo size comparison. Black bar indicates 5mm. H+E and *in situ* end labelling of fragment DNA (ISEL) staining of embryonic foetal liver sections at E16.5. Black bar =100 μ m. **g.** Total viable cells and percentage of apoptotic cells (Annexin-V+ and 7AAD+) in embryonic foetal livers at day E14.5 and E16.5, with mean $\pm$ standard deviation. n=9-25 *p<0.0001, ** p=0.031. **h.** Number and ratio of Aih2^{+/-} and MyD88^{+/-} double heterozygous crosses. A = Aih2, m=MyD88, p=0.035. † mice have reduced viability, with death occurring at day 11 and day 88. E=embryonic day; PND=post-natal day, wk=week. The data was reproduced in three independent mouse lines; n=32

[0046] FIG. 2 shows that loss of Aih2 in DCs leads to a premature activation state.

a. Proportions of CD80, CD83, CD86, CD40 and MHCII (I-A/I-E) in Aih2^{+/-} and Aih2^{-/-} foetal liver derived DCs following incubation with media, LPS or CpG.

Representative flow cytometry plots with mean $\pm$ standard deviation are shown. n=3-8 per group. **b.** Cytokine production from DCs incubated with media or CpG.

Black=Aih2^{+/-} and grey=Aih2^{-/-}. Bar graphs show means $\pm$ standard deviation from 3 independent experiments. **c.** Means $\pm$ standard deviation of cytokine production from Aih2^{+/-} and Aih2^{-/-} DCs. Black=Aih2^{+/-} and grey=Aih2^{-/-}. Data is a triplicate, representative of 3 independent sets. *p<0.001. **d.** Diabetes onset as determined by random blood glucose (mmol/L) in RIP-GP mice injected with DCs. DCs were incubated with media or CpG and pulsed with LCMV gp33, gp61 gp276 peptides. d=days. **e.** The number of mice with diabetes determined by random blood glucose levels $\geq$ 15mmol/L for a minimum of 3 consecutive days. The denominator indicates

group size of 3 independent experimental sets. * $p=0.011$, ** p value=0.035. **f.** Time to event curves demonstrating the number of mice with diabetes defined as random blood glucose levels ≥ 15 mmol/L for a minimum of 3 consecutive days. The denominator indicates group size of 3 independent experimental sets.

[0047] FIG. 3 shows that loss of Aih2 leads to hyperactive and sustained NF κ B signaling in DCs. **a.** Aih2^{+/-} and Aih2^{-/-} foetal liver derived DCs were stimulated with CpG 10 μ M for western blot and gel mobility shift analyses. All number timepoints are in minutes. Total cell lysates were immunoblotted with MyD88, phospho(473)-Akt (pAkt) and total Akt. **b.** p(Ser32)I κ B and total I κ B levels in DCs and mEFs treated with TNF α 10 μ g/ml. **c.** Nuclear fraction lysates were immunoblotted for p(536)p65/RelA, total p65/RelA, and RelB expression **d.** Nuclear fractions were subjected to gel mobility shift assays with NF κ B probes. **e.** Total cell lysates were immunoblotted with p(Thr202/Tyr204)ERK, total ERK and p(Thr180/Tyr182)p38 and total p38. **f.** Nuclear fractions were subjected to gel mobility shift assays with AP-1 probes. β -Actin levels are used as loading control for cytoplasmic fractions and H2AX levels are used as loading control for nuclear fractions. These results were reproducible in three independent experiments.

[0048] FIG. 4 shows that Aih2^{-/-} chimeric mice have reduced viability and display signs of a lymphoproliferative disorder. **a.** Chimeric mice at 6-7 weeks postreconstitution. **b.** Weight change (g) in chimeras. Significant weight loss is shaded in purple. $n=7$. * $p=0.003$, ** $p=0.005$. **c.** Illness onset curve for Aih2^{+/-} and Aih2^{-/-} chimeras. $n=6-30$. $p<0.0001$. **d.** Serum immunoglobulin levels. $n=6-18$. **e.** Serum cytokine levels. $n=7-20$. **f.** Peripheral T cell, B cell and innate cell populations from spleen and lymph nodes of Aih2^{+/-} and Aih2^{-/-} chimeras as determined by FACS analysis, with mean $\pm$ standard deviation. CD25+CD69+ cells are early activated T cells, CD44hiCD62Llo are 'memorylike'/ effector T cells, Gran=granulocyte, Mp=macrophage. $n=8-20$. * $p<0.01$, ** $p<0.00003$ **g.** H+ E staining of heart, liver, lung, GI (gastrointestinal tract/small intestine) and colon from Aih2^{+/-} and Aih2^{-/-} chimeras. Black bar indicates 100 μ m. ec=endocardium; my=myocardium; a=alveoli and alveolar duct; b=respiratory bronchiole; at=an artery;

hv=portal venules, bile ductule, hepatic artery and vein; *hc*=hepatocytes; *v*=villi; *lp*=lamina propria; *mm*=muscularis mucosae; *v-gb*=villi with goblet cells; *sm*=submucosa and *mm* muscularis.

[0049] FIG. 5 show that *Arih2* is highly expressed in immune cells. **a.** Expression of *Arih2*. *Arih2* mRNA expression in adult mouse tissues assessed by Northern blot and compared to *Gapdh* expression. **b.** Protein expression of *ARIH2* in adult mouse tissues, compared to β -Actin expression. **c.** Protein expression of *ARIH2* in mouse embryo day 14.5 (E14.5), T cells, and dendritic cell (DC) cytoplasmic (cyto) and nuclear (nuc) fractions, compared to β -Actin and H2AX respectively. mEF $+/+$ = wild type mouse embryonic fibroblast. B= brain; H=heart; K=kidney; L=lung; Li=liver; P=pancreas; Sp=spleen; M=skeletal muscle; Sm=smooth muscle; Ty=thymus; T=testis. **d.** *Arih2* gene targeting strategy. Structure of the mouse *Arih2* wild type (wt) locus (top), the *Arih2* targeting vector construct and the predicted mutant (mt) *Arih2* allele (bottom). Exons (black box) 6, 7 and 8 were replaced by the PGKNeomycin resistance cassette (pgk-Neo). LA=long arm; SA=short arm; fp=3' flanking probe; np=Neomycin probe. **e.** NcoI digest and 3' fp Southern blot confirmation of correct targeting of the *Arih2* locus. **f.** PCR analysis of *Arih2* $+/+$, *Arih2* $^{+/-}$ and *Arih2* $^{-/-}$ genomic DNA. The wild type band is 1kb and the mutant band is 400bp. **g.** *ARIH2* protein expression in *Arih2* $+/+$, *Arih2* $^{+/-}$ and *Arih2* $^{-/-}$ mEFs compared to β -actin expression. **h.** Number and ratio of offspring from intercrosses of *Arih2* $^{+/-}$ and (T) *TNFA* $^{+/-}$, **i.** *TNFA* $^{-/-}$, or **j.** *IFNY* $^{-/-}$ mice.

[0050] FIG. 6 shows results of fetal liver haematopoietic stem cell methylcellulose colony formation assay in media containing **a.** IL-3, IL-6, SCF and negative for erythropoietin (EPO); **b.** IL-3, IL-6, SCF, and EPO. Colonies were counted on day 8. **c.** Lethally irradiated *Rag1* $^{-/-}$ mice repopulated with *Arih2* $^{+/-}$ and *Arih2* $^{-/-}$ E14.5 foetal liver cells. Representative FACS profile of peripheral blood from at 4-5 weeks post reconstitution, with means $\pm$ standard deviation. * p value <0.001. **d.** Population of cDCs and pDCs differentiated in vitro using GM-CSF or Flt3L cultures as previously described. Lutz M.B. et al. *J Immunol Methods* 223, 77-92 (1999); Naik, S.H. et al. *Methods Mol Biol* 595, 167-176 (2010); Naik, S.H. et al. *Nat Immunol* 8, 1217-1226

(2007). DCs were collected on day 8-9 and stained for FACS analysis as in Naik, S.H. et al. *Methods Mol Biol* 595, 167-176 (2010).

[0051] FIG. 7 shows that loss of Aih2 leads to hyperactive and sustained NF κ B signaling in DCs. **a.** Aih2^{+/-} and Aih2^{-/-} foetal liver derived DCs were stimulated with LPS 10 μ g/mL for western blot and gel mobility shift analyses. All numbered timepoints are in minutes. Total cell lysates were immunoblotted with MyD88, phospho(473)-Akt (pAkt) and total Akt. **b.** p(Ser32)I κ B and total I κ B levels in DCs. **c.** Nuclear fraction lysates were immunoblotted with p(536)p65/RelA, total p65/RelA, and RelB expression **d.** Nuclear fractions were subjected to gel mobility shift assays with NF κ B probes. **e.** Total cell lysates were immunoblotted with p(Thr202/Tyr204)ERK, total ERK and p(Thr180/Tyr182)p38 and total p38 **f.** Nuclear fractions were subjected to gel mobility shift assays with AP-1 probes. β -Actin levels are used as loading control for cytoplasmic fractions and H2AX levels are used as loading control for nuclear fractions. These results were reproducible in three independent experiments.

[0052] FIG. 8 shows results of chimeric mice examined at 6-7 weeks post-reconstitution. **a.** Aih2^{+/-} and Aih2^{-/-} B cell proliferation 48 hours post-stimulation determined by tritiated [H3] thymidine incorporation (x10⁴ counts per minute (cpm)). B cells were stimulated with media, 5 μ g/mL IgM, 5 μ g/mL IgM + 5 μ g/mL CD40, LPS (011B5)20ng/mL, CpG 10nM, and PolyI:C 100ng/mL. n=8. *p<0.001. **b.** Aih2^{+/-} and Aih2^{-/-} T cells stimulated with plate bound CD3 ϵ (10 μ g/mL) + CD28 (5 μ g/mL) or PMA (10ng/mL) + Ionomycin (50ng/mL) measured by CFSE dilution determined by FACS with mean $\pm$ standard deviation. n=5-11. *p=0.001 and **p=0.0005. **c.** Aih2^{+/-} and Aih2^{-/-} T cells were stimulated with CD3 (10 μ g/mL) + CD28 (5 μ g/mL) or PMA(10ng/mL) + Ionomycin (50ng/mL) total lysates were used for Western blot analysis and immunoblotted p(Ser32)-I κ B, total I κ B, p(473)pAkt, total Akt and p(Thr180/Tyr182)p38 and compared to β -Actin levels. **d.** Aih2^{+/-} and Aih2^{-/-} T cells were stained for surface markers CD4, CD25 and intracellular Foxp3. n=6. * p =0.012 and **p=0.0098. All number timepoints are in minutes. **e.** Regulatory T cell induced colitis model. Weight monitoring over 14 weeks postinjection of CD4+CD25+

(Te) and CD4⁺CD25⁻ (Tr) T cells. n=3-4. **f.** H+ E and CD3 immunohistology on transverse colon sections at 14 weeks. Histological grading was recorded as previously described¹. **g.** Black bar indicates 100µm.

[0053] FIG. 9 shows H+ E and immunohistochemical staining of **a.** heart, **b.** lung, **c.** liver, **d.** small intestine and **e.** colon from Arih2^{+/-} and Arih2^{-/-} chimeras. Sections were immunostained with CD3 (Dako), B220 (BD Pharmingen) and F4/80 (Serotec). Black bar indicates 100µm. *ec*=endocardium; *my*=myocardium; *a*=alveoli and alveolar duct; *b*=respiratory bronchiole; *at*=an artery; *hv*=portal venules, bile ductule, hepatic artery and vein; *hc*=hepatocytes; *v*=villi; *lp*=lamina propria; *mm*=muscularis mucosae; *v-gb*=villi with goblet cells; *sm*=submucosa and *mm* muscularis. **f.** Table of mean ± standard deviation of infiltrating immune cells (x10⁶) cells from lung and liver of Arih2^{+/-} and Arih2^{-/-} chimeras analysed by FACS. *p<0.02 and **p<0.005.

[0054] FIG. 10 shows a re-analysis of the results of the experiment shown in Fig. 1h after completion of the experiment in a larger number of mice. Number and ratio of Arih2^{+/-} and MyD88^{+/-} double heterozygous crosses. A = Arih2, m=MyD88, p<0.00001. † mice have reduced viability, with death occurring between day 1 and day 88. E=embryonic day; PND=post-natal day, wk=week. The data was reproduced in three independent mouse lines; n=89.

[0055] FIG. 11 shows that loss of Arih2 led to hyperactive and sustained NF κ B signaling in DCs. **a-c.** Arih2^{+/-} and Arih2^{-/-} fetal liver derived macrophages and mEFs with 10ng/mL LPS and 10µg/mL TNF α respectively for western blot analysis. Total cell lysates were immunoblotted with I κ B α and β -actin. **d and f.** AriH2^{+/-} and Arih2^{-/-} foetal liver derived DCs were stimulated with 10µM CpG or LPS for western blot analysis. Total cell lysates were immunoblotted with p(Thr202/Tyr204)ERK, total ERK and p(Thr180/Tyr182)p38 and total p38. **e and g.** Nuclear fractions were subjected to gel mobility shift assays with AP-1 probes. All numbered timepoints are in minutes. These results were reproduced in three independent experiments.

[0056] FIG. 12 shows that loss of Aih2 led to hyperactive and sustained NFκB signalling in DCs. **a.** Aih2^{+/-} and Aih2^{-/-} mEFs were stimulated with 10ng/mL LPS for western blot analysis. Total cell lysates were immunoblotted with total IκBα and β-Actin. **b.** Aih2^{+/-} and Aih2^{-/-} fetal liver derived DCs were stimulated with CpG 10μM for western blot and gel mobility shift analyses. All number timepoints are in minutes. Total cell lysates were immunoblotted with MyD88, phospho(473)-Akt (pAkt) and total Akt. **c.** p(Ser32)IκB and total IκB levels in DCs treated with TNFα 10μg/ml. **d.** Nuclear fraction lysates were immunoblotted for p(536)p65/RelA, total p65/RelA, and IκBβ expression. **e.** Nuclear fractions were subjected to gel mobility shift assays with NFκB probes. **f.** Fetal liver derived DCs were left untreated or incubated with 10 ng/mL of LPS for 30 min and then harvested or treated for a further 2-4 hr (as indicated) with 25μM MG132 proteasome inhibitor. Nuclear extracts were prepared and immunoprecipitated with IκBβ antibodies and Western blots were then probed for the presence of Aih2 and ubiquitin. β-Actin and H2AX levels were used as loading control for cytoplasmic and nuclear fractions respectively. These results were reproduced in three independent experiments.

DETAILED DESCRIPTION

[0057] As used herein, certain terms may have the following defined meanings. As used in the specification and claims, the singular form “a,” “an” and “the” include singular and plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a single cell as well as a plurality of cells, including mixtures thereof.

[0058] As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or

method. "Consisting of" shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

[0059] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". The term "about" also includes the exact value "X" in addition to minor increments of "X" such as "X + 0.1" or "X - 0.1." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0060] A "composition" is also intended to encompass a combination of active agent and another carrier, e.g., compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Carriers also include pharmaceutical excipients and additives proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. Carbohydrate excipients are also intended within the scope

of this invention, examples of which include but are not limited to monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

[0061] The term “pharmaceutically acceptable carrier” (or medium), which may be used interchangeably with the term biologically compatible carrier or medium, refers to reagents, cells, compounds, materials, compositions, and/or dosage forms that are not only compatible with the cells and other agents to be administered therapeutically, but also are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other complication commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers suitable for use in the present invention include liquids, semi-solid (e.g., gels) and solid materials (e.g., cell scaffolds and matrices, tubes sheets and other such materials as known in the art and described in greater detail herein). These semi-solid and solid materials may be designed to resist degradation within the body (non-biodegradable) or they may be designed to degrade within the body (biodegradable, bioerodable). A biodegradable material may further be bioresorbable or bioabsorbable, i.e., it may be dissolved and absorbed into bodily fluids (water-soluble implants are one example), or degraded and ultimately eliminated from the body, either by conversion into other materials or breakdown and elimination through natural pathways.

[0062] As used herein, the term “patient” intends an animal, a mammal or yet further a human patient. For the purpose of illustration only, a mammal includes but is not limited to a human, a simian, a murine, a bovine, an equine, a porcine or an ovine.

[0063] As used herein, the term “oligonucleotide” or “polynucleotide” refers to a short polymer composed of deoxyribonucleotides, ribonucleotides or any combination thereof. Oligonucleotides are generally at least about 10, 15, 20, 25,

30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides in length. An oligonucleotide may be used as a primer or as a probe.

[0064] The term “isolated” as used herein refers to molecules or biological or cellular materials being substantially free from other materials, e.g., greater than 70%, or 80%, or 85%, or 90%, or 95%, or 98%. In one aspect, the term “isolated” refers to nucleic acid, such as DNA or RNA, or protein or polypeptide, or cell or cellular organelle, or tissue or organ, separated from other DNAs or RNAs, or proteins or polypeptides, or cells or cellular organelles, or tissues or organs, respectively, that are present in the natural source and which allow the manipulation of the material to achieve results not achievable where present in its native or natural state, e.g., recombinant replication or manipulation by mutation. The term “isolated” also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polypeptides which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides, e.g., with a purity greater than 70%, or 80%, or 85%, or 90%, or 95%, or 98%. The term “isolated” is also used herein to refer to cells or tissues that are isolated from other cells or tissues and is meant to encompass both cultured and engineered cells or tissues.

[0065] A “recombinant” nucleic acid refers an artificial nucleic acid that is created by combining two or more sequences that would not normally occur together. In one embodiment, it is created through the introduction of relevant DNA into an existing organismal DNA, such as the plasmids of bacteria, to code for or alter different traits for a specific purpose, such as antibiotic resistance. A “recombinant” polypeptide is a polypeptide that is derived from a recombinant nucleic acid.

[0066] As used herein, the term “promoter” refers to a nucleic acid sequence sufficient to direct transcription of a gene. Also included in the invention are those

promoter elements which are sufficient to render promoter dependent gene expression controllable for cell type specific, tissue specific or inducible by external signals or agents.

[0067] In some embodiments, a promoter is an inducible promoter or a discrete promoter. Inducible promoters can be turned on by a chemical or a physical condition such as temperature or light. Examples of chemical promoters include, without limitation, alcohol-regulated, tetracycline-regulated, steroid-regulated, metal-regulated and pathogenesis-related promoters. Examples of discrete promoters can be found in, for examples, Wolfe et al. (2002) *Molecular Endocrinology* 16(3): 435-49.

[0068] As used herein, the term “regulatory element” refers to a nucleic acid sequence capable of modulating the transcription of a gene. Non-limiting examples of regulatory element include promoter, enhancer, silencer, poly-adenylation signal, transcription termination sequence. Regulatory element may be present 5' or 3' regions of the native gene, or within an intron.

[0069] Various proteins are also disclosed herein with their GenBank Accession Numbers for their human proteins and coding sequences. However, the proteins are not limited to human-derived proteins having the amino acid sequences represented by the disclosed GenBank Accession numbers, but may have an amino acid sequence derived from other animals, particularly, a warm-blooded animal (e.g., rat, guinea pig, mouse, chicken, rabbit, pig, sheep, cow, monkey, etc.).

[0070] As used herein, the term “Arih2”, or “ARIH2 ariadne homolog 2” refers to a protein having an amino acid sequence substantially identical to any of the representative Arih2 sequences of GenBank Accession Nos. NP_006312 (human), NP_035920 (mouse) or NP_001012275 (rat). Suitable cDNA encoding Arih2 are provided at GenBank Accession Nos. NM_006321 (human), NM_011790 (mouse) or NM_001012275.

[0071] As used herein, the term “biological activity of Arih2” refers to any biological activity associated with the full length native Arih2 protein. In one embodiment, the

biological activity of Aih2 refers to inhibition of NF- κ B signaling. In another aspect, the biological activity of Aih2 refers to inhibiting dendritic cell activation. In suitable embodiments, the Aih2 biological activity is equivalent to the activity of a protein having an amino acid sequence represented by GenBank Accession No. NP_006312, NP_035920 or NP_001012275. Measurement of transcriptional activity can be performed using any known method, such as immunohistochemistry, reporter assay or RT-PCR.

[0072] A patient in need of decreased nuclear expression of I κ B β is a patient who can benefit from increased biological activity of Aih2. A patient in need of decreased nuclear expression of I κ B β is, for example, a patient suffering from an autoimmune disease, diabetes, an inflammatory disease, or is a patient in need of immunosuppression (e.g., for a graft versus host disease). Preferably, the inflammatory disease is a disease involving chronic inflammation. Examples of an inflammatory disease include but are not limited to rheumatoid arthritis, osteoarthritis, sepsis, asthma, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, psoriasis, dermatitis, scleroderma, hepatitis, nephritis, and acquired immunodeficiency syndrome.

[0073] A patient in need of increased nuclear expression of I κ B β is a patient who can benefit from decreased biological activity of Aih2. A patient in need of decreased nuclear expression of I κ B β includes, for example, a patient suffering from cancer or an infection, or a patient in need of an enhanced immune response.

[0074] As used herein, the term "MyD88" or "myeloid differentiation primary response gene (88)" refers to a protein having an amino acid sequence substantially identical to the representative MyD88 sequence of GenBank Accession No. NP_001166038. A suitable cDNA encoding CD18 is provided at GenBank Accession No. NM_001172567.

[0075] As used herein, the term "biological activity of MyD88" refers to any biological activity associated with the full length native MyD88 protein. In one embodiment, the biological activity of MyD88 refers to enhanced immune response

or dendritic cell activation. In suitable embodiments, the MyD88 biological activity is equivalent to the activity of a protein having an amino acid sequence represented by GenBank Accession No. NP_001166038. Measurement of transcriptional activity can be performed using any known method, such as immunohistochemistry, reporter assay or RT-PCR.

[0076] As used herein, the term “treating” is meant administering a pharmaceutical composition for the purpose of improving the condition of a patient by reducing, alleviating, reversing, or preventing at least one adverse effect or symptom.

[0077] As used herein, the term “preventing” is meant identifying a subject (i.e., a patient) having an increased susceptibility to a disease but not yet exhibiting symptoms of the disease, and administering a therapy according to the principles of this disclosure. The preventive therapy is designed to reduce the likelihood that the susceptible subject will later become symptomatic or that the disease will be delay in onset or progress more slowly than it would in the absence of the preventive therapy. A subject may be identified as having an increased likelihood of developing the disease by any appropriate method including, for example, by identifying a family history of the disease or other degenerative brain disorder, or having one or more diagnostic markers indicative of disease or susceptibility to disease.

[0078] As used herein, the term “sample” or “test sample” refers to any liquid or solid material containing nucleic acids. In suitable embodiments, a test sample is obtained from a biological source (i.e., a “biological sample”), such as cells in culture or a tissue sample from an animal, most preferably, a human.

[0079] As used herein, the term “substantially identical”, when referring to a protein or polypeptide, is meant one that has at least 80%, 85%, 90%, 95%, or 99% sequence identity to a reference amino acid sequence. The length of comparison is preferably the full length of the polypeptide or protein, but is generally at least 10, 15, 20, 25, 30, 40, 50, 60, 80, or 100 or more contiguous amino acids. A “substantially identical” nucleic acid is one that has at least 80%, 85%, 90%, 95%, or 99% sequence identity to a reference nucleic acid sequence. The length of comparison is

preferably the full length of the nucleic acid, but is generally at least 20 nucleotides, 30 nucleotides, 40 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 125 nucleotides, or more.

[0080] A “biological equivalent” of a protein or nucleic acid refers to a protein or nucleic acid that is substantially identical to the protein or nucleic acid.

[0081] As used herein, the term “effective amount” refers to a quantity of compound (e.g., a Aih2 protein or biologically active fragment thereof) delivered with sufficient frequency to provide a medical benefit to the patient. In one embodiment, an effective amount of a protein is an amount sufficient to treat or ameliorate a symptom of a neurological disease.

[0082] A population of cells intends a collection of more than one cell that is identical (clonal) or non-identical in phenotype and/or genotype.

[0083] “Substantially homogeneous” describes a population of cells in which more than about 50%, or alternatively more than about 60 %, or alternatively more than 70 %, or alternatively more than 75 %, or alternatively more than 80%, or alternatively more than 85 %, or alternatively more than 90%, or alternatively, more than 95 %, of the cells are of the same or similar phenotype. Phenotype can be determined by a pre-selected cell surface marker or other marker.

[0084] The terms autologous transfer, autologous transplantation, autograft and the like refer to treatments wherein the cell donor is also the recipient of the cell replacement therapy. The terms allogeneic transfer, allogeneic transplantation, allograft and the like refer to treatments wherein the cell donor is of the same species as the recipient of the cell replacement therapy, but is not the same individual. A cell transfer in which the donor's cells and have been histocompatibly matched with a recipient is sometimes referred to as a syngeneic transfer. The terms xenogeneic transfer, xenogeneic transplantation, xenograft and the like refer to treatments wherein the cell donor is of a different species than the recipient of the cell replacement therapy.

[0085] As used herein, an “antibody” includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0086] The antibodies can be polyclonal or monoclonal and can be isolated from any suitable biological source, e.g., murine, rat, sheep and canine.

[0087] A monoclonal antibody is an antibody produced by a single clone of cells or a hybridoma, and therefore is a single pure homogeneous type of antibody.

[0088] A hybridoma is a cell that is produced in the laboratory from the fusion of an antibody-producing lymphocyte and a non-antibody producing cancer cell, usually a myeloma or lymphoma. A hybridoma proliferates and produces a continuous supply of a specific monoclonal antibody.

[0089] The term “human antibody” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term “human antibody” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Thus, as used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_{H1}, C_{H2}, C_{H3}), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent

(mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

[0090] As used herein, a human antibody is “derived from” a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, e.g., by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library. A human antibody that is “derived from” a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequence of human germline immunoglobulins. A selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may

display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[0091] A "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. The term also intends recombinant human antibodies. Methods to making these antibodies are described herein.

[0092] The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. Methods to making these antibodies are described herein.

[0093] As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

[0094] The terms "polyclonal antibody" or "polyclonal antibody composition" as used herein refer to a preparation of antibodies that are derived from different B-cell lines. They are a mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope.

[0095] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0096] As used herein, the term "label" intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, e.g., N-terminal histidine tags (N-His), magnetically active isotopes, e.g., ^{115}Sn , ^{117}Sn and ^{119}Sn , a non-radioactive isotopes such as ^{13}C and ^{15}N , polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to magnetically active isotopes, non-radioactive isotopes, radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it may be quantified. A response that is simply detected generally comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component.

[0097] Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescently labeling assay

components are known in the art and described for example in Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.).

Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

[0098] Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade BlueTM, and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.).

[0099] In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

Modes for Carrying out the Invention

[0100] One embodiment of the present disclosure provides a method for inhibiting activation of a dendritic cell, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, contacting the dendritic cell with an effective amount of an agent that increases the biological activity of Aih2 in the dendritic cell, thereby inhibiting activation of the dendritic cell.

[0101] In one aspect, the dendritic cell underexpresses the Aih2. In another aspect, the method further comprises contacting the dendritic cell with an agent that decreases the biological activity of MyD88. Such contacting, in some aspects, can be in vitro, ex vivo or in vivo.

[0102] Another embodiment of the present disclosure provides a method for suppressing an immune response in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of ARIH2, thereby suppressing the immune response. In some aspects, the patient is in need of immunosuppression for a graft versus host disease.

[0103] Also provided, in one embodiment, is a method for reducing inflammation in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of ARIH2, thereby reducing inflammation. In one aspect, the inflammation is chronic inflammation.

[0104] Further provided, in yet another embodiment, is a method for treating an autoimmune disease or condition in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of ARIH2, thereby treating the autoimmune disease.

[0105] Yet another embodiment of the present disclosure provides a method for preventing diabetes in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that increases the biological activity of ARIH2, thereby preventing diabetes. In one aspect, the patient is suffering from an autoimmune reaction or disorder.

[0106] In any of the above embodiments, ARIH2 can be underexpressed on dendritic cells in the patient. In another aspect, the method of any of the embodiments can further comprise administering to the patient an agent that decreases the biological activity of MYD88.

[0107] In any of the above embodiments, the agent that increases the biological activity of ARIH2 comprises an ARIH2 protein, a transcription regulator of ARIH2, a polynucleotide encoding the ARIH2 protein, a vector comprising the polynucleotide, a

small molecule Arih2 activator or an equivalent of each thereof or a stem cell comprising each thereof.

[0108] Methods of increasing the biological activity of a gene or protein are known in the art and are further described below.

Methods for Increasing the Biological Activity of Arih2 in a Cell

[0109] Methods for increasing the level of a protein, or polypeptide or peptide, such as Arih2, in a cell are known in the art, however, the therapeutic and diagnostic benefit is provided herein. In one aspect, the Arih2 level is increased by increasing the amount of a polynucleotide encoding Arih2, as provided above, wherein that polynucleotide is expressed such that new Arih2 is produced. In another aspect, increasing the Arih2 level is increased by increasing the transcription of a polynucleotide encoding Arih2, or alternatively translation of Arih2, or alternatively post-translational modification, activation or appropriate folding of Arih2. In yet another aspect, increasing Arih2 level is increased by increasing the binding of the protein to appropriate cofactor, receptor, activator, ligand, or any molecule that is involved in the protein's biological functioning. In some embodiments, increasing the binding of Arih2 to the appropriate molecule is increasing the amount of the molecule. In one aspect of the embodiments, the molecule is the Arih2 protein. In another aspect of the embodiments, the molecule is a small molecule. In a further aspect of the embodiments, the molecule is a polynucleotide.

[0110] Methods of increasing the amount of polynucleotide in a cell are known in the art and can be modified for increasing the amount of a polynucleotide encoding Arih2. In one aspect, the polynucleotide can be introduced to the cell and expressed by a gene delivery vehicle that can include a suitable expression vector.

[0111] Suitable expression vectors are well-known in the art, and include vectors capable of expressing a polynucleotide operatively linked to a regulatory element, such as a promoter region and/or an enhancer that is capable of regulating expression of such DNA. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector

that, upon introduction into an appropriate host cell, results in expression of the inserted DNA. Appropriate expression vectors include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

[0112] As used herein, the term “vector” refers to a non-chromosomal nucleic acid comprising an intact replicon such that the vector may be replicated when placed within a cell, for example by a process of transformation. Vectors may be viral or non-viral. Viral vectors include retroviruses, adenoviruses, herpesvirus, papovirus, or otherwise modified naturally occurring viruses. Exemplary non-viral vectors for delivering nucleic acid include naked DNA; DNA complexed with cationic lipids, alone or in combination with cationic polymers; anionic and cationic liposomes; DNA-protein complexes and particles comprising DNA condensed with cationic polymers such as heterogeneous polylysine, defined-length oligopeptides, and polyethylene imine, in some cases contained in liposomes; and the use of ternary complexes comprising a virus and polylysine-DNA.

[0113] Non-viral vector may include plasmid that comprises a heterologous polynucleotide capable of being delivered to a target cell, either in vitro, in vivo or ex-vivo. The heterologous polynucleotide can comprise a sequence of interest and can be operably linked to one or more regulatory elements and may control the transcription of the nucleic acid sequence of interest. As used herein, a vector need not be capable of replication in the ultimate target cell or subject. The term vector may include expression vector and cloning vector.

[0114] A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either in vivo, ex vivo or in vitro. Examples of viral vectors include retroviral vectors, adenovirus vectors, adeno-associated virus vectors, alphavirus vectors and the like. Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger and Dubensky (1999) *Curr. Opin. Biotechnol.* 5:434-439 and Ying et al. (1999) *Nat. Med.* 5(7):823-827. In aspects where gene transfer is mediated by a retroviral

vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and a therapeutic gene. As used herein, "retroviral mediated gene transfer" or "retroviral transduction" carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

[0115] Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form which integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus.

[0116] In aspects where gene transfer is mediated by a DNA viral vector, such as an adenovirus (Ad) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a transgene.

Adenoviruses (Ads) are a relatively well characterized, homogenous group of viruses, including over 50 serotypes. See, e.g., International PCT Application No. WO 95/27071. Ads do not require integration into the host cell genome.

Recombinant Ad derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. See, International PCT Application Nos. WO 95/00655 and WO 95/11984. Wild-type AAV has high infectivity and specificity integrating into the host cell's genome. See, Hermonat and Muzyczka (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470 and Lebkowski et al. (1988) *Mol. Cell. Biol.* 8:3988-3996.

[0117] Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). In

order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression.

[0118] Gene delivery vehicles also include DNA/liposome complexes, micelles and targeted viral protein-DNA complexes. Liposomes that also comprise a targeting antibody or fragment thereof can be used in the methods of this invention. To enhance delivery to a cell, the nucleic acid or proteins of this invention can be conjugated to antibodies or binding fragments thereof which bind cell surface antigens, e.g., a cell surface marker found on stem cells or cardiomyocytes. In addition to the delivery of polynucleotides to a cell or cell population, direct introduction of the proteins described herein to the cell or cell population can be done by the non-limiting technique of protein transfection, alternatively culturing conditions that can enhance the expression and/or promote the activity of the proteins of this invention are other non-limiting techniques.

[0119] Proteins have been described that have the ability to translocate desired nucleic acids across a cell membrane. Typically, such proteins have amphiphilic or hydrophobic subsequences that have the ability to act as membrane-translocating carriers. For example, homeodomain proteins have the ability to translocate across cell membranes. The shortest internalizable peptide of a homeodomain protein, Antennapedia, was found to be the third helix of the protein, from amino acid position 43 to 58 (see, e.g., Prochiantz (1996) *Current Opinion in Neurobiology* 6:629-634. Another subsequence, the h (hydrophobic) domain of signal peptides, was found to have similar cell membrane translocation characteristics (see, e.g., Lin et al. (1995) *J. Biol. Chem.* 270:14255-14258). Such subsequences can be used to translocate oligonucleotides across a cell membrane. Oligonucleotides can be conveniently derivatized with such sequences. For example, a linker can be used to link the

oligonucleotides and the translocation sequence. Any suitable linker can be used, e.g., a peptide linker or any other suitable chemical linker.

[0120] Methods of delivering a protein to a cell, either to increase the biological activity of itself or a protein positively regulated by this protein, or to decrease the biological activity of a protein negatively regulated by this protein, are generally known in the art. For example, Aih2 can be delivered to a eukaryotic cell by a type III secretion machine. See, e.g., Galan and Wolf-Watz (2006) *Nature* 444:567-73. Biologically active and full length protein, for another example, can also be delivered into a cell using cell penetrant peptides (CPP) as delivery vehicles. The trans-activating transcriptional activator (TAT) from human immunodeficiency virus 1 (HIV-1) is such a CPP, which is able to deliver different proteins, such as horseradish peroxidase and RNase A across cell membrane into the cytoplasm in different cell lines. Wadia et al. (2004) *Nat. Med* 10:310-15. Accordingly, in one aspect, Aih2 can be delivered to a cell using TAT as a vehicle to increase the biological activity of Aih2 in the cell.

[0121] Liposomes, microparticles and nanoparticles are also known to be able to facilitate delivery of proteins or peptides to a cell by encapsulating the peptides (reviewed in Tan et al. (2010) *Peptides* 31(1):184-93). The liposomes, microparticles or nanoparticles can also comprise a targeting antibody or fragment thereof can be used in the methods of this invention. To enhance delivery to a cell, the proteins can be conjugated to antibodies or binding fragments thereof which bind cell surface antigens, e.g., a cell surface marker found on progenitor cells.

[0122] In another aspect, non-covalent method which forms CPP/protein complexes has also been developed to address the limitations in covalent method such as chemical modification before crosslinking and denaturation of proteins before delivery. For example, a short amphipathic peptide carrier, Pep-1 and protein complexes have proven effective for delivery. It was shown that Pep-1 could facilitate rapid cellular uptake of various peptides, proteins and even full-length antibodies with high efficiency and less toxicity. Cheng et al. (2001) *Nat. Biotechnol.* 19:1173-6.

[0123] Proteins can be synthesized for delivery. Nucleic acids that encode a protein or fragment thereof may be introduced into various cell types or cell-free systems for expression, thereby allowing purification of Aih2 or other proteins, for large-scale production and patient therapy.

[0124] Eukaryotic and prokaryotic expression systems may be generated in which a gene sequence is introduced into a plasmid or other vector, which is then used to transform living cells. Constructs in which the cDNA contains the entire open reading frame inserted in the correct orientation into an expression plasmid may be used for protein expression. Prokaryotic and eukaryotic expression systems allow for the protein to be recovered, if desired, as fusion proteins or further containing a label useful for detection and/or purification of the protein. Typical expression vectors contain regulatory elements that direct the synthesis of large amounts of mRNA corresponding to the inserted nucleic acid in the plasmid-bearing cells. They may also include a eukaryotic or prokaryotic origin of replication sequence allowing for their autonomous replication within the host organism, sequences that encode genetic traits that allow vector-containing cells to be selected for in the presence of otherwise toxic drugs, and sequences that increase the efficiency with which the synthesized mRNA is translated. Stable long-term vectors may be maintained as freely replicating entities by using regulatory elements of, for example, viruses (e.g., the OriP sequences from the Epstein Barr Virus genome). Cell lines may also be produced that have integrated the vector into the genomic DNA, and in this manner the gene product is produced on a continuous basis.

[0125] Expression of foreign sequences in bacteria, such as *Escherichia coli*, requires the insertion of the nucleic acid sequence into a bacterial expression vector. Such plasmid vectors contain several elements required for the propagation of the plasmid in bacteria, and for expression of the DNA inserted into the plasmid. Propagation of only plasmid-bearing bacteria is achieved by introducing, into the plasmid, selectable marker-encoding sequences that allow plasmid-bearing bacteria to grow in the presence of otherwise toxic drugs. The plasmid also contains a transcriptional promoter capable of producing large amounts of mRNA from the

cloned gene. Such promoters may be (but are not necessarily) inducible promoters that initiate transcription upon induction. The plasmid also preferably contains a polylinker to simplify insertion of the gene in the correct orientation within the vector.

[0126] Stable or transient cell line clones of mammalian cells can also be used to express a protein. Appropriate cell lines include, for example, COS, HEK293T, CHO, or NIH cell lines.

[0127] Once the appropriate expression vectors containing a gene, fragment, fusion, or mutant are constructed, they are introduced into an appropriate host cell by transformation techniques, such as, but not limited to, calcium phosphate transfection, DEAE-dextran transfection, electroporation, microinjection, protoplast fusion, or liposome-mediated transfection. The host cells that are transfected with the vectors of this invention may include (but are not limited to) E. coli or other bacteria, yeast, fungi, insect cells (using, for example, baculoviral vectors for expression in SF9 insect cells), or cells derived from mice, humans, or other animals (e.g., mammals). In vitro expression of a protein, fusion, polypeptide fragment, or mutant encoded by cloned DNA may also be used. Those skilled in the art of molecular biology will understand that a wide variety of expression systems and purification systems may be used to produce recombinant proteins and fragments thereof.

[0128] Once a recombinant protein is expressed, it can be isolated from cell lysates using protein purification techniques such as affinity chromatography. Once isolated, the recombinant protein can, if desired, be purified further by e.g., by high performance liquid chromatography (HPLC; e.g., see Fisher, Laboratory Techniques In Biochemistry And Molecular Biology, Work and Burdon, Eds., Elsevier, 1980).

Methods for Decreasing the Biological Activity of ARIH2 in a Cell

[0129] Further, one embodiment of the present disclosure provides a method for promoting activation of a dendritic cell, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, contacting the dendritic cell with an

agent that decreases the biological activity of Aih2, thereby promoting activation of the dendritic cell.

[0130] In one aspect, the dendritic cell overexpresses Aih2. In another aspect, the method further comprises contacting the dendritic cell with an agent that increases the biological activity of MyD88. In yet another aspect, the contacting is in vitro or in vivo.

[0131] Yet another embodiment of the present disclosure provides a method for enhancing an immune response in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that decreases the biological activity of Aih2, thereby enhancing the immune response in the patient.

[0132] One embodiment of the present disclosure provides a method for treating an infection in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that decreases the biological activity of Aih2, thereby treating the infection in the patient. In one aspect, the infection is a bacterial infection.

[0133] Another embodiment of the present disclosure provides a method for treating a cancer patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of an agent that decreases the biological activity of Aih2, thereby treating cancer in the patient.

[0134] In one aspect, the patient suffers one or more cancer selected from an adenocarcinoma, a leukemia, a lymphoma, a melanoma, a myeloma, a sarcoma or a teratocarcinoma. In another aspect, the patient suffers from a cancer in one or more of adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid or uterus.

[0135] In one aspect of these embodiments, the method further comprises administering to the patient a chemotherapy, a biological cancer therapy or a radiation therapy. In another aspect, Aih2 is overexpressed in the patient.

[0136] Still further provided is a method for enhancing potency of a vaccine in a patient, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient an effective amount of the vaccine and an effective amount of an agent that decreases the biological activity of Aih2, thereby enhancing vaccination of the vaccine in the patient.

[0137] An agent that decreases the biological activity of siRNA can be a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to Aih2 DNA or mRNA, or a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA, a vector comprising the polynucleotide, an antibody or an antibody fragment that specifically recognizes the Aih2 protein, a small molecule Aih2 inhibitor or an equivalent of each thereof or a stem cell comprising each thereof.

[0138] Methods of decreasing the biological activity or inhibiting a protein are known in the art. Non-limiting examples include siRNA, dsRNA, miRNA, antisense polynucleotide, ribozymes, triplex polynucleotide, antibody, antibody variant, antibody derivative or a fragment thereof, and other inhibitory polypeptides.

[0139] "Short interfering RNAs" (siRNA) refer to double-stranded RNA molecules (dsRNA), generally, from about 10 to about 30 nucleotides in length that are capable of mediating RNA interference (RNAi). "RNA interference" (RNAi) refers to sequence-specific or gene specific suppression of gene expression (protein synthesis) that is mediated by short interfering RNA (siRNA). As used herein, the term siRNA includes short hairpin RNAs (shRNAs). A siRNA directed to a gene or the mRNA of a gene may be a siRNA that recognizes the mRNA of the gene and directs a RNA-induced silencing complex (RISC) to the mRNA, leading to degradation of the mRNA. A siRNA directed to a gene or the mRNA of a gene may also be a siRNA that recognizes the mRNA and inhibits translation of the mRNA. A siRNA may be chemically modified to increase its stability and safety. See, e.g.

Dykxhoorn and Lieberman (2006) *Annu. Rev. Biomed. Eng.* 8:377-402 and U.S. Patent Application Publication No.: 2008/0249055.

[0140] “Double stranded RNAs” (dsRNA) refer to double stranded RNA molecules that may be of any length and may be cleaved intracellularly into smaller RNA molecules, such as siRNA. In cells that have a competent interferon response, longer dsRNA, such as those longer than about 30 base pair in length, may trigger the interferon response. In other cells that do not have a competent interferon response, dsRNA may be used to trigger specific RNAi.

[0141] “MicroRNAs” (miRNA) refer to single-stranded RNA molecules of 21-23 nucleotides in length, which regulate gene expression. miRNAs are encoded by genes from whose DNA they are transcribed but miRNAs are not translated into protein (non-coding RNA); instead each primary transcript (a pri-miRNA) is processed into a short stem-loop structure called a pre-miRNA and finally into a functional miRNA. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to down-regulate gene expression.

[0142] siRNA, dsRNA, and miRNA to inhibit gene expression can be designed following procedures known in the art. See, e.g., Dykxhoorn and Lieberman (2006) *Annu. Rev. Biomed. Eng.* 8:377-402; Dykxhoorn et al. (2006) *Gene Therapy* 13:541-52; Aagaard and Rossi (2007) *Adv. Drug Delivery Rev.* 59:75-86; de Fougères et al. (2007) *Nature Reviews Drug Discovery* 6:443-53; Krueger et al. (2007) *Oligonucleotides* 17:237-250; U.S. Patent Application Publication No.: 2008/0188430; and U.S. Patent Application Publication No.: 2008/0249055.

[0143] Delivery of siRNA, dsRNA or miRNA to a cell can be made with methods known in the art. See, e.g., Dykxhoorn and Lieberman (2006) *Annu. Rev. Biomed. Eng.* 8:377-402; Dykxhoorn et al. (2006) *Gene Therapy* 13:541-52; Aagaard and Rossi (2007) *Adv. Drug Delivery Rev.* 59:75-86; de Fougères et al. (2007) *Nature Reviews Drug Discovery* 6:443-53; Krueger et al. (2007) *Oligonucleotides* 17:237-

250; U.S. Patent Application Publication No.: 2008/0188430; and U.S. Patent Application Publication No.: 2 008/0249055.

[0144] “Antisense” oligonucleotides have nucleotide sequences complementary to the protein coding or “sense” sequence. Antisense RNA sequences function as regulators of gene expression by hybridizing to complementary mRNA sequences and arresting translation (Mizuno et al. (1984) PNAS 81:1966; Heywood et al. (1986) Nucleic Acids Res. 14:6771). An antisense polynucleotide comprising the entire sequence of the target transcript or any part thereof can be synthesized with methods known in the art. See e.g., Ferretti et al. (1986) PNAS 83:599. The antisense polynucleotide can be placed into vector constructs, and effectively introduced into cells to inhibit gene expression (Izant et al. (1984) Cell 36:1007). Generally, to assure specific hybridization, the antisense sequence is substantially complementary to the target sequence. In certain embodiments, the antisense sequence is exactly complementary to the target sequence. The antisense polynucleotides may also include, however, nucleotide substitutions, additions, deletions, transitions, transpositions, or modifications, or other nucleic acid sequences or non-nucleic acid moieties so long as specific binding to the relevant target sequence corresponding to the gene is retained as a functional property of the polynucleotide.

[0145] The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein and known to one of skill in the art. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by de novo chemical synthesis or by cloning. For example, an antisense RNA can be made by inserting (ligating) a gene sequence in reverse orientation operably linked to a promoter in a vector (e.g., plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention.

[0146] It will be appreciated that the oligonucleotides can be made using nonstandard bases (e.g., other than adenine, cytidine, guanine, thymine, and uridine) or nonstandard backbone structures to provides desirable properties (e.g., increased nuclease-resistance, tighter-binding, stability or a desired T_m). Techniques for rendering oligonucleotides nuclease-resistant include those described in PCT Publication WO 94/12633. A wide variety of useful modified oligonucleotides may be produced, including oligonucleotides having a peptide-nucleic acid (PNA) backbone (Nielsen et al. (1991) Science 254:1497) or incorporating 2'-O-methyl ribonucleotides, phosphorothioate nucleotides, methyl phosphonate nucleotides, phosphotriester nucleotides, phosphorothioate nucleotides, phosphoramidates. Another example of the modification is replacement of a non-bridging phosphoryl oxygen atom with a sulfur atom which increases resistance to nuclease digestion. Increased antisense polynucleotide stability can also be achieved using molecules with 2-methoxyethyl substituted backbones. See e.g., U.S. Patent Nos. 6,451,991 and 6,900,187.

[0147] In another embodiment, ribozymes can be used (see, e.g., Cech (1995) Biotechnology 13:323; and Edgington (1992) Biotechnology 10:256 and Hu et al., PCT Publication WO 94/03596). A ribonucleic acid enzyme ("ribozymes", "RNA enzyme", or "catalytic RNA") is an RNA molecule that catalyzes a chemical reaction. Many natural ribozymes catalyze either the hydrolysis of one of their own phosphodiester bonds, or the hydrolysis of bonds in other RNAs, but they have also been found to catalyze the aminotransferase activity of the ribosome. Methods of making and using ribozymes can be found in e.g., U.S. Patent Application Publication No. 2006/0178326.

[0148] "Triplex ribozymes" configurations allow for increased target cleavage relative to conventionally expressed ribozymes. Examples of triplex ribozymes include hairpin ribozymes and hammerhead ribozymes. Methods of making and using triplex ribozymes are found in, e.g., Aguino-Jarguin et al. (2008) Oligonucleotides 18(3):213-24 and U.S. Patent Application Publication No. 2005/0260163.

[0149] Proteins have been described that have the ability to translocate desired nucleic acids across a cell membrane. Typically, such proteins have amphiphilic or hydrophobic subsequences that have the ability to act as membrane-translocating carriers. For example, homeodomain proteins have the ability to translocate across cell membranes. The shortest internalizable peptide of a homeodomain protein, Antennapedia, was found to be the third helix of the protein, from amino acid position 43 to 58 (see, e.g., Prochiantz (1996) *Current Opinion in Neurobiology* 6:629-634. Another subsequence, the h (hydrophobic) domain of signal peptides, was found to have similar cell membrane translocation characteristics (see, e.g., Lin et al. (1995) *J. Biol. Chem.* 270:14255-14258). Such subsequences can be used to translocate oligonucleotides across a cell membrane. Oligonucleotides can be conveniently derivatized with such sequences. For example, a linker can be used to link the oligonucleotides and the translocation sequence. Any suitable linker can be used, e.g., a peptide linker or any other suitable chemical linker.

Antibodies and Antibody Compositions

[0150] The disclosure, in another embodiment, provides an antibody that binds the Arih2 protein for decreasing the biological activity of Arih2. Methods of preparing an antibody are generally known in the art. For example, United States Patent No. 6,727,350 discloses an antibody directed to Arih2.

[0151] The antibody can be a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a derivative or fragment thereof as defined below. In one aspect, the fragment comprises, or alternatively consists essentially of, or yet further consists of the CDR of the antibody. In one aspect, the antibody is detectably labeled or further comprises a detectable label conjugated to it. Also provided is a hybridoma cell line that produces a monoclonal antibody of this invention. Compositions comprising one or more of the above embodiments are further provided herein.

[0152] Also provided is a composition comprising the antibody and a carrier. Further provided is a biologically active fragment of the antibody, or a composition comprising the antibody fragment. Suitable carriers are defined supra.

[0153] Further provided is an antibody-peptide complex comprising, or alternatively consisting essentially of, or yet alternatively consisting of, the antibody and a polypeptide specifically bound to the antibody. In one aspect, the polypeptide is the chimeric polypeptide against which the antibody is raised.

[0154] This invention also provides an antibody capable of specifically forming a complex with Aih2, which are useful in the therapeutic methods of this invention. The term "antibody" includes polyclonal antibodies and monoclonal antibodies, antibody fragments, as well as derivatives thereof (described above). The antibodies include, but are not limited to mouse, rat, and rabbit or human antibodies. Antibodies can be produced in cell culture, in phage, or in various animals, including but not limited to cows, rabbits, goats, mice, rats, hamsters, guinea pigs, sheep, dogs, cats, monkeys, chimpanzees, apes, etc. The antibodies are also useful to identify and purify therapeutic polypeptides.

[0155] This invention also provides an antibody-peptide complex comprising, or alternatively consisting essentially of, or yet alternatively consisting of, antibodies described above and a polypeptide specifically bound to the antibody. In one aspect the polypeptide is the polypeptide against which the antibody was raised. In one aspect the antibody-peptide complex is an isolated complex. In a further aspect, the antibody of the complex is, but not limited to, a polyclonal antibody, a monoclonal antibody, a humanized antibody or an antibody derivative described herein. Either or both of the antibody or peptide of the antibody-peptide complex can be detectably labeled or further comprises a detectable label conjugated to it. In one aspect, the antibody-peptide complex of the invention can be used as a control or reference sample in diagnostic or screening assays.

[0156] Polyclonal antibodies of the invention can be generated using conventional techniques known in the art and are well-described in the literature. Several

methodologies exist for production of polyclonal antibodies. For example, polyclonal antibodies are typically produced by immunization of a suitable mammal such as, but not limited to, chickens, goats, guinea pigs, hamsters, horses, mice, rats, and rabbits. An antigen is injected into the mammal, which induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This IgG is purified from the mammals serum. Variations of this methodology include modification of adjuvants, routes and site of administration, injection volumes per site and the number of sites per animal for optimal production and humane treatment of the animal. For example, adjuvants typically are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site antigen depot, which allows for a slow release of antigen into draining lymph nodes. Other adjuvants include surfactants which promote concentration of protein antigen molecules over a large surface area and immunostimulatory molecules. Non-limiting examples of adjuvants for polyclonal antibody generation include Freund's adjuvants, Ribi adjuvant system, and Titermax. Polyclonal antibodies can be generated using methods described in U.S. Patent Nos. 7,279,559; 7,119,179; 7,060,800; 6,709,659; 6,656,746; 6,322,788; 5,686,073; and 5,670,153.

[0157] The monoclonal antibodies of the invention can be generated using conventional hybridoma techniques known in the art and well-described in the literature. For example, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U397, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, CHO, PerC.6, YB2/O) or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art (see, e.g., www.atcc.org, www.lifetech.com, last accessed on November 26, 2007, and the like), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or

endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing-heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods.

[0158] In one embodiment, the antibodies described herein can be generated using a Multiple Antigenic Peptide (MAP) system. The MAP system utilizes a peptidyl core of three or seven radially branched lysine residues, on to which the antigen peptides of interest can be built using standard solid-phase chemistry. The lysine core yields the MAP bearing about 4 to 8 copies of the peptide epitope depending on the inner core that generally accounts for less than 10% of total molecular weight. The MAP system does not require a carrier protein for conjugation. The high molar ratio and dense packing of multiple copies of the antigenic epitope in a MAP has been shown to produce strong immunogenic response. This method is described in U.S. Patent No. 5,229,490 and is herein incorporated by reference in its entirety.

[0159] Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from various commercial vendors such as Cambridge Antibody Technologies (Cambridgeshire, UK), MorphoSys (Martinsreid/Planegg, Del.), Biovation (Aberdeen, Scotland, UK) BioInvent (Lund, Sweden), using methods known in the art. See U.S.

Patent Nos. 4,704,692; 5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862. Alternative methods rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al. (1997) *Microbiol. Immunol.* 41:901-907; Sandhu et al. (1996) *Crit. Rev. Biotechnol.* 16:95-118; Eren et al. (1998) *Immunol.* 93:154-161 that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:4937-4942; Hanes et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:14130-14135); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Patent No. 5,627,052, Wen et al. (1987) *J. Immunol.* 17:887-892; Babcook et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848); gel microdroplet and flow cytometry (Powell et al. (1990) *Biotechnol.* 8:333-337; One Cell Systems, (Cambridge, Mass); Gray et al. (1995) *J. Imm. Meth.* 182:155-163; and Kenny et al. (1995) *Bio. Technol.* 13:787-790); B-cell selection (Steenbakkers et al. (1994) *Molec. Biol. Reports* 19:125-134.

[0160] Antibody derivatives of the present invention can also be prepared by delivering a polynucleotide encoding an antibody of this invention to a suitable host such as to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. These methods are known in the art and are described for example in U.S. Patent Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489.

[0161] The term "antibody derivative" includes post-translational modification to linear polypeptide sequence of the antibody or fragment. For example, U.S. Patent No. 6,602,684 B1 describes a method for the generation of modified glycol-forms of antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having enhanced Fc-mediated cellular toxicity, and glycoproteins so generated.

[0162] Antibody derivatives also can be prepared by delivering a polynucleotide of this invention to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified

portions or variants in the plant parts or in cells cultured therefrom. For example, Cramer et al. (1999) *Curr. Top. Microbol. Immunol.* 240:95-118 and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter. Transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al. (1999) *Adv. Exp. Med. Biol.* 464:127-147 and references cited therein. Antibody derivatives have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al. (1998) *Plant Mol. Biol.* 38:101-109 and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to known methods.

[0163] Antibody derivatives also can be produced, for example, by adding exogenous sequences to modify immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

[0164] In general, the CDR residues (an example of an antibody fragment) are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method such as, but not limited to, those described in U.S. Patent Nos. 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567.

[0165] Techniques for making partially to fully human antibodies are known in the art and any such techniques can be used. According to one embodiment, fully human antibody sequences are made in a transgenic mouse which has been engineered to express human heavy and light chain antibody genes. Multiple strains

of such transgenic mice have been made which can produce different classes of antibodies. B cells from transgenic mice which are producing a desirable antibody can be fused to make hybridoma cell lines for continuous production of the desired antibody. (See for example, Russel et al. (2000) *Infection and Immunity* 68(4):1820-1826; Gallo et al. (2000) *European J. of Immun.* 30:534-540; Green (1999) *J. of Immun. Methods* 231:11-23; Yang et al. (1999A) *J. of Leukocyte Biology* 66:401-410; Yang (1999B) *Cancer Research* 59(6):1236-1243; Jakobovits (1998) *Advanced Drug Delivery Reviews* 31:33-42; Green & Jakobovits (1998) *J. Exp. Med.* 188(3):483-495; Jakobovits (1998) *Exp. Opin. Invest. Drugs* 7(4):607-614; Tsuda et al. (1997) *Genomics* 42:413-421; Sherman-Gold (1997) *Genetic Engineering News* 17(14); Mendez et al. (1997) *Nature Genetics* 15:146-156; Jakobovits (1996) *Weir's Handbook of Experimental Immunology, The Integrated Immune System Vol. IV*, 194.1-194.7; Jakobovits (1995) *Current Opinion in Biotechnology* 6:561-566; Mendez et al. (1995) *Genomics* 26:294-307; Jakobovits (1994) *Current Biology* 4(8):761-763; Arbones et al. (1994) *Immunity* 1(4):247-260; Jakobovits (1993) *Nature* 362(6417):255-258; Jakobovits et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(6):2551-2555; and U.S. Patent No. 6,075,181.)

[0166] The antibodies of this invention also can be modified to create chimeric antibodies. Chimeric antibodies are those in which the various domains of the antibodies' heavy and light chains are coded for by DNA from more than one species. See, e.g., U.S. Patent No. 4,816,567.

[0167] Alternatively, the antibodies of this invention can also be modified to create veneered antibodies. Veneered antibodies are those in which the exterior amino acid residues of the antibody of one species are judiciously replaced or "veneered" with those of a second species so that the antibodies of the first species will not be immunogenic in the second species thereby reducing the immunogenicity of the antibody. Since the antigenicity of a protein is primarily dependent on the nature of its surface, the immunogenicity of an antibody could be reduced by replacing the exposed residues which differ from those usually found in another mammalian species antibodies. This judicious replacement of exterior residues should have

little, or no, effect on the interior domains, or on the interdomain contacts. Thus, ligand binding properties should be unaffected as a consequence of alterations which are limited to the variable region framework residues. The process is referred to as “veneering” since only the outer surface or skin of the antibody is altered, the supporting residues remain undisturbed.

[0168] The procedure for “veneering” makes use of the available sequence data for human antibody variable domains compiled by Kabat et al. (1987) Sequences of Proteins of Immunological Interest, 4th ed., Bethesda, Md., National Institutes of Health, updates to this database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Non-limiting examples of the methods used to generate veneered antibodies include EP 519596; U.S. Patent No. 6,797,492; and described in Padlan et al. (1991) Mol. Immunol. 28(4-5):489-498.

[0169] The term “antibody derivative” also includes “diabodies” which are small antibody fragments with two antigen-binding sites, wherein fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain. (See for example, EP 404,097; WO 93/11161; and Hollinger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448.) By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. (See also, U.S. Patent No. 6,632,926 to Chen et al. which discloses antibody variants that have one or more amino acids inserted into a hypervariable region of the parent antibody and a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for the antigen.)

[0170] The term “antibody derivative” further includes “linear antibodies”. The procedure for making linear antibodies is known in the art and described in Zapata et al. (1995) Protein Eng. 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H 1-VH -C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0171] The antibodies of this invention can be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.

[0172] Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells, or alternatively from a prokaryotic cells as described above.

[0173] If a monoclonal antibody being tested binds with protein or polypeptide, then the antibody being tested and the antibodies provided by the hybridomas of this invention are equivalent. It also is possible to determine without undue experimentation, whether an antibody has the same specificity as the monoclonal antibody of this invention by determining whether the antibody being tested prevents a monoclonal antibody of this invention from binding the protein or polypeptide with which the monoclonal antibody is normally reactive. If the antibody being tested competes with the monoclonal antibody of the invention as shown by a decrease in binding by the monoclonal antibody of this invention, then it is likely that the two antibodies bind to the same or a closely related epitope. Alternatively, one can pre-incubate the monoclonal antibody of this invention with a protein with which it is normally reactive, and determine if the monoclonal antibody being tested is inhibited in its ability to bind the antigen. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or a closely related, epitopic specificity as the monoclonal antibody of this invention.

[0174] The term "antibody" also is intended to include antibodies of all isotypes. Particular isotypes of a monoclonal antibody can be prepared either directly by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection

technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) Proc. Natl. Acad. Sci. USA 82:8653 or Spira et al. (1984) J. Immunol. Methods 74:307.

[0175] The isolation of other hybridomas secreting monoclonal antibodies with the specificity of the monoclonal antibodies of the invention can also be accomplished by one of ordinary skill in the art by producing anti-idiotypic antibodies. Herlyn et al. (1986) Science 232:100. An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the monoclonal antibody produced by the hybridoma of interest.

[0176] Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody it is possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

[0177] It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the mirror image of the epitope bound by the first monoclonal antibody. Thus, in this instance, the anti-idiotypic monoclonal antibody could be used for immunization for production of these antibodies.

[0178] In some aspects of this invention, it will be useful to detectably or therapeutically label the antibody. Suitable labels are described supra. Methods for conjugating antibodies to these agents are known in the art. For the purpose of illustration only, antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either in vivo, or in an isolated test sample.

[0179] The coupling of antibodies to low molecular weight haptens can increase the sensitivity of the antibody in an assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens

such as biotin, which reacts avidin, or dinitrophenol, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies. See, Harlow & Lane (1988) *supra*.

[0180] The antibodies of the invention also can be bound to many different carriers. Thus, this invention also provides compositions containing the antibodies and another substance, active or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

Vectors Suitable for Delivery to Humans

[0181] This disclosure features methods and compositions for increasing or decreasing the biological activity of Aih2 in a cell. In one aspect, the disclosure features methods of gene therapy to express a gene or protein in a cell, such as a granulocyte of a patient. Gene therapy, including the use of viral vectors as described herein, seeks to transfer new genetic material (e.g., polynucleotides encoding Aih2 or other proteins or a biologically active fragment thereof) to the cells of a patient with resulting therapeutic benefit to the patient.

[0182] For in vivo gene therapy, expression vectors encoding the gene of interest is administered directly to the patient. The vectors are taken up by the target cells (e.g., neurons or pluripotent stem cells) and the gene expressed. Recent reviews discussing methods and compositions for use in gene therapy include Eck et al., in Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, Ninth Edition, Hardman et al., eds., McGraw-Hill, New York, 1996, Chapter 5, pp. 77-101; Wilson (1997) *Clin. Exp. Immunol.* 107 (Suppl. 1):31-32; Wivel et al. (1998) *Hematology/Oncology Clinics of North America*, Gene Therapy, S.L. Eck, ed., 12(3):483-501; Romano et al. (2000) *Stem Cells* 18:19-39, and the references cited therein. U.S. Patent No. 6,080,728 also provides a discussion of a wide variety of gene delivery methods and compositions.

[0183] Adenoviruses are able to transfect a wide variety of cell types, including non-dividing cells. There are more than 50 serotypes of adenoviruses that are known in the art, but the most commonly used serotypes for gene therapy are type 2 and type 5. Typically, these viruses are replication-defective; and genetically-modified to prevent unintended spread of the virus. This is normally achieved through the deletion of the E1 region, deletion of the E1 region along with deletion of either the E2 or E4 region, or deletion of the entire adenovirus genome except the cis-acting inverted terminal repeats and a packaging signal (Gardlik et al. (2005) Med. Sci. Monit. 11: RA110-121).

[0184] Retroviruses are also useful as gene therapy vectors and usually (with the exception of lentiviruses) are not capable of transfecting non-dividing cells. Accordingly, any appropriate type of retrovirus that is known in the art may be used, including, but not limited to, HIV, SIV, FIV, EIAV, and Moloney Murine Leukaemia Virus (MoMLV). Typically, therapeutically useful retroviruses including deletions of the gag, pol, or env genes.

[0185] In another aspect, the invention features the methods of gene therapy that utilize a lentivirus vectors to express Aih2, or other proteins in a patient. Lentiviruses are a type of retroviruses with the ability to infect both proliferating and quiescent cells. An exemplary lentivirus vector for use in gene therapy is the HIV-1 lentivirus. Previously constructed genetic modifications of lentiviruses include the deletion of all protein encoding genes except those of the gag, pol, and rev genes (Moreau-Gaudry et al. (2001) Blood 98:2664-2672).

[0186] Adeno-associated virus (AAV) vectors can achieve latent infection of a broad range of cell types, exhibiting the desired characteristic of persistent expression of a therapeutic gene in a patient. The invention includes the use of any appropriate type of adeno-associated virus known in the art including, but not limited to AAV1, AAV2, AAV3, AAV4, AAV5, and AAV6 (Lee et al. (2005) Biochem. J. 387: 1-15; U.S. Patent Publication 2006/0204519).

[0187] Herpes simplex virus (HSV) replicates in epithelial cells, but is able to stay in a latent state in non-dividing cells such as the midbrain dopaminergic neurons. The gene of interest may be inserted into the LAT region of HSV, which is expressed during latency. Other viruses that have been shown to be useful in gene therapy include parainfluenza viruses, poxviruses, and alphaviruses, including Semliki forest virus, Sinbis virus, and Venezuelan equine encephalitis virus (Kennedy (1997) Brain 120: 1245-1259).

[0188] Exemplary non-viral vectors for delivering nucleic acid include naked DNA; DNA complexed with cationic lipids, alone or in combination with cationic polymers; anionic and cationic liposomes; DNA-protein complexes and particles comprising DNA condensed with cationic polymers such as heterogeneous polylysine, defined-length oligopeptides, and polyethylene imine, in some cases contained in liposomes; and the use of ternary complexes comprising a virus and polylysine-DNA. In vivo DNA-mediated gene transfer into a variety of different target sites has been studied extensively. Naked DNA may be administered using an injection, a gene gun, or electroporation. Naked DNA can provide long-term expression in muscle. See Wolff et al. (1992) Human Mol. Genet. 1:363-369; Wolff et al. (1990) Science 247: 465-1468. DNA-mediated gene transfer has also been characterized in liver, heart, lung, brain and endothelial cells. See Zhu et al. (1993) Science 261:209-211; Nabel et al. (1989) Science 244:1342-1344. DNA for gene transfer also may be used in association with various cationic lipids, polycations and other conjugating substances. See Przybylska et al. (2004) J. Gene Med. 6:85-92; Svahn et al. (2004) J. Gene Med. 6:S36-S44.

[0189] Methods of gene therapy using cationic liposomes are also well known in the art. Exemplary cationic liposomes for use in this invention are DOTMA, DOPE, DOSPA, DOTAP, DC-Chol, Lipid GL-67.TM., and EDMPC. These liposomes may be used in vivo or ex vivo to encapsulate a vector for delivery into target cells (e.g., neurons or pluripotent stem cells).

[0190] Typically, vectors made in accordance with the principles of this disclosure will contain regulatory elements that will cause constitutive expression of the coding

sequence. Desirably, neuron-specific regulatory elements such as neuron-specific promoters are used in order to limit or eliminate ectopic gene expression in the event that the vector is incorporated into cells outside of the target region. Several regulatory elements are well known in the art to direct neuronal specific gene expression including, for example, the neural-specific enolase (NSE), and synapsin-1 promoters (Morelli et al. (1999) J. Gen. Virol. 80: 571-583).

Direct Protein Administration

[0191] In some embodiments, the biological activity of Arih2 is increased by directly administering Arih2 to the cells in a manner in which Arih2 is taken up by the cell (i.e., transits across the cell membrane into the cytoplasm). Alternatively, a mutant Arih2 protein, which does not have the Arih2 activity or does not have the complete activity of Arih2 and maintains the capability to bind to cofactors or ligands, can be administered to the cells to compete with the wildtype Arih2 so as to decrease the biological activity in the cells. To help facilitate the delivery of Arih2 into a cell and across the cell membrane, Arih2 may be fused chemically or recombinantly, or otherwise associated with a peptide that facilitates the delivery, such as a cell penetrating peptides (CPP) or protein transduction domain (PTD).

[0192] Cell penetrating peptides, or “CPPs”, as used herein, refer to short peptides that facilitate cellular uptake of various molecular cargos (from small chemical molecules to nanosize particles and large fragments of DNA). A “cargo”, such as a protein, is associated with the peptides either through chemical linkage via covalent bonds or through non-covalent interactions. The function of the CPPs are to deliver the cargo into cells, a process that commonly occurs through endocytosis with the cargo delivered to the endosomes of living mammalian cells. CPPs typically have an amino acid composition containing either a high relative abundance of positively charged amino acids such as lysine or arginine, or have sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids. In 1988, Frankel and Pabo found that the human immunodeficiency virus transactivator of transcription (HIV-TAT) protein can be delivered to cells using a CPP (Frankel et al. 1988a and Frankel et al. 1988b).

[0193] A CPP employed in accordance with one aspect of the invention may include 3 to 35 amino acids, preferably 5 to 25 amino acids, more preferably 10 to 25 amino acids, or even more preferably 15 to 25 amino acids.

[0194] A CPP may also be chemically modified, such as prenylated near the C-terminus of the CPP. Prenylation is a post-translation modification resulting in the addition of a 15 (farnesyl) or 20 (geranylgeranyl) carbon isoprenoid chain on the peptide. A chemically modified CPP can be even shorter and still possess the cell penetrating property. Accordingly, a CPP, pursuant to another aspect of the invention, is a chemically modified CPP with 2 to 35 amino acids, preferably 5 to 25 amino acids, more preferably 10 to 25 amino acids, or even more preferably 15 to 25 amino acids.

[0195] A CPP suitable for carrying out one aspect of the invention may include at least one basic amino acid such as arginine, lysine and histidine. In another aspect, the CPP may include more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, or more such basic amino acids, or alternatively about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50% of the amino acids are basic amino acids. In one embodiment, the CPP contains at least two consecutive basic amino acids, or alternatively at least three, or at least five consecutive basic amino acids. In a particular aspect, the CPP includes at least two, three, four, or five consecutive arginine. In a further aspect, the CPP includes more arginine than lysine or histidine, or preferably includes more arginine than lysine and histidine combined.

[0196] CPPs may include acidic amino acids but the number of acidic amino acids should be smaller than the number of basic amino acids. In one embodiment, the CPP includes at most one acidic amino acid. In a preferred embodiment, the CPP does not include acidic amino acid. In a particular embodiment, a suitable CPP is the HIV-TAT peptide.

[0197] CPPs can be linked to a protein recombinantly, covalently or non-covalently. A recombinant protein having a CPP peptide can be prepared in bacteria, such as *E. coli*, a mammalian cell such as a human HEK293 cell, or any

cell suitable for protein expression. Covalent and non-covalent methods have also been developed to form CPP/protein complexes. A CPP, Pep-1, has been shown to form a protein complex and proven effective for delivery (Kameyama et al. (2006) *Bioconjugate Chem.* 17:597–602).

[0198] CPPs also include cationic conjugates which also may be used to facilitate delivery of the proteins into the progenitor or stem cell. Cationic conjugates may include a plurality of residues including amines, guanidines, amidines, N-containing heterocycles, or combinations thereof. In related embodiments, the cationic conjugate may comprise a plurality of reactive units selected from the group consisting of alpha-amino acids, beta-amino acids, gamma-amino acids, cationically functionalized monosaccharides, cationically functionalized ethylene glycols, ethylene imines, substituted ethylene imines, N-substituted spermine, N-substituted spermidine, and combinations thereof. The cationic conjugate also may be an oligomer including an oligopeptide, oligoamide, cationically functionalized oligoether, cationically functionalized oligosaccharide, oligoamine, oligoethyleneimine, and the like, as well as combinations thereof. The oligomers may be oligopeptides where amino acid residues of the oligopeptide are capable of forming positive charges. The oligopeptides may contain 5 to 25 amino acids; preferably 5 to 15 amino acids; more preferably 5 to 10 cationic amino acids or other cationic subunits.

[0199] Recombinant proteins anchoring CPP to the proteins can be generated to be used for delivery to neural progenitor cells or stem cells to prepare mature and functional DA neurons.

Delivery by Stem Cells

[0200] Compositions described here for therapeutic uses can be administered to a patient with a stem cell. For example, mesenchymal stem cells and monoblast stem cells have been shown as therapeutics and vehicles for gene and drug delivery. See, for example, Porada et al. (2010) *Adv. Drug Deliv. Rev.* 2010 Sep 7. [Epub ahead of print].

[0201] As used herein, “stem cell” defines a cell with the ability to divide for indefinite periods in culture and give rise to specialized cells. Stem cells include, for example, somatic (adult) and embryonic stem cells. A somatic stem cell is an undifferentiated cell found in a differentiated tissue that can renew itself (clonal) and (with certain limitations) differentiate to yield all the specialized cell types of the tissue from which it originated. An embryonic stem cell is a primitive (undifferentiated) cell derived from the embryo that has the potential to become a wide variety of specialized cell types. An embryonic stem cell is one that has been cultured under in vitro conditions that allow proliferation without differentiation. Non-limiting examples of embryonic stem cells are the HES2 (also known as ES02) cell line available from ESI, Singapore and the H1 (also known as WA01) cell line available from WiCells, Madison, WI. In addition, for example, there are 40 embryonic stem cell lines that are recently approved for use in NIH-funded research including CHB-1, CHB-2, CHB-3, CHB-4, CHB-5, CHB-6, CHB-8, CHB-9, CHB-10, CHB-11, CHB-12, RUES1, HUES1, HUES2, HUES3, HUES4, HUES5, HUES6, HUES7, HUES8, HUES9, HUES10, HUES11, HUES12, HUES13, HUES14, HUES15, HUES16, HUES17, HUES18, HUES19, HUES20, HUES21, HUES22, HUES23, HUES24, HUES26, HUES27, and HUES28. Pluripotent embryonic stem cells can be distinguished from other types of cells by the use of markers including, but not limited to, Oct-4, alkaline phosphatase, CD30, TDGF-1, GCTM-2, Genesis, Germ cell nuclear factor, SSEA1, SSEA3, and SSEA4.

[0202] As used herein, a “pluripotent cell” broadly refers to stem cells with similar properties to embryonic stem cells with respect to the ability for self-renewal and pluripotency (i.e., the ability to differentiate into cells of multiple lineages). Pluripotent cells refer to cells both of embryonic and non-embryonic origin. For example, pluripotent cells includes Induced Pluripotent Stem Cells (iPSCs).

[0203] An “induced pluripotent stem cell” or “iPSC” or “iPS cell” refers to an artificially derived stem cell from a non-pluripotent cell, typically an adult somatic cell, produced by inducing expression of one or more reprogramming genes or corresponding proteins or RNAs. Such stem cell specific genes include, but are not

limited to, the family of octamer transcription factors, i.e. Oct-3/4; the family of Sox genes, i.e. Sox1, Sox2, Sox3, Sox 15 and Sox 18; the family of Klf genes, i.e. Klf1, Klf2, Klf4 and Klf5; the family of Myc genes, i.e. c-myc and L-myc; the family of Nanog genes, i.e. OCT4, NANOG and REX1; or LIN28. Examples of iPSCs and methods of preparing them are described in Takahashi et al. (2007) Cell 131(5):861-72; Takahashi & Yamanaka (2006) Cell 126:663-76; Okita et al. (2007) Nature 448:260-262; Yu et al. (2007) Science 318(5858):1917-20; and Nakagawa et al. (2008) Nat. Biotechnol. 26(1):101-6.

[0204] A “precursor” or “progenitor cell” intends to mean cells that have a capacity to differentiate into a specific type of cell. A progenitor cell may be a stem cell. A progenitor cell may also be more specific than a stem cell. A progenitor cell may be unipotent or multipotent. Compared to adult stem cells, a progenitor cell may be in a later stage of cell differentiation. Examples of progenitor cells include, but are not limited to, satellite cells found in muscles, intermediate progenitor cells formed in the subventricular zone, bone marrow stromal cells, periosteum progenitor cells, pancreatic progenitor cells and angioblasts or endothelial progenitor cells. Examples of progenitor cells may also include, but are not limited to, epidermal and dermal cells from neonatal organisms.

[0205] Mesenchymal stem cells, or MSCs, are multipotent stem cells. Mesenchymal stem cells can differentiate into a variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells) and adipocytes (fat cells).

Therapeutic Compositions

[0206] The present disclosure, in one embodiment, provides a cell comprising, or alternatively consisting essentially of, or yet alternatively consisting of, a recombinant Arih2 protein or polynucleotide. In one aspect, the Arih2 protein is fused to a cell penetrating peptide.

[0207] The present disclosure, in another embodiment, provides a cell comprising, or alternatively consisting essentially of, or yet alternatively consisting of, an agent that decreases the biological activity of Arih2. In one aspect, the cell is a stem cell.

In another aspect, the cell is a stem cell. In yet another aspect, the stem cell is a monoblast stem cell.

[0208] Agents that increase or decrease the biological activity of Arih2 are described above. A cell can be an animal cell, a mammal or yet further a human cell. For the purpose of illustration only, a mammal includes but is not limited to a human, a simian, a murine, a bovine, an equine, a porcine or an ovine.

Treatments

[0209] In some embodiments, the present disclosure provides methods for treating a condition or disease such as a bacterial infection, chronic inflammation or cancer by increasing or decreasing the biological activity of Arih2 in cells of a patient. The methods are applicable to animals, mammals or yet further human patients. For the purpose of illustration only, a mammal includes but is not limited to a human, a simian, a murine, a bovine, an equine, a porcine or an ovine.

[0210] In some embodiments, the present disclosure also provides uses of the agents that increase or decrease the biological activity of Arih2 in a cell for the manufacture of a medicament in treating such conditions or diseases. Yet in some embodiments, an agent that increases or decreases the biological activity of Arih2 is provided for use in treating a condition or disease. Suitable agents, patients, conditions and diseases are further described within the present disclosure.

[0211] Any compositions described herein for a therapeutic use may be administered with an acceptable pharmaceutical carrier. Acceptable "pharmaceutical carriers" are well known to those of skill in the art and can include, but not be limited to any of the standard pharmaceutical carriers, such as phosphate buffered saline, water and emulsions, such as oil/water emulsions and various types of wetting agents.

[0212] As used herein, the term "administering" for in vivo and ex vivo purposes means providing the subject with an effective amount of the nucleic acid molecule or polypeptide effective to prevent or inhibit a disease or condition in the subject.

Methods of administering pharmaceutical compositions are well known to those of skill in the art and include, but are not limited to, microinjection, intravenous or parenteral administration. The compositions are intended for topical, oral, or local administration as well as intravenously, subcutaneously, or intramuscularly. Administration can be effected continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the vector used for therapy, the polypeptide or protein used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. For example, the compositions can be administered prior to a subject already suffering from a disease or condition that is linked to apoptosis.

Diagnostics and Screens

[0213] The present disclosure, in another embodiment of the present disclosure provides a method for determining whether a subject is likely to develop an infection or cancer, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, determining in a sample isolated from the subject the expression level of Arih2, wherein an overexpression of Arih2 determines that the subject is likely to develop an infection or cancer.

[0214] Further provided is a method for determining whether a patient is suitable for a treatment comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administration of an effective amount of an agent that decreases the biological activity of Arih2, wherein the patient suffers from an infection or cancer comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an overexpression of Arih2 determines that the patient is suitable for the treatment.

[0215] The present disclosure, in another embodiment, provides a method for identifying an agent suitable for increasing or decreasing the biological activity of

Arih2, comprising contacting a granulocyte with a candidate agent and determining the activation threshold of activation of the granulocyte, wherein an elevated activation threshold indicates that the candidate agent is suitable for increasing the biological activity of Arih2 or a reduced activation threshold indicates that the candidate agent is suitable for decreasing the biological activity of Arih2.

[0216] Candidate agents that modulate the activation threshold of a granulocyte by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or more relative to an untreated control not contacted with the candidate agent are identified as agents suitable for increasing or decreasing the biological activity of Arih2 and thus suitable for treating certain conditions or diseases as disclosed here.

Kits

[0217] Further, the present disclosure also provides a kit for use in inhibiting dendritic cell activation, reducing chronic inflammation or treating an autoimmune disease, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, an effective amount of an agent that increases the biological activity of Arih2 and instructions to use.

[0218] In another embodiment, the present disclosure provides a kit for use in promoting dendritic cell activation, treating an infection or treating a cancer, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, an effective amount of an agent that decreases the biological activity of Arih2 and instructions to use.

[0219] Also provided is a method for determining whether a subject is likely to develop a chronic inflammation, an autoimmune disease or diabetes, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, determining in a sample isolated from the subject the expression level of Arih2, wherein an underexpression of Arih2 determines that the subject is likely to develop a chronic inflammation, an autoimmune disease or diabetes.

[0220] Yet also provided is a method for determining whether a patient is suitable for a treatment comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administration of an effective amount of an agent that increases the biological activity of Arih2, wherein the patient suffers from a chronic inflammation, an autoimmune disease or diabetes, comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an underexpression of Arih2 determines that the patient is suitable for the treatment. Suitable agents, patients, conditions and diseases are further described within the present disclosure.

EXPERIMENTAL

Example 1

[0221] This example shows that Arih2 involved in immune regulation by antagonizing DC activation. This establishes Arih2 as having a critical role in the maintenance of peripheral tolerance and the pathogenesis of autoimmunity and has implications for immunotherapies and dendritic cell-based vaccines.

Methods

[0222] Generation of Arih2^{-/-} mice. Genomic Arih2 clones were isolated from 129/J BAC library and used to generate genomic fragments or used as PCR template to construct the targeting vector for electroporation into E14K ES cells. Homologous recombination replaces the genomic fragment containing exons 6, 7 and 8 containing the first RING finger of the Ring Finger-B-Box-RING Finger (RBR) domain with a neomycin-resistance cassette and G418 resistant clones were selected and identified by PCR and confirmed by southern analysis. Three independent clones were injected into C57B/6 E3.5 blastocysts to generate chimeric mice and Arih2^{+/-} mice as previously described^{S2} Confirmation of germline transmission of mutant allele was determined by PCR and Southern blot of tail DNA. In this example, F₁ Arih2^{-/-} and littermates on the 129JOla x C57BL/6 background and backcrossed F₁₀ to F₁₂ C57BL/6 heterozygous crosses were used.

[0223] Mice. C57BL/6 Rag1^{-/-} mice were purchased from Jackson Laboratory (Bar Harbour, ME). The derivation of RIP-GP transgenic mice, IFN γ , TNF α , MyD88 mice have been have been described previously^{S3-S7}. All animals were housed in a specific-pathogen-free facility and all mice generated were bred and maintained in compliance with the Canadian Council on Animal Care and the Princess Margaret Hospital Animal Care Committee.

[0224] Histopathology. All tissues and organs were dissected and fixed in 10% phosphate buffered Formalin (Fisher) and embedded in paraffin. Organs were sectioned at various depths in serial sections of 3-6 μ m thickness, stained with H+E. For immunohistochemistry, sections were incubated with primary antibodies CD3 (Dako), B220 (BD Pharmingen) and F4/80 (Serotec) and secondary antibody HRP-conjugated ultrastreptavidin (ID Labs Inc) and counterstained with Mayer's haematoxylin. In situ end-labeling of fragment DNA (ISEL) on paraffin embedded tissue slides were performed as previously described^{S8}.

[0225] Fetal liver dendritic cell preparation. Single cell suspension of fetal liver cells were seeded at 1×10^5 cells/mL in untreated dishes using supplemented RPMI (10% FCS w/v, 20 ng/mL GM-CSF (PeproTech) and additional media was added on day 3, and on days 6 and 8 fresh media was exchanged from half the suspension culture, as previously described^{S9}. DC purity was determined by flow cytometry for greater than 90% CD11c, and MHCII, or CD86 expression. Non-adherent DCs were collected on day 10 and seeded at 2×10^5 cells/mL and incubated in media alone, or stimulated with 10 μ g/mL LPS (O111:B4; Sigma) or 10 μ M CpG ODN1826 (Invivogen).

[0226] For plasmacytoid dendritic cells, single cell suspension of E14.5 fetal liver cells were seeded at 1.5×10^6 cells/mL using supplemental RPMI (10%FCS w/v, 100 ng/mL Flt3L (PeproTech) as previously described in Esashi, E. *et al. Immunity*, 28, 509-520 (2008). DC populations were determined by flow cytometry on day 8-9 and stained for CD11c, CD11b, B220, SIRP α and CD103 expression.

[0227] Fetal liver macrophage preparation. Single cell suspension of fetal liver cells were seeded at 1×10^5 cells/mL with supplemented Iscoves Media (10% FCS w/v, 50 ng/mL M-CSF (PeproTech) using standard protocols. Adherent cells were collected on day 4-5.

[0228] Diabetes induction. Naïve DCs or DCs stimulated with LPS or CpG were replated at 2×10^6 cells/mL and incubated with the LCMV peptides GP33 10^{-6} M (KAVYNFATM), GP276 10^{-6} M (SGVENPGGYCL) and GP61 (GLNGPDIYKGVQFKSVEFD (Anasepec) $1 \mu\text{g/mL}$ for 2-3 hours and thoroughly washed in Hank's buffered saline solution. 1×10^5 cells were injected into tail veins of RIP-GP mice, as previously described^{S10}. RIP-GP mice random blood glucose was measured from tail vein bleed using the Accu-chek Advantage® test strips and meter (Accu-chek, Roche). Diabetes was determined by a minimum of 3 consecutive measurements of random blood glucose $\geq 15\text{mmol/L}$.

[0229] Western Blot. Total cell lysates were harvested in Triton-X-100 lysis buffer (0.4% Triton-X-100, 50mM Tris pH8.0, 100nM NaCl, 2mM EDTA pH8.0, 10mM NaF, 20mM β -Glycerophosphate, Na₄VO₃ 1mM, 1mM PMSF and PhosSTOP phosphatase and complete protease inhibitor tablets (Roche)). Nuclear and cytoplasmic fractions from unstimulated and stimulated DCs were prepared as previously described^{S2}.

[0230] For proteasome inhibition, cells were treated with 25 μM MG132 as previously described Lee, E.G. *et al. Science*, 289, 2350-2353 (2000). Immunoprecipitation experiments were performed using $\mu\text{MACS}^{\text{TM}}$ protein G microbeads and columns (Miltenyi Biotec) and I κ B β antibody (Santa Cruz).

[0231] Gel mobility shift assay. 5 μg of nuclear protein extracts and the Odessey Infrared EMSA Kit and IRDye® 700 EMSA oligonucleotides for NF κ B (5' AGT TGA GGG GAC TTT CCC AGG G 3' (SEQ ID NO: 1) and 3' TCA ACT GGG CTG AAA GGG TCC G 5' (SEQ ID NO: 2)) and AP-1 (5' CGC TTG ATG ACT CAG CCG GAA 3' (SEQ ID NO: 3) and 3' GCG AAC TAC TGA GTC GGG CTT 5' (SEQ ID NO: 4)),

resolved on 5% TBE native acrylamide gels and quantified using the Odessey Infrared Imaging system (Li-cor Biosciences).

[0232] Cell enrichment. T and B cells were enriched from spleen and peripheral lymph nodes from chimeric mice by magnetic depletion (Miltenyi, BD IMag) at minimum purity of 90% Thy1.2 or B220 surface staining determined by flow cytometry. Isolation of infiltrating lymphocytes from liver, lung, heart and gastrointestinal tract was as follows. Organs were harvested from chimeric mice and mechanically minced, strained and subjected to red cell lysis followed by Percoll gradient separation for lymphocyte isolation, as previously described^{S11}.

[0233] T cell stimulation and proliferation. CFSE labeled T cells (2×10^5 cells/mL) were stimulated with plate bound CD3 ϵ (BD Biosciences) 10 μ g/mL with CD28 (BD Biosciences) 5 μ g/mL, or PMA (Sigma) 10ng/mL with ionomycin (Sigma) 50ng/mL, as previously described^{S2}. CFSE labeled T cells were assessed 24 and 48 hours post stimulation by flow cytometry. Non-CFSE labeled T cells (2×10^6 to 1×10^7 cells/mL) were stimulated as described for western analysis.

[0234] B cell stimulation and proliferation. A total of 2×10^6 B cells/mL were stimulated with plate bound IgM (BD Pharmingen) 5 μ g/mL, CD40 (eBioscience) 5 μ g/mL, or LPS (OD111:B4, Sigma) 20 μ g/mL, as previously described^{S2}. 48 hours post-stimulation, B cells pulsed for 5 hours with 1 μ Ci/mL of H³ thymidine and incorporation was measured with UniFilter 96-well plate nad TopCount NXTTM Microplate Scintillation and Luminescence Couter (Perkin Elmer).

[0235] TNF stimulation of mouse embryonic fibroblasts. Early passage primary Arih2^{+/-} and Arih2^{-/-} mouse embryonic fibroblasts (mEFs) were plated at 3×10^5 cells/ml and treated with 10ng/ml TNF α (Sigma). Total cell lysates were harvested for Western blot analysis.

[0236] LPS stimulation of mouse embryonic fibroblasts. Early passage primary Arih2^{+/-} and Arih2^{-/-} mouse embryonic fibroblasts (mEFs) were plated at 3×10^5 cells/ml and treated with 10ng/ml LPS (Sigma). Total cell lysates were harvested for Western blot analysis.

[0237] Flow cytometric analysis. Surface marker expression was analyzed using the FACSCanto or FACSCalibur instruments (Becton Dickson) and data was analyzed using FlowJo software (Treestar).

[0238] Antibodies. The following antibodies for western blotting were purchased from Cell Signaling Technologies (Danvers, MA): Akt, p(473)-Akt, I κ B, p(S32)-I κ B, flow cytometry RelB, H2AX, p65/RelA, p(536)p65/RelA, I κ B β , ERK(42/44), p(Thr202/Tyr204)-ERK, p38 and p(Thr180/Tyr182)-p38. MyD88 was purchased from Anaspec (Fremont, CA). Ariadne 2 specific antibody was generated in rabbits against peptides specific to the C terminus (Sigma Genosys). The following flow cytometry antibodies were purchased from eBioscience (San Diego, CA) or BD Pharmingen (San Jose, CA): Annexin-V, B220, CD3, CD4, CD8, CD11b, CD11c, CD25, CD40, CD44, CD69, CD80, CD83, CD86, Foxp3, Gr-1, IgD, IgM, MHCII(I-A/I-E), Thy1.2.

[0239] Serum immunoglobulin and cytokine measurement. Serum immunoglobulin and cytokine levels were measured using the multiplex Searchlight assay system (Aushon Biosystems). Serum IgE levels were determined by ELISA (BD OptEIA). DC cytokine secretion in culture supernatants was quantified for IL-6, IL-12 and TNF α using ELISA (BD OptEIA; eBiosciences).

[0240] Methlycellulose assay. E14.5 fetal liver single cell suspensions were seeded at 1×10^4 cells/mL in methylcellulose media with or without erythropoietin (Stemcell Technologies). Colonies were counted based on morphology on day 9.

[0241] Fetal liver adoptive transfer. Single cell suspension of 2×10^6 cells Arih2^{-/-} fetal liver were prepared from E13.5-E14.5 embryos and injected intravenously into Rag1^{-/-} mice subjected to 600 rads of γ -irradiation as previously described^{S12}. Successful repopulation of all compartments was determined at 5 weeks post-injection by tail vein bleed flow cytometry for CD3, B220 and Gr1/Mac-1. Analysis of chimeras was performed 6-7 weeks post-injection.

[0242] Foxp3 staining and colitis model. CD4⁺CD25⁺ regulatory T cells (Tr) and CD4⁺CD25⁻ effector T cells (Te) were isolated from spleen and peripheral lymph

nodes from chimeric mice using the CD4⁺ T cell Isolation Kit (Miltenyi) and the BD FACSVantage SE cell sorter, as previously described^{S1}. A total of 4x10⁵ Te and 1x10⁵ Tr cells in various genotype combinations were injected into the tail vein of Rag1^{-/-} mice. Colons were fixed in 10% buffered formalin. Histological preparation, examination and scoring for colitis and inflammation were performed as previously described^{S1}.

[0243] Statistical analysis. *P* values were calculated using Student's T-test for all data sets except, χ^2 for Mendelian ratios, ANOVA for cytokine assays, or Log-Rank (Mantel Cox) test for survival curves with *p*<0.05 indicating a significant difference. Error bars in graphs represent standard deviation of the mean.

Results

[0244] Aih2 was highly expressed in immune cells including T cells, and DCs (**FIG. 5a, b, c**). Aih2 deficient mice were generated to study the function and possible role of this E3 ligase in immune response regulation (**FIG. 5d, e, f, g**).

[0245] Intercrosses of Aih2 heterozygous mice (Aih2^{+/-}) demonstrated that germline deletion of Aih2 is perinatally lethal. In a mixed background, Aih2 deficient (Aih2^{-/-}) pups were born at Mendelian ratio (**FIG. 1a**) but the majority of Aih2^{-/-} pups die in the first week of life with 3.3% survival at 3 weeks of age (**FIG. 1a, b**). Those reaching maturity are severely runted (**FIG. 1c**) and they display overt autoimmune phenotypes including debilitating dermatitis and immune cell infiltrates in various organs including liver, lung and heart causing mortality (**FIG. 1d**). On the C57BL/6 background there were no Aih2^{-/-} live births (**FIG. 1e**) with lethality occurring at E16.5 (**FIG. 1f**). It was found that Aih2^{-/-} foetal liver cellularity was reduced at E14.5 and E16.5 however, increased liver apoptosis only became overtly detectable at E16.5 by histopathology and FACS staining, correlating with lethality at this time point (**FIG. 1f, g**). This decrease in foetal liver cellularity and increased apoptosis does not affect the haematopoietic stem potential as Aih2^{-/-} foetal liver was capable of in vitro methylcellulose colony formation (**FIG. 6a, b**) and complete repopulation in adoptive transfer models (**FIG. 6c**).

[0246] The *Arih2*^{-/-} phenotype is reminiscent of the immune dysregulation seen in mice deficient in key regulators, including TRAF2, TRAF6, A20 and SOCS1. Embryonic and perinatal lethality in these mice is caused by uncontrolled proinflammatory responses and signalling that cause hepatotoxicity and shock. Lethality in these cases was circumvented by the compound loss of signalling mediators, such as IFN- γ , TNFR1 and MyD88. It was investigated whether the *Arih2*^{-/-} lethality could be abrogated with the deletion of similar proinflammatory mediators. Loss of MyD88, but not TNF α or IFN γ , partially rescued the lethality caused by the loss of *Arih2* (**FIG. 1h, FIG. 10 and FIG. 5h, i, j**). This implicates an aberrant TLR-MyD88 signalling pathway as a cause of early lethality and lymphoproliferation observed in *Arih2*^{-/-} mice.

[0247] TLR signalling is an integral component of DC activation so DC cultures were prepared by differentiating foetal liver progenitor cells, to allow investigation of the role of *Arih2* in innate immune cell homeostasis and to determine the cell intrinsic consequences of the loss of *Arih2* in DCs 24. Foetal livers were harvested from mice at day E13.5-E14.5 for DC differentiation. At this time *Arih2*^{-/-} embryos did not show any significant abnormalities.

[0248] Conventional and plasmacytoid DC cultures (cDC, pDC respectively) were prepared by differentiating DCs *in vitro* using GM-CSF to obtain predominately conventional DCs (cDCs defined as CD11c⁺C11b⁺) and Flt3L to obtain a combination of cDCs and plasmacytoid DCs (pDCs defined as CD11c^{lo}B220⁺). In all instances the proportions of pDCs and cDCs were equivalent in *Arih2*^{+/-} and *Arih2*^{-/-} foetal liver derived DC cultures (**FIG. 6d**). Similarly there were no differences when the various subsets of cDCs (CD103⁺ and SIRP α ⁺) were quantified. Collectively these data indicate that *Arih2* does not play a role in early DC development and differentiation.

0249] Naïve foetal liver differentiated *Arih2*^{-/-} DCs were found to express abundant maturation markers that promote T cell activation including CD80/B7.1, CD86/B7.2, CD83, CD40 and MHCII far exceeding levels observed in control *Arih2*^{+/-} DCs (**FIG. 2a**). Stimulation of *Arih2*^{+/-} DCs with TLR4 and TLR9 ligands, LPS and CpG

respectively, increased maturation marker expression but levels did not reach those expressed by naïve Aih2^{-/-} DCs. In fact, the addition of TLR stimulation to Aih2^{-/-} DCs did not significantly increase surface marker expression beyond naïve Aih2^{-/-} DC levels. Similar to the saturation effect observed with activation markers, in the absence of exogenous stimuli, naïve Aih2^{-/-} DCs produce higher levels of DC and T cell activating cytokines IL-6, IL-12 and TNF α which did not significantly increase with the addition of LPS or CpG (**FIG. 2b, c**). Following the addition of LPS or CpG, Aih2^{+/-} DC cytokine production increased to equal those of naïve Aih2^{-/-} DCs with the exception of TNF α .

[0250] The isotype staining controls in **FIG. 2a** show that the differentiation system is not causing activation of control DCs as defined by the acquisition of maturation markers and co-stimulatory molecules.

[0251] To investigate the effectiveness of these Aih2^{-/-} DC in promoting in vivo functional T cell activation, foetal liver differentiated DCs were pulsed with CD8 LCMV glycoprotein specific epitopes, gp33 and gp276 and CD4 LCMV specific glycoprotein epitope, gp61. These pulsed dendritic cells were injected into transgenic mice that express the LCMV glycoprotein under the control of the rat insulin promoter, RIP-GP mice²⁵⁻²⁶. All β -islet cells in these mice expressed the LCMV glycoprotein but the animals remained in an immunologically ignorant state and did not develop insulinitis or diabetes. Adoptive transfer of activated matured and LCMV peptide pulsed DC into RIP-GP mice infrequently initiates an adaptive immune response where specific autoreactive T cells directed against the target GP antigen are activated and cause overt diabetes. β -islet cell destruction can be assessed in these mice by the measurement of random blood glucose with repeated values in excess of 15 mmol/L indicating a greater than 90% destruction of β -islet cell mass. It is apparent that successful induction of autoimmune diabetes is critically dependant on the maturation state of the injected DC and typically requires prior activation with TLR stimulation or the simultaneous addition of agents such as anti-CD40 antibody 27. However, naïve Aih2^{-/-} DCs were able to induce diabetes. In total, 5 of the 10 RIP-GP mice injected with peptide pulsed naïve Aih2^{-/-} DCs developed diabetes

were observed in contrast to 0 of 10 RIP-GP mice injected with peptide pulsed naïve Aih2^{+/-} DCs (**FIG. 2d, e, f**). With CpG stimulation, all RIP-GP mice injected with peptide pulsed Aih2^{-/-} DCs developed diabetes, in contrast to only 2 of the 5 in the Aih2^{+/-} cohort (**FIG. 2d, e, f**). This clearly demonstrates that in the absence of exogenous stimuli, naïve Aih2^{-/-} DCs are prematurely activated and capable of activating T cells in vivo. Moreover, the induction of T cell responses was robust, overcoming the host immunoregulatory networks to reliably induce diabetes in RIP-GP mice. This demonstrates a critical role of Aih2 in maintaining DCs in a quiescent state and regulating the adaptive immune response to avert spontaneous autoimmunity.

[0252] The partial reversal of embryonic lethality in Aih2^{-/-} mice with the compound loss of MyD88, indicated that the DC and autoimmune phenotypes in Aih2^{-/-} mice were most likely a consequence of aberrant TLR signalling. Key signaling events downstream of TLR activation in dendritic cells were investigated. Compared to naïve Aih2^{+/-} DCs, Aih2^{-/-} DCs exhibited normal levels of MyD88, Akt and IκB phosphorylation however Aih2^{-/-} DCs have an increase in basal IκB levels causing less pronounced degradation of IκB upon TLR stimulation (**FIG. 3a, b and 7a, b**). This appears to be unique to DCs and TLR activation as Aih2^{+/-} and Aih2^{-/-} mEF stimulation by TNFα shows similar kinetics of IκB degradation (**FIG. 3b**). Nuclear p65/RelA levels along with phospho-p65/RelA and RelB were dramatically increased and sustained following TLR stimulation, accompanied by an increase in NFκB binding suggesting that the loss of Aih2 leads to an increase in nuclear and activated NFκB subunits and sustained NFκB signalling (**FIG. 3c, d and 7c, d**). Also, Applicants observed Aih2^{-/-} DCs exhibited increased and sustained ERK phosphorylation correlating with increased nuclear AP-1 a likely consequence of excessive NFκB signalling and cross-talk between the two pathways (**FIG. 3e, f and 7e, f**).

[0253] Applicants proceeded to investigate the NFκB pathway in more detail. The results of these investigations demonstrated that in the absence of Aih2, IκBβ accumulates in the nucleus; furthermore, the accumulation of IκBβ in the nucleus

was recapitulated if the ultimate function of Aih2 was interrupted by inhibiting the proteasomal degradation pathway. In the presence of Aih2, I κ B β did not accumulate in the nucleus. Applicants showed that nuclear Aih2 binds nuclear I κ B β and is responsible for its removal.

[0254] In Aih2^{-/-} DCs, increased basal I κ B reduced degradation was unique to DCs downstream of TLR signalling, as LPS stimulation of fetal liver derived macrophages and TNF α stimulation of mEFs showed similar I κ B kinetics (See **FIG. 11**). Nuclear p65/RelA levels along with phospho-p65/RelA and RelB were dramatically increased and sustained following CpG stimulation compared to controls. This was accompanied by an increase in NF κ B binding, indicating that the loss of Aih2 leads to an increase in nuclear and activated NF κ B subunits and sustained NF κ B signaling (**FIG. 12 d, e**). The hyperactivation effects observed in Aih2 deficient DCs were not restricted to CpG stimulation as similar results were obtained with TNF α and LPS treatment (**FIG. 11 b,f,g**). Additionally, it was observed that Aih2^{-/-} DCs exhibited increased and sustained ERK phosphorylation correlating with increased nuclear AP-1 levels, indicating excessive NF κ B signalling and cross-talk between the two pathways (**FIG. 11 d, e**) (Ten Embo 11:195 1992, Paya 1992 PNAS 89:7826, Waterfield Mol Cell 2003 11:685, Tomczak 2006 J Immunol 176:1244). Aih2^{-/-} DCs had increased and sustained nuclear I κ B β levels following CpG stimulation (**FIG. 12d**). The accumulation of nuclear I κ B β and increased and sustained levels of phosphorylated p65/RelA was recapitulated in Aih2 sufficient DCs when cells were treated, 30 min after stimulation, with the proteasome inhibitor MG132 for 4 hours (**FIG. 12f**). In the absence of stimulation, Aih2 sufficient DCs treated for 2 hours with MG132 still accumulated nuclear I κ B β but without obvious changes in cytoplasmic I κ B β or nuclear phosphorylated p65 levels; these latter changes indicate upstream NF κ B signalling events. When proteasomal degradation was blocked, we detected physical interaction between Aih2 and I κ B β in the nucleus (**FIG. 12f**). Collectively these data show that Aih2 is required for the removal of nuclear I κ B β and the termination of nuclear p65 activity. In the absence of Aih2, NF κ B signalling was aberrantly sustained with the continued high level expression of nuclear I κ B β

which is required for the maintenance and transcriptional activity of p65. Rao, P. *et al. Nature* 466, 1115-1119 (2010); Scheibel, M. *et al. J Exp Med* 207, 2621-2630 (2010).

[0255] Embryonic lethality precluded a thorough dissection of the role of Aih2 in immune homeostasis and regulation. To address this issue Aih2^{+/-} and Aih2^{-/-} chimeras were generated by transferring E14.5 foetal liver haematopoietic progenitor cells into lethally irradiated Rag1^{-/-} mice (**FIG. 6c**). Comparison of the Aih2^{-/-} and Aih2^{+/-} chimeras revealed an obvious collection of phenotypic changes that recapitulated the lymphoproliferative and early mortality and phenotypes observed in the surviving Aih2^{-/-} mice in a mixed background (**FIG. 4a, b, c**). Consistent with immune dysfunction and a breakdown in tolerance and progression to autoimmunity, hyperactive humoral activity was found in Aih2^{-/-} chimeras with high levels of serum immunoglobulins and circulating proinflammatory cytokines, including IFN γ , IL-1, IL-2, IL-10, IL-12, IL-4, IL-6 and IL-18 (**FIG. 4d, e**). The peripheral lymphoid and myeloid compartments were examined and an increase in peripheral T cells was found, in particular early activated CD25⁺CD69⁺ T cells and CD62L^{lo}CD44^{hi} 'memory-like'/effector T cells however B cells were not affected (**FIG. 4f, 8a**). In vitro, Aih2^{-/-} B cell proliferation in response to IgM crosslinking or TLR stimulation was not altered (**FIG. 8a**). Aih2^{-/-} T cells showed no aberrations in signalling pathways downstream of CD3 + CD28 and PMA + Ionomycin but at early time points Aih2^{-/-} T cells proliferated with increased kinetics (**FIG. 8b, c**). This enhanced early proliferative phenotype is likely a consequence of in vivo activation rather than an intrinsic signalling anomaly. No defects were identified in the suppressive function of regulatory T cells or effector T cell response to suppression using an adoptive transfer induced colitis model, despite an increase in total peripheral CD4⁺CD25⁺Foxp3⁺ T cells (**FIG. 8d-g**). Collectively these data indicate that the break in tolerance observed in Aih2^{-/-} chimeric mice is not due to a T cell intrinsic defect. Further supporting the role of spontaneously activated Aih2^{-/-} DC in causing the immune phenotype that drives the break in tolerance, a striking increase was also observed in myeloid cells, including granulocytes, macrophages and dendritic cells (**FIG. 4f**) which was not confined to lymphoid organs alone. Similar to Aih2^{-/-}

mice, Aih2^{-/-} chimeras had massive immune cell infiltrates in numerous organs (**FIG. 4g, 9a-e**). This is consistent with other mice that have aberrant NFκB activation such as relB^{-/-} and A20^{-/-17,28}. Infiltrates consisted of a heterogeneous population dominated by T cells and myeloid cells including granulocytes, macrophages and dendritic cells (**FIG. 4g, 9a-f**).

[0256] In the absence of functional NFκB activity DC development is impaired²⁹⁻³⁰. Conversely, excessive NFκB activation in immune cells causes overt autoimmunity partly due to hyperactive DCs^{1,17}. Recent work highlights the importance of balanced regulation by key inhibitors of DC activation including STAT3 induction of cytokine production³¹ and HPK1 mediated NFκB activation³². Loss of these DC regulators leads to a break in tolerance and progression to autoimmunity. Given the importance of NFκB in DC development, Aih2 may be critical in preventing spontaneous activation by acting as a novel negative regulator of NFκB activation in DCs. The posttranslational modification and nuclear translocation of p65 is an additional means of NFκB regulation that is distinct from the classical cytoplasmic regulation of IκB degradation³³⁻³⁹. This example demonstrates Aih2 functions in both cytoplasmic and the nuclear compartments to enhance the transport and stabilize the binding activity of p65/RelA in DCs.

[0257] The present experiments demonstrated that in the nucleus, Aih2 binds to IκBβ and is required for the degradation of nuclear IκBβ. The loss of Aih2 led to increased nuclear expression of IκBβ and sustained p65/RelA transcriptional activity. Thus, Aih2 is required for the regulation of IκBβ levels and the termination of p65/RelA activity in DCs.

[0258] Overall, these observations demonstrate that Aih2 has a crucial role in maintaining immune homeostasis by negatively regulating DC activation. The loss of Aih2 leads to a premature activation state such that naïve Aih2^{-/-} DCs are capable of inducing an autoreactive T cell response. The critical role of Aih2 in maintaining DCs in a quiescent state and regulating the adaptive immune response to avert spontaneous autoimmunity is also demonstrated. These data have therapeutic implications for the inhibition of DC activity in autoimmune disease. Conversely

therapeutics that repress Aih2 may promote the activity of DC vaccines targeting infectious diseases and cancer.

[0259] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All nucleotide sequences provided herein are presented in the 5' to 3' direction.

[0260] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

[0261] Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this invention. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[0262] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0263] In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is

also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0264] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

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WHAT IS CLAIMED IS:

1. A method for inhibiting activation of a dendritic cell, comprising contacting the dendritic cell with an effective amount of an agent that increases the biological activity of Aih2 in the dendritic cell, thereby inhibiting activation of the dendritic cell.
2. The method of claim 1, wherein the dendritic cell underexpresses the Aih2.
3. The method of claim 1 or 2, further comprising contacting the dendritic cell with an agent that decreases the biological activity of MyD88.
4. The method of any one of claims 1-3, wherein the contacting is in vitro or in vivo.
5. A method for suppressing an immune response in a patient, comprising administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby suppressing the immune response.
6. The method of claim 5, wherein the patient is in need of immunosuppression for a graft versus host disease.
7. A method for reducing inflammation in a patient, comprising administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby reducing inflammation.
8. The method of claim 7, wherein the inflammation is chronic inflammation.
9. A method for treating an autoimmune disease or condition in a patient, comprising administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby treating the autoimmune disease.
10. A method for preventing diabetes in a patient, comprising administering to the patient an effective amount of an agent that increases the biological activity of Aih2, thereby preventing diabetes.

11. The method of claim 10, wherein the patient is suffering from an autoimmune reaction or disorder.
12. The method of any one of claims 6-11, wherein Arih2 is underexpressed in dendritic cells in the patient.
13. The method of any one of claims 6-12, further comprising administering to the patient an agent that decreases the biological activity of MyD88.
14. The method of any one of claims 1-13, wherein the agent that increases the biological activity of Arih2 comprises an Arih2 protein, a transcription regulator of Arih2, a polynucleotide encoding the Arih2 protein, a small molecule Arih2 activator or an equivalent of each thereof.
15. The method of claim 14, wherein the polynucleotide encoding the Arih2 protein further comprises a transcription regulator.
16. The method of claim 14 or 15, wherein the agent is administered in a stem cell.
17. The method of claim 15 or 16, wherein the viral vector is selected from the group of an adenovirus, adeno-associated virus, lentivirus or retrovirus.
18. The method of claim 14, wherein the Arih2 protein or polynucleotide is administered in an encapsulated form.
19. The method of claim 14, wherein the Arih2 protein further comprises a cell penetrating peptide (CPP).
20. The method of claim 19, wherein the cell penetrating peptide comprises a HIV-TAT peptide.
21. A method for promoting activation of a dendritic cell, comprising contacting the dendritic cell with an agent that decreases the biological activity of Arih2, thereby promoting activation of the dendritic cell.

22. The method of claim 21, wherein the dendritic cell overexpresses Arih2.
23. The method of claim 21 or 22, further comprising contacting the dendritic cell with an agent that increases the biological activity of MyD88.
24. The method of any one of claims 21-23, wherein the contacting is in vitro or in vivo.
25. A method for enhancing an immune response in a patient, comprising administering to the patient an effective amount of an agent that decreases the biological activity of Arih2, thereby enhancing the immune response in the patient.
26. A method for treating an infection in a patient, comprising administering to the patient an effective amount of an agent that decreases the biological activity of Arih2, thereby treating the infection in the patient.
27. The method of claim 26, wherein the infection is a bacterial infection.
28. A method for treating a cancer patient, comprising administering to the patient an effective amount of an agent that decreases the biological activity of Arih2, thereby treating the cancer patient.
29. The method of claim 28, wherein the cancer patient suffers one or more cancer selected from an adenocarcinoma, a leukemia, a lymphoma, a melanoma, a myeloma, a sarcoma or a teratocarcinoma.
30. The method of claim 28 or 29, wherein the cancer patient suffers from a cancer in one or more of adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid or uterus.

31. The method of any one of claims 28 to 30, further comprising administering to the patient an effective amount of a chemotherapy, a biological cancer therapy or a radiation therapy.
32. The method of any one of claims 25-31, wherein Arih2 is overexpressed in the patient.
33. The method of any one of claims 25-32, wherein the agent comprises: a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to Arih2 DNA or mRNA; a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA; an antibody that specifically recognizes the Arih2 protein; a small molecule Arih2 inhibitor or an equivalent of each thereof.
34. The method of claim 33, wherein the agent comprises an antibody that specifically recognizes the Arih2 protein.
35. The method of claim 33, wherein the agent is administered in a stem cell.
36. A cell comprising a recombinant Arih2 protein or polynucleotide.
37. The cell of claim 36, wherein the Arih2 protein further comprises a cell penetrating peptide.
38. A cell comprising an agent that decreases the biological activity of Arih2.
39. The cell of claim 38, wherein the cell is a stem cell.
40. A kit for use in inhibiting dendritic cell activation, reducing chronic inflammation or treating an autoimmune disease, comprising an effective amount of an agent that increases the biological activity of Arih2 and instructions to use.
41. A kit for use in promoting dendritic cell activation, treating an infection or treating a cancer, comprising an effective amount of an agent that decreases the biological activity of Arih2 and instructions to use.

42. A method for determining whether a subject is likely to develop a chronic inflammation, an autoimmune disease or diabetes, comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an underexpression of Arih2 determines that the subject is likely to develop a chronic inflammation, an autoimmune disease or diabetes.
43. A method for determining whether a patient is suitable for a treatment comprising administration of an effective amount of an agent that increases the biological activity of Arih2, wherein the patient suffers from a chronic inflammation, an autoimmune disease or diabetes, comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an underexpression of Arih2 determines that the patient is suitable for the treatment.
44. A method for determining whether a subject is likely to develop an infection or cancer, comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an overexpression of Arih2 determines that the subject is likely to develop an infection or cancer.
45. A method for determining whether a patient is suitable for a treatment comprising administration of an effective amount of an agent that decreases the biological activity of Arih2, wherein the patient suffers from an infection or cancer comprising determining in a sample isolated from the subject the expression level of Arih2, wherein an overexpression of Arih2 determines that the patient is suitable for the treatment.
46. A method for identifying an agent suitable for increasing or decreasing the biological activity of Arih2, comprising contacting a dendritic cell with a candidate agent and determining the activation of the dendritic cell, wherein an increased activation indicates that the candidate agent is suitable for decreasing the biological activity of Arih2 or a decreased activation indicates that the candidate agent is suitable for increasing the biological activity of Arih2.

47. A method for enhancing potency of a vaccine in a patient, comprising administering to the patient an effective amount of the vaccine and an effective amount of an agent that decreases the biological activity of Aih2, thereby enhancing vaccination of the vaccine in the patient.
48. The method of claim 47, wherein the administration of the vaccine and the agent is concurrent.
49. The method of claim 47, wherein the administration of the vaccine and the agent is sequential.
50. The method of any one of claims 47 to 49, wherein the agent comprises: a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to Aih2 DNA or mRNA; a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA; an antibody that specifically recognizes the Aih2 protein, a small molecule Aih2 inhibitor or an equivalent of each thereof.
51. A method for decreasing nuclear expression of I κ B β in a patient in need thereof, comprising administering to the patient in need thereof an effective amount of an agent that increases the biological activity of Aih2, thereby decreasing nuclear expression of I κ B β .
52. A method for increasing nuclear expression of I κ B β in a patient in need thereof, comprising administering to the patient in need thereof an effective amount of an agent that decreases the biological activity of Aih2, thereby increasing nuclear expression of I κ B β .
53. A use of an effective amount of an agent that increases the biological activity of Aih2, for suppressing an immune response in a patient.
54. A use of an effective amount of an agent that increases the biological activity of Aih2, for the preparation of a medicament for suppressing an immune response in a patient.

55. A use of an effective amount of an agent that increases the biological activity of Arih2, for reducing inflammation in a patient.
56. A use of an effective amount of an agent that increases the biological activity of Arih2, for the preparation of a medicament for reducing inflammation in a patient.
57. A use of an effective amount of an agent that increases the biological activity of Arih2, for treating an autoimmune disease or condition in a patient.
58. A use of an effective amount of an agent that increases the biological activity of Arih2, for the preparation of a medicament for treating an autoimmune disease or condition in a patient.
59. A use of an effective amount of an agent that increases the biological activity of Arih2, for preventing diabetes in a patient.
60. A use of an effective amount of an agent that increases the biological activity of Arih2, for the preparation of a medicament for preventing diabetes in a patient.
61. A use an effective amount of an agent that decreases the biological activity of Arih2, for enhancing an immune response in a patient.
62. A use an effective amount of an agent that decreases the biological activity of Arih2, for the preparation of a medicament for enhancing an immune response in a patient.
63. A use of an effective amount of an agent that decreases the biological activity of Arih2, for treating an infection in a patient.
64. A use of an effective amount of an agent that decreases the biological activity of Arih2, for the preparation of a medicament for treating an infection in a patient.

65. A use of an effective amount of an agent that decreases the biological activity of Arih2, for treating a cancer patient.
66. A use of an effective amount of an agent that decreases the biological activity of Arih2, for the preparation of a medicament for treating a cancer patient.
67. A use of an effective amount of the vaccine and an effective amount of an agent that decreases the biological activity of Arih2, for enhancing potency of a vaccine in a patient.
68. A use of an effective amount of the vaccine and an effective amount of an agent that decreases the biological activity of Arih2, for the preparation of a medicament for enhancing potency of a vaccine in a patient.
69. A use of an effective amount of an agent that increases the biological activity of Arih2, for decreasing nuclear expression of I κ B β in a patient in need thereof.
70. A use of an effective amount of an agent that increases the biological activity of Arih2, for the preparation of a medicament for decreasing nuclear expression of I κ B β in a patient in need thereof.
71. A use of an effective amount of an agent that decreases the biological activity of Arih2, for increasing nuclear expression of I κ B β in a patient in need thereof.
72. A use of an effective amount of an agent that decreases the biological activity of Arih2, for the preparation of a medicament for increasing nuclear expression of I κ B β in a patient in need thereof.

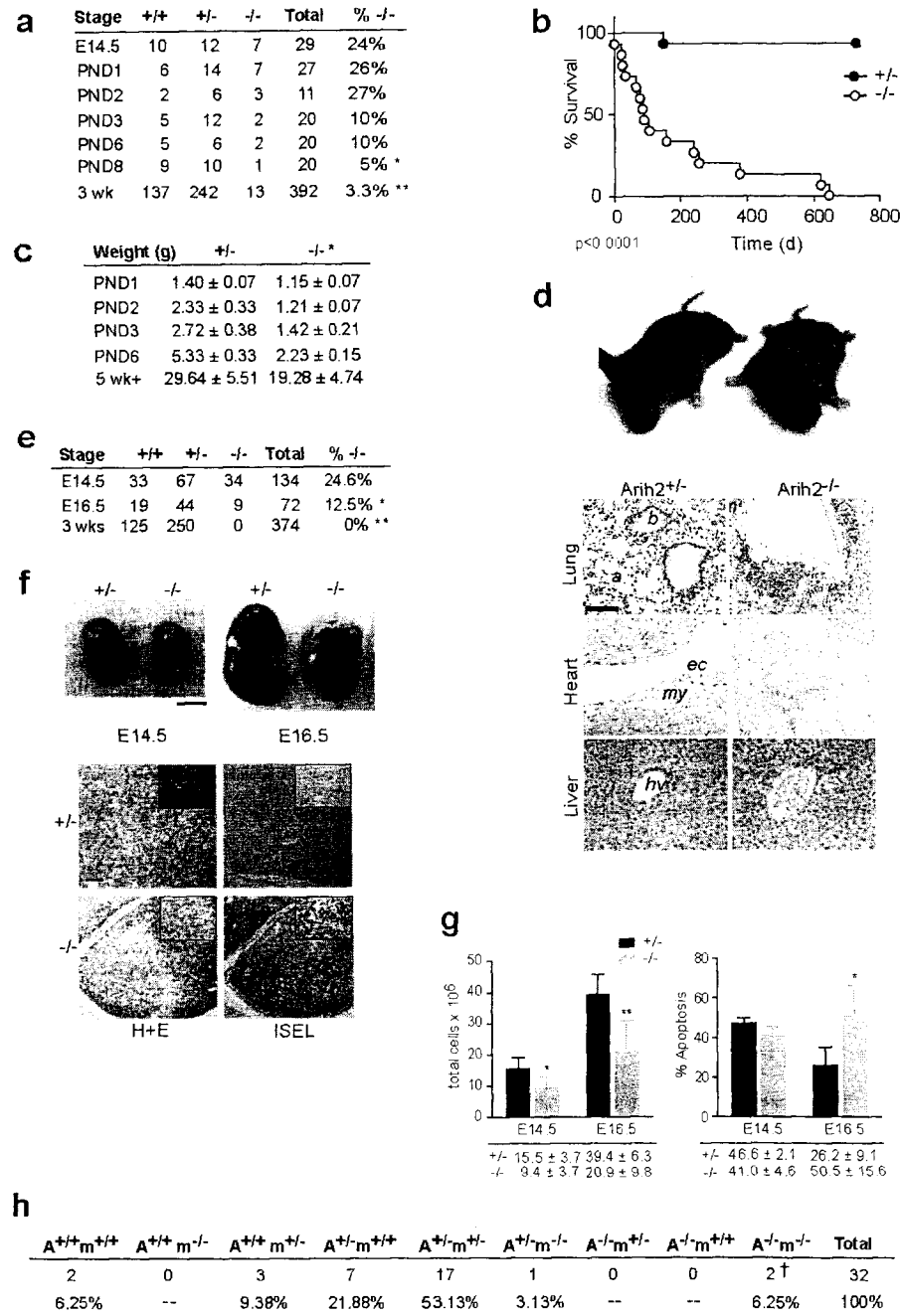
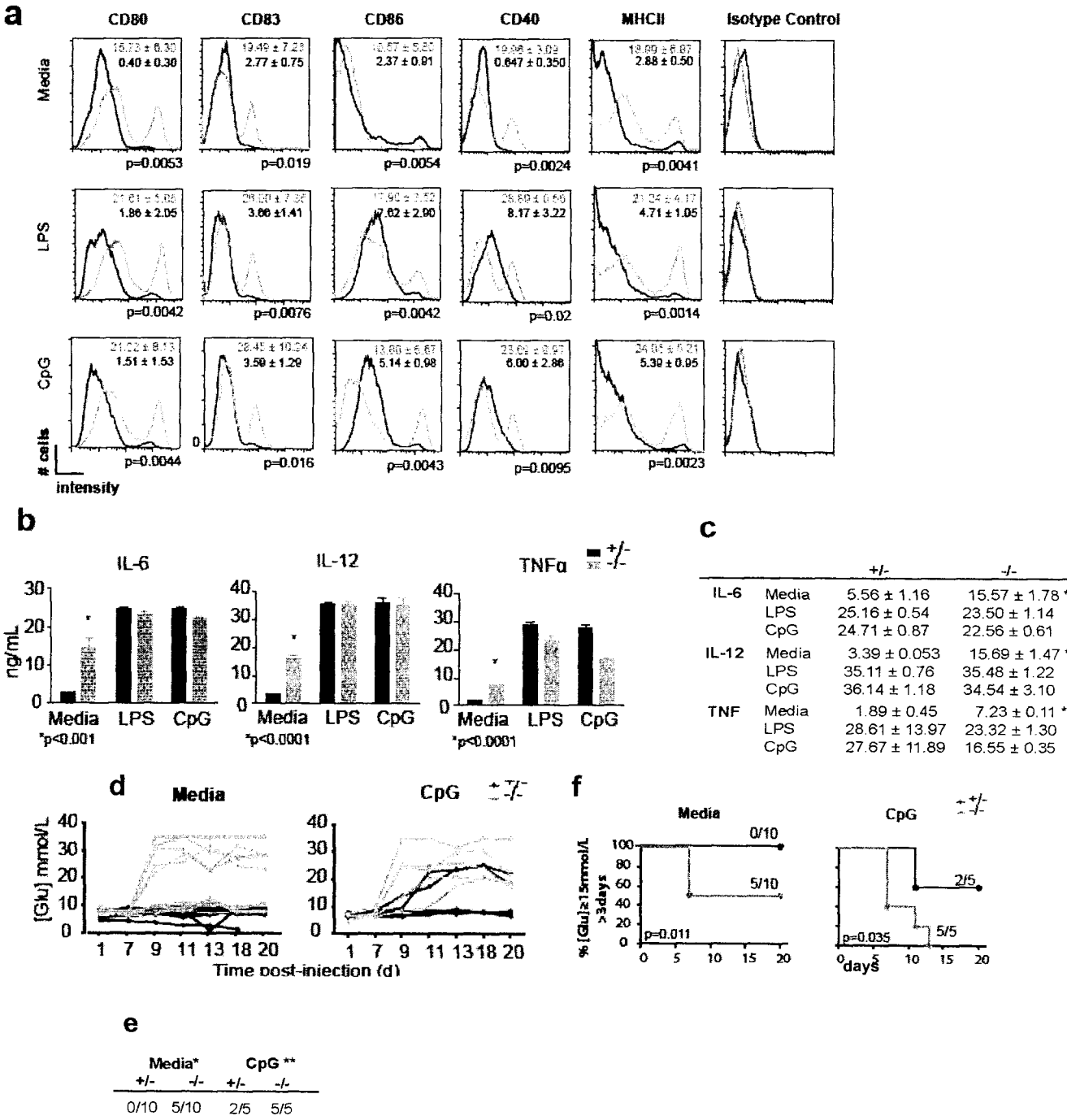


FIG. 1



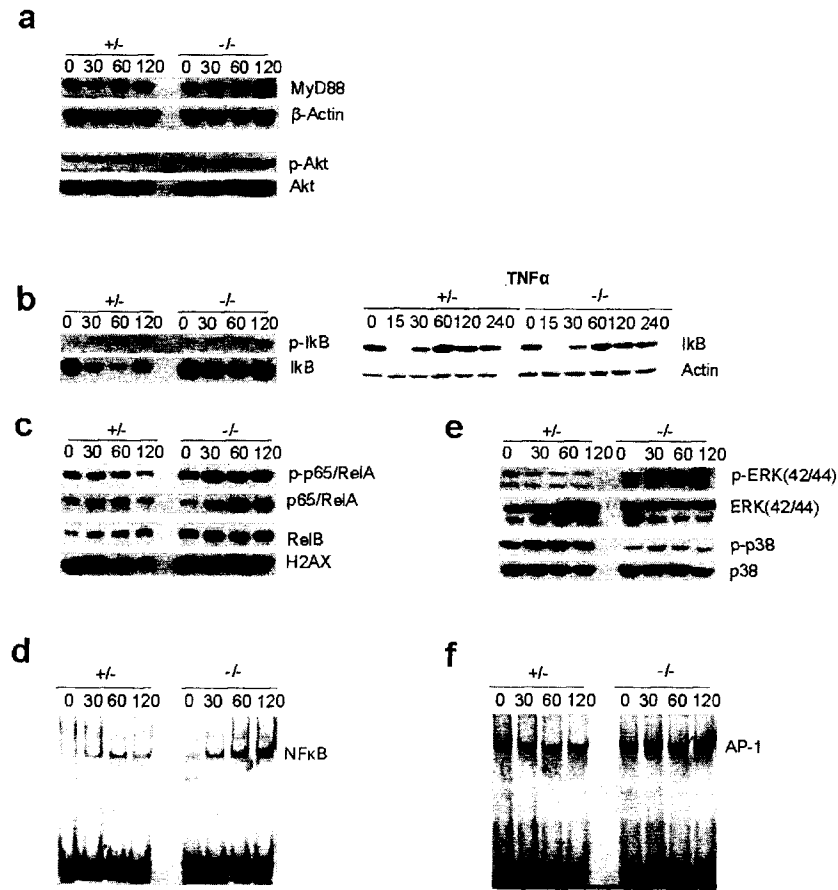


FIG. 3

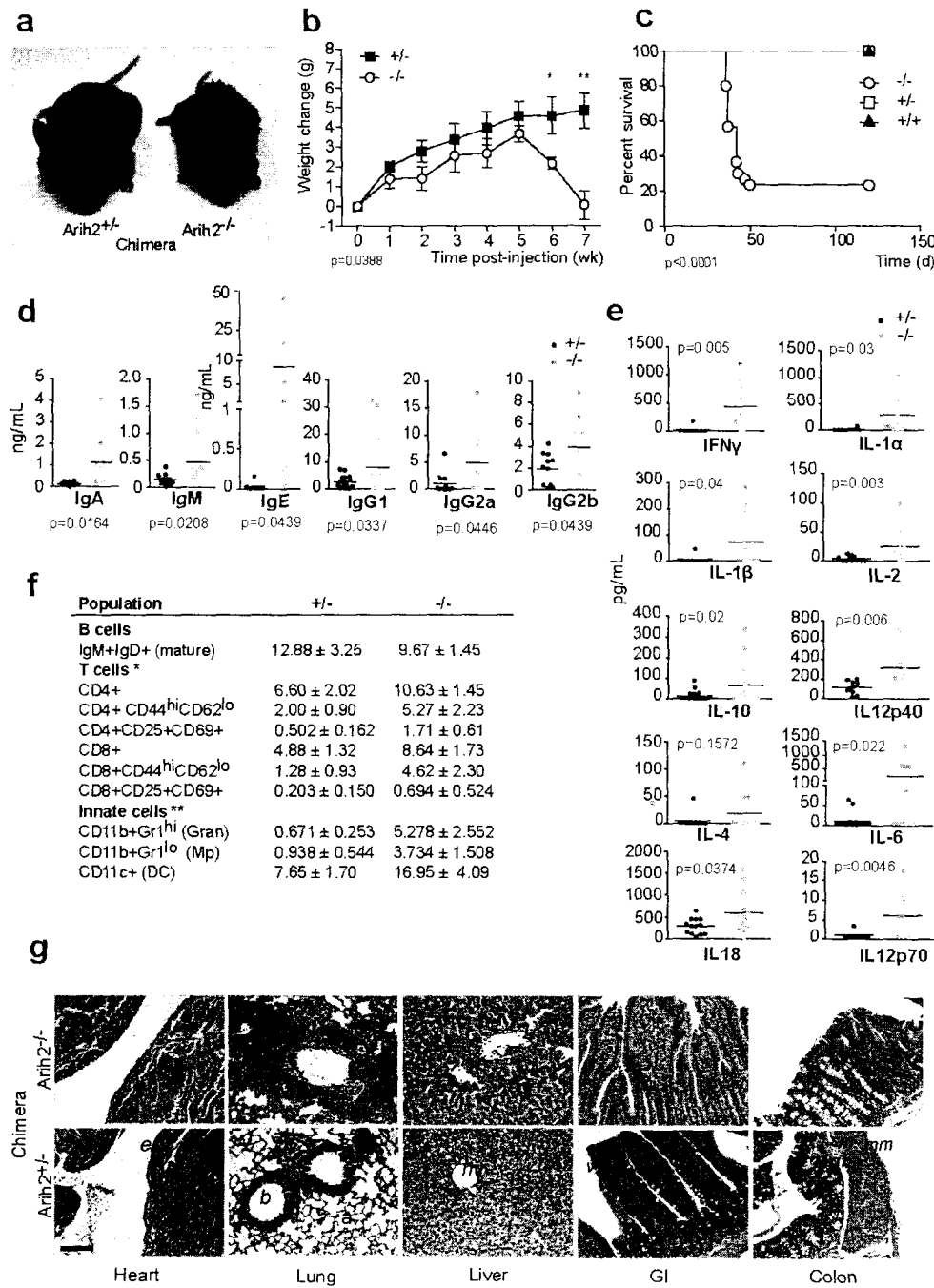


FIG. 4

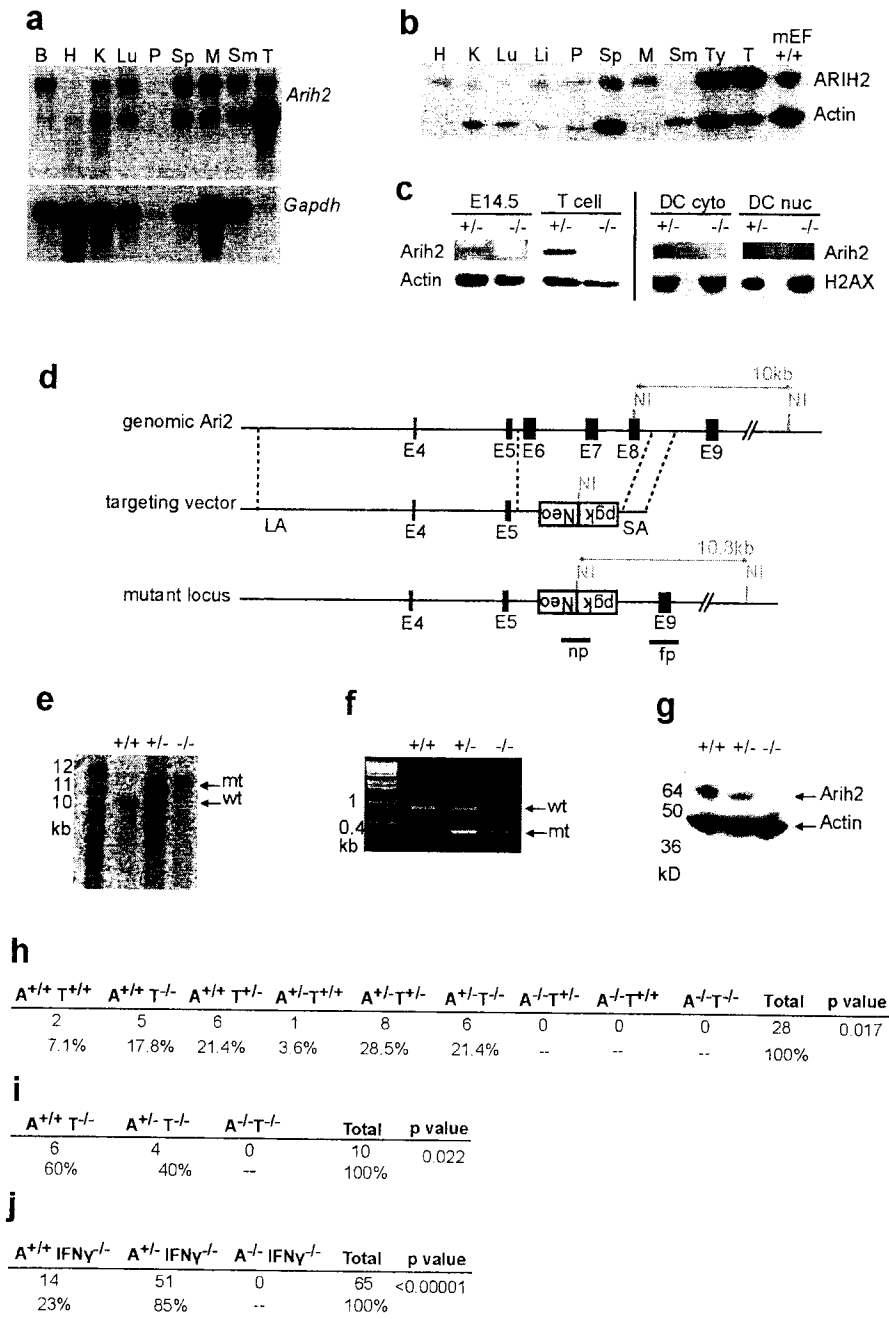


FIG. 5

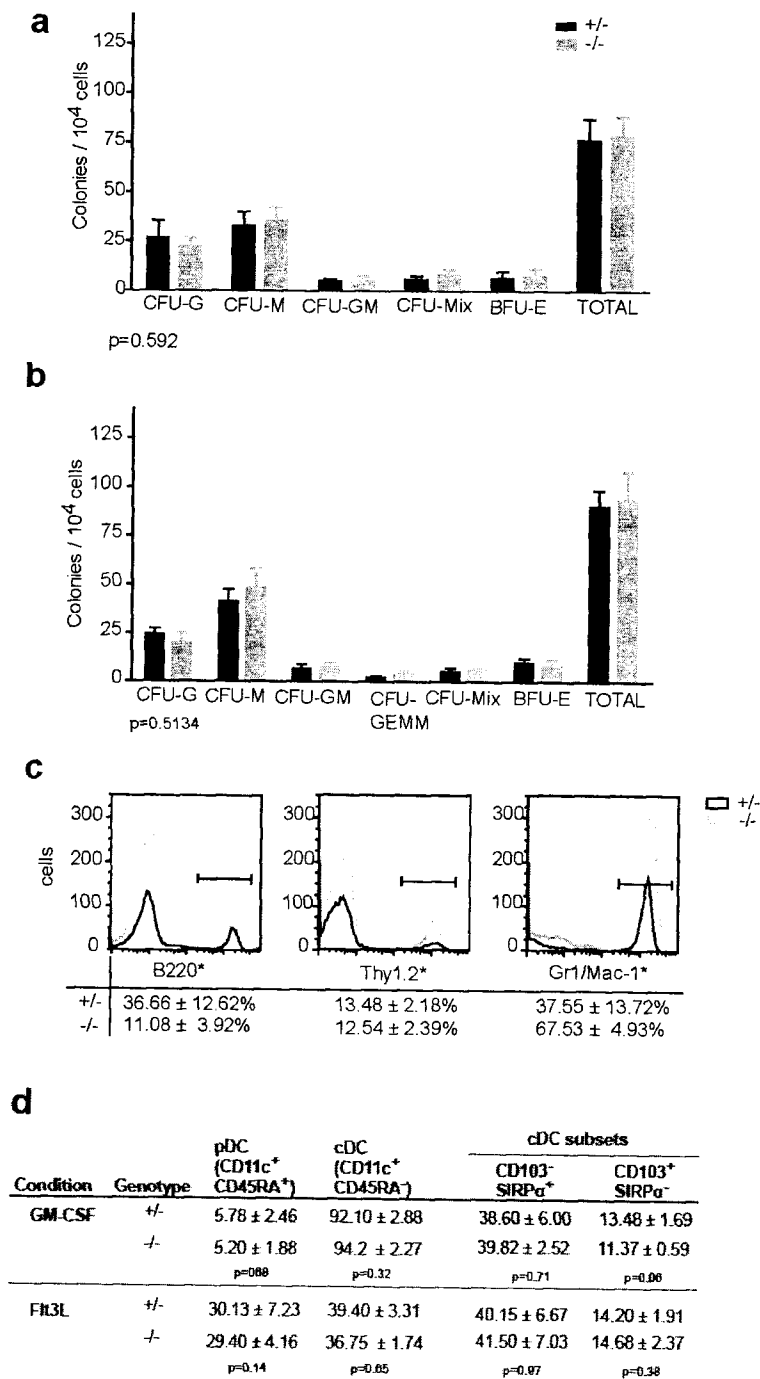


FIG. 6

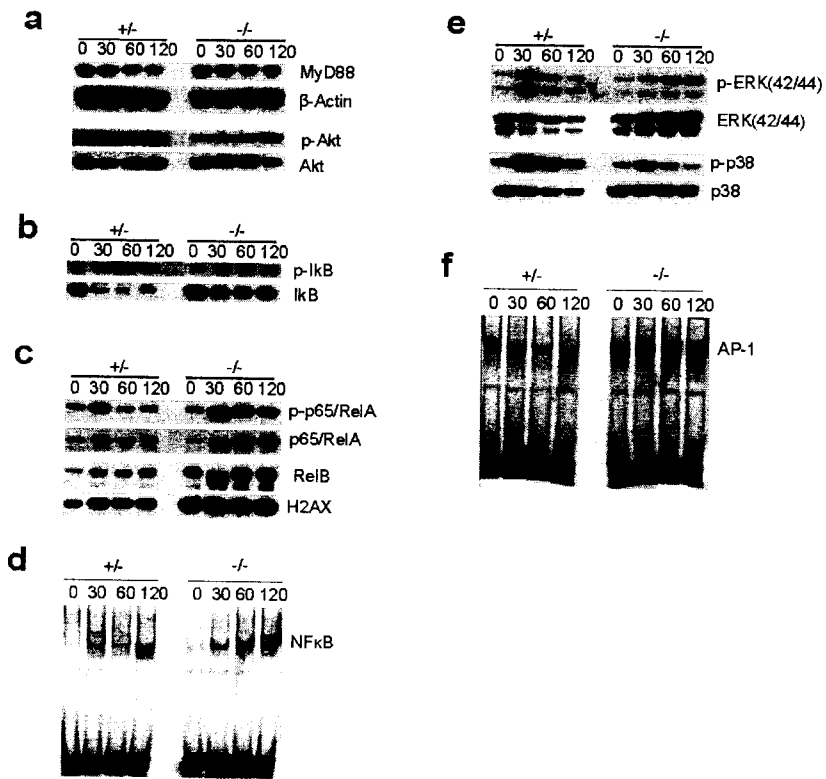


FIG. 7

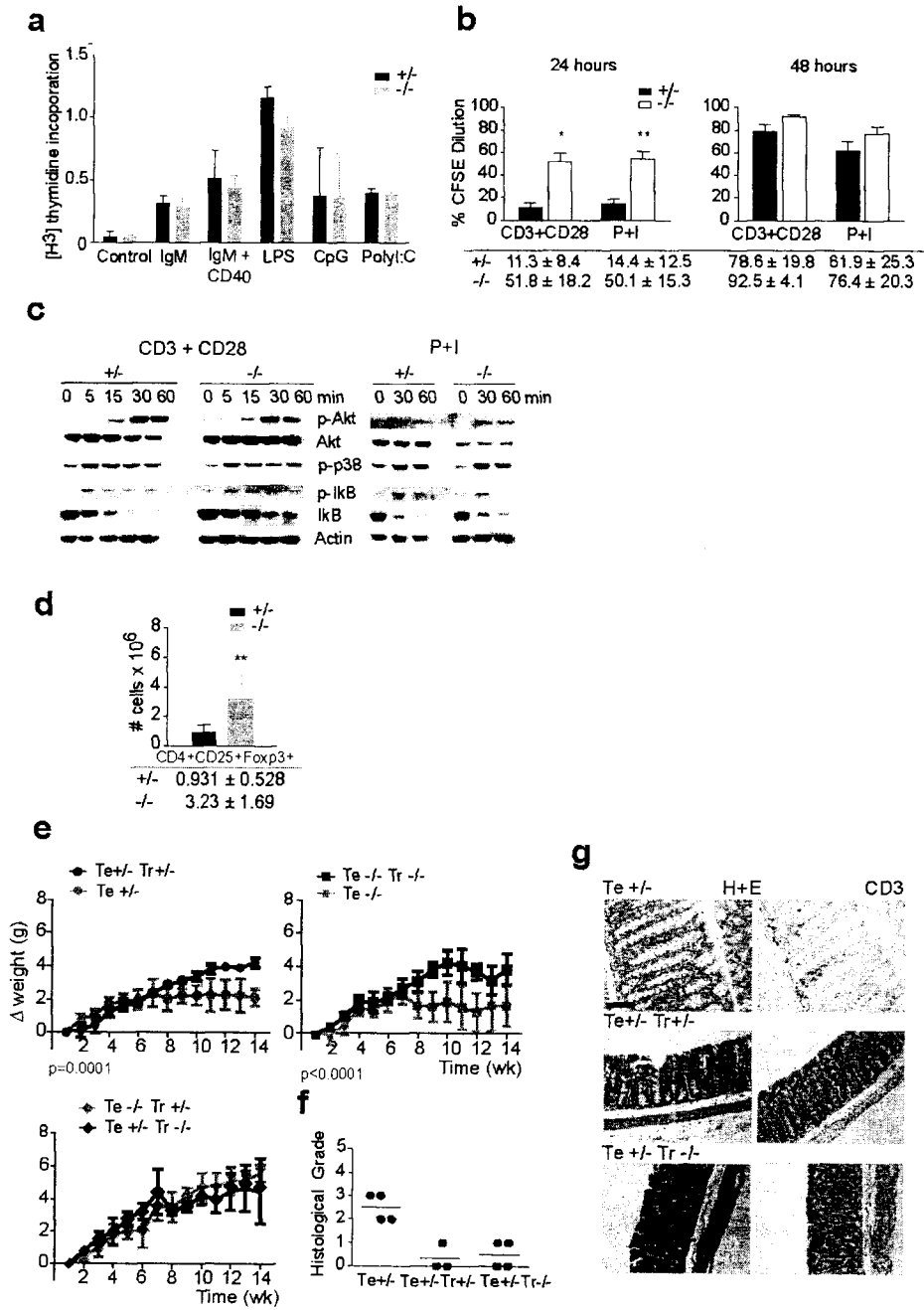


FIG. 8

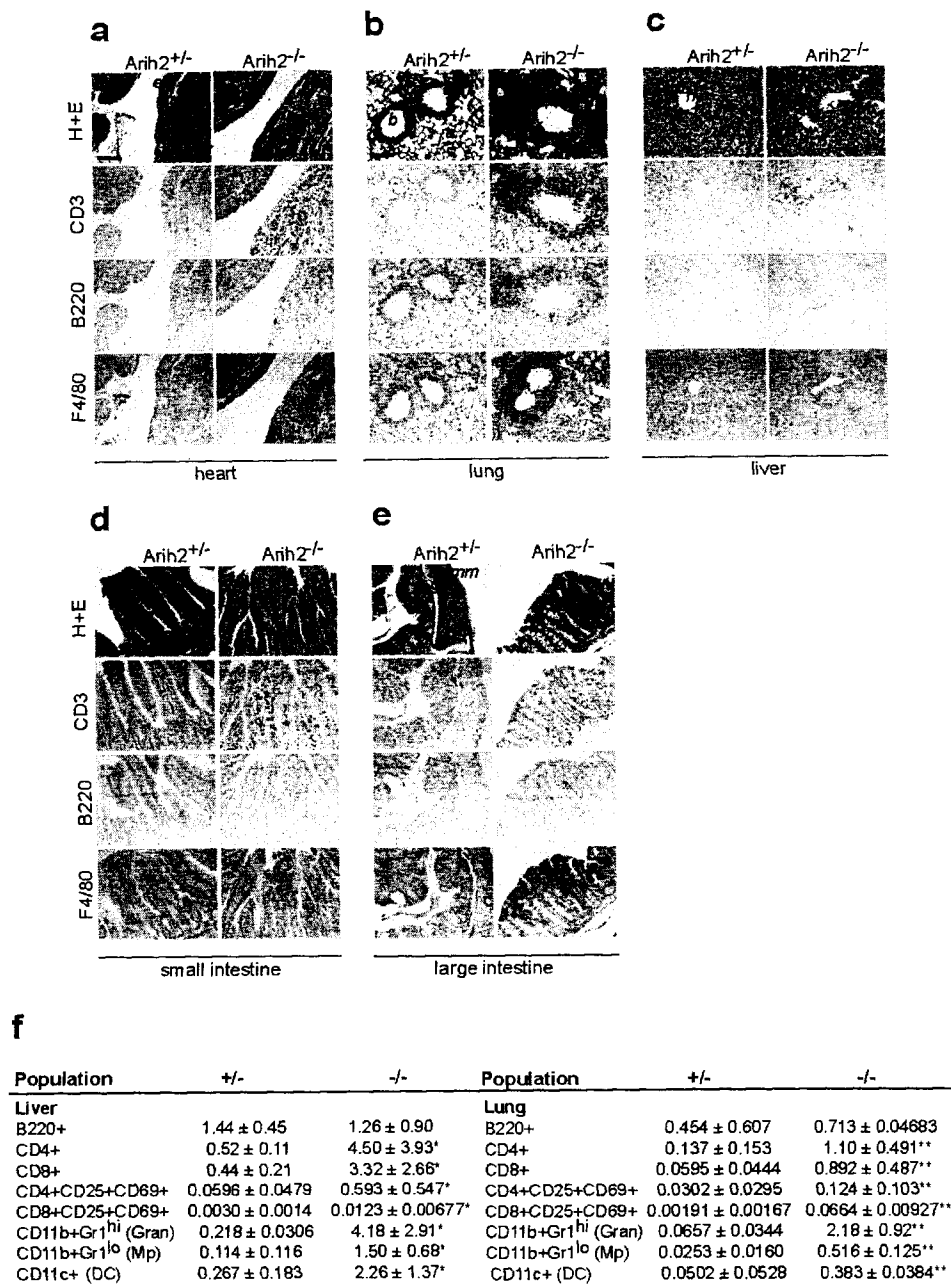


FIG. 9

$A^{+/+}m^{+/+}$	$A^{+/+}m^{-/-}$	$A^{+/+}m^{+/-}$	$A^{+/-}m^{+/+}$	$A^{+/-}m^{+/-}$	$A^{+/-}m^{-/-}$	$A^{-/-}m^{+/+}$	$A^{-/-}m^{+/-}$	$A^{-/-}m^{-/-}$	Total	p value
3	3	11	17	50	2	0	0	3 †	89	<0.00001
5.4%	5.4%	12.3%	15.2%	56.2%	2.2%	—	—	5.4%	100%	

FIG. 10

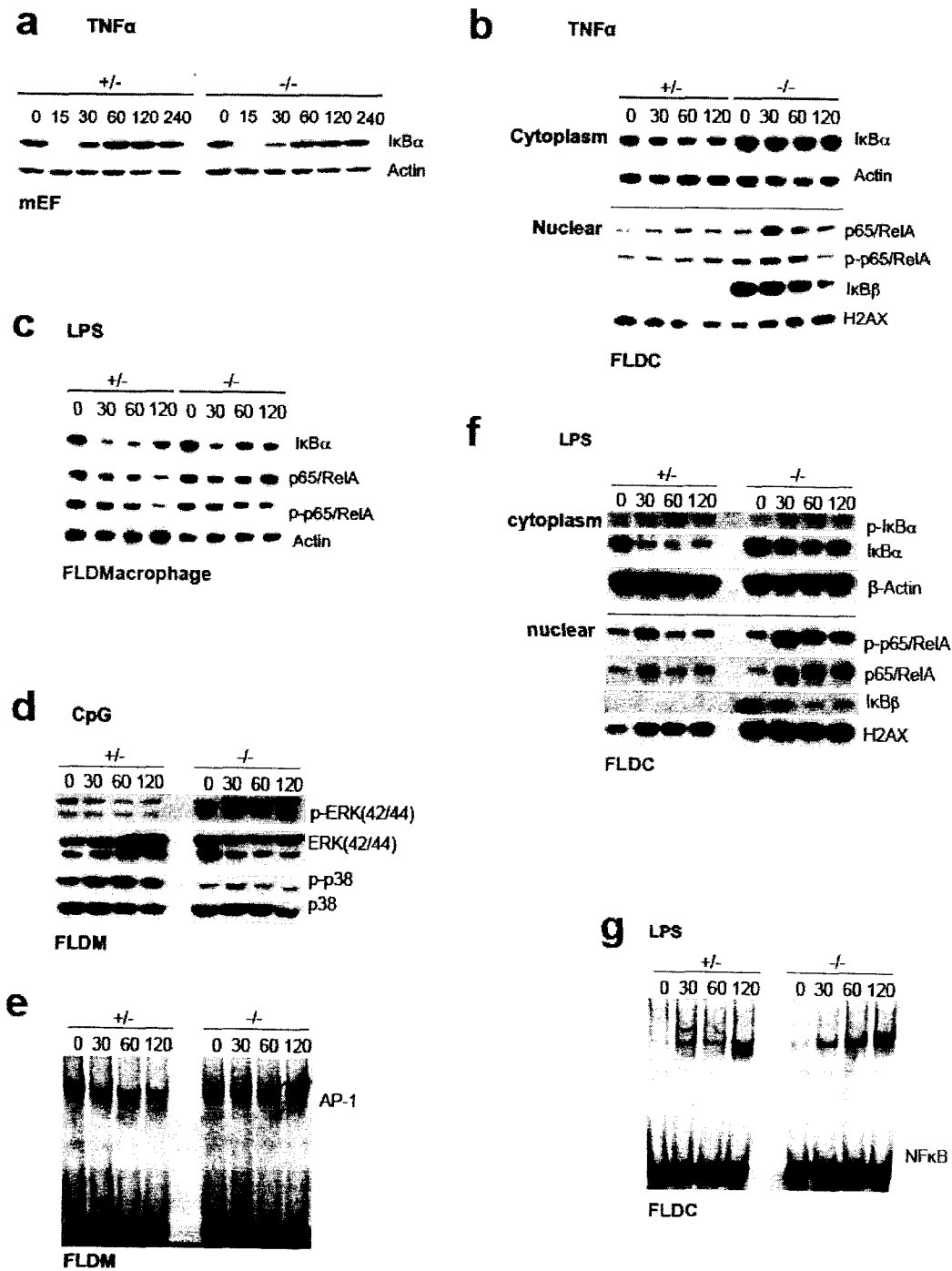


FIG. 11

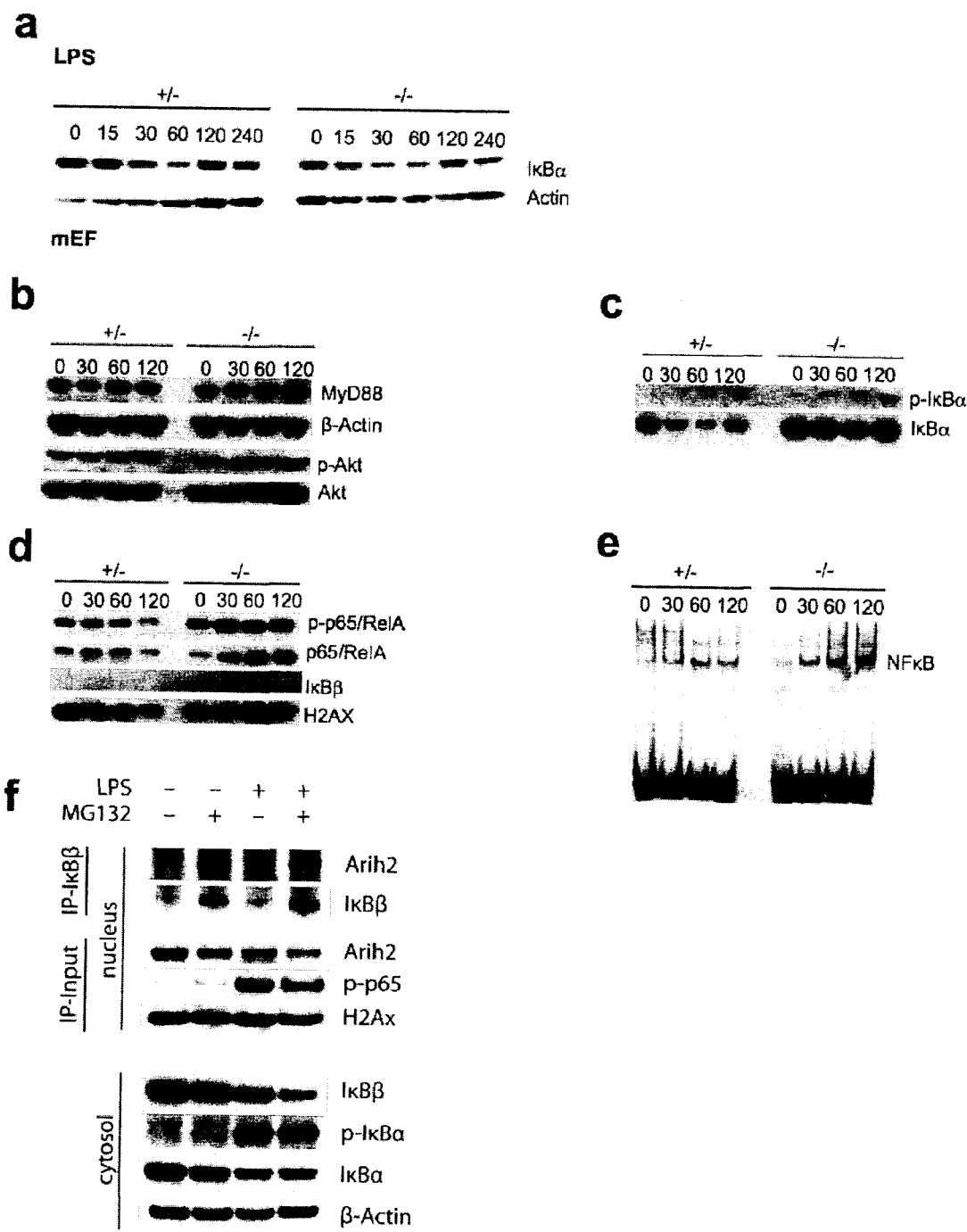


FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/001122

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12N 5/10* (2006.01) , *A61K 31/7088* (2006.01) , *A61K 38/17* (2006.01) , *A61K 39/39* (2006.01) , *A61K 39/395* (2006.01) , *A61K 47/48* (2006.01) (more IPCs on the last page)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 5/10 (2006.01) , *A61K 31/7088* (2006.01) , *A61K 38/17* (2006.01) , *A61K 39/39* (2006.01) , *A61K 39/395* (2006.01) , *A61K 47/48* (2006.01), *A61P 31/04* (2006.01) , *A61P 35/00* (2006.01) , *A61P 37/06* (2006.01) *G01N 33/48* (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: PubMed, Total Patent, Biosis, Scopus

Keywords: TRIAD1, Arih2, ariadne, dendritic, ligase, Gfi1, MyD88

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTELIN, J.A.F., et al. "Gfi1 ubiquitination and proteasomal degradation is inhibited by the ubiquitin ligase Triad1" BLOOD. 1 November 2007 (01-11-2007), V.110(9), p.3128-3135. ISSN: 0006-4971.	40 and 41
Y	whole document	1-4, 14-24 and 46
A	US2006/0179497 A1 (LI, HAO) 10 August 2006 (10-08-2006)	
	whole document	
Y	RATHINAM, C. et al. "The Transcriptional Repressor Gfi1 Controls STAT3-Dependent Dendritic Cell Development and Function" IMMUNITY. June 2005 (06-2005), V.22, p. 717-728. ISSN: 1074-7613.	1-4, 14-24 and 46
	whole document	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 December 2011 (19-12-2011)

Date of mailing of the international search report

17 January 2012 (17-01-2012)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Rena Oulton (819) 997-4567

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☒ on paper
☒ in electronic form
 - b. (time)
☐ in the international application as filed
☐ together with the international application in electronic form
☒ subsequently to this Authority for the purposes of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments :

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 1-4, 14-24, and 46

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-4, 14-24 and 46 are directed to a method for treatment of the human or animal body by surgery or therapy, and are not required to be searched nor is a written opinion required by this Authority. Regardless, this Authority has conducted a search based on the alleged effect or purpose/use of the product defined in claims 1-4, 14-24 and 46.

2. ☒ Claim Nos. : 1-4, 14-24, 40, and 41

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

See extra sheet.

3. ☐ Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

1-4, 21-24, 46 (all wholly); 14-20, 40, and 41 (all partially) relating to Group A

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/001122

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WANG, H. et al. "HoxA10 influences protein ubiquitination by activating transcription of ARIH2, the gene encoding Triad1" J. BIOL. CHEM. 13 May 2011 (13-05-2011), V. 286(19), p.16832-45. ISSN: 00219258. whole document	
Y	KRUMMEN, M. et al. "Release of IL-12 by dendritic cells activated by TLR ligation is dependent on MyD88 signaling, whereas TRIF signaling is indispensable for TLR synergy" J. LEUK. BIOL. July 2010 (07-2010), V. 88(1), p. 189-99. ISSN: 07415400. whole document	3 and 23

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2011/001122

<u>Patent Document Cited in Search Report</u>	<u>Publication Date</u>	<u>Patent Family Member(s)</u>	<u>Publication Date</u>
US20060179497A1	10-08-2006	AU2003289315 (A1)	22-07-2004
		AU2003298176 (A1)	30-06-2004
		EP1571903 (A1)	14-09-2005
		EP1728424 (A2)	06-12-2006
		EP1728424 (A3)	28-02-2007
		JP4479961 (B2)	09-06-2010
		JP2006509795 (A)	23-03-2006
		US2006111587 (A1)	25-05-2006
		US7271288 (B2)	18-09-2007
		US2006179497 (A1)	10-08-2006
		WO2004052094 (A1)	24-06-2004
		WO2004058684 (A1)	15-07-2004

Box No. II continued:

Claims 1-4, 14-24, 40, and 41 are so inadequately supported by the description that no meaningful opinion can be formed. The instant application does not disclose and characterize all possible agents that increase or decrease the biological activity of Arih2. Therefore, the international search report has been established with regard to the following specific agents: an Arih2 protein (an agent that increases the biological activity of Arih2) and siRNA (an agent that decreases the biological activity of Arih2).

Box No. III continued:

The claims are directed to a plurality of inventions as follows:

Group A - Claims 1-4, 21-24, 46 (all wholly); 14-20, 40, and 41 (all partially), featuring methods and kits relating to inhibiting or promoting activation of a dendritic cell.

Group B - Claims 5, 6, 25, 53, 54, 61, 62 (all wholly); 12-20, and 32-35 (all partially), featuring methods and uses relating to suppressing or enhancing an immune response.

Group C - Claims 7, 8, 55, 56 (all wholly); 12-20, 40, 42, and 43 (all partially) featuring methods, kits and uses relating to inflammation.

Group D - Claims 9, 57, 58 (all wholly); 12-20, 40, 42, and 43 (all partially) featuring methods, kits and uses relating to an autoimmune disease.

Group E - Claims 10, 11, 59, 60 (all wholly); 12-20, 42, and 43 (all partially); featuring methods and uses relating to diabetes.

Group F - Claims 26, 27, 63, 64 (all wholly); 32-35, 41, 44, and 45 (all partially), featuring methods, kits and uses relating to infection.

Group G - Claims 28-31, 65, 66 (all wholly); 32-35, 41, 44, and 45 (all partially); featuring methods, kits and uses relating to cancer.

Group H - Claims 36 and 37 (all wholly) featuring a cell comprising recombinant Arih2 protein or polynucleotide.

Group I - Claims 38 and 39 (all wholly) featuring an agent that decreases the biological activity of Arih2.

Group J - Claims 47-50, 67, and 68 (all wholly) featuring a method for enhancing potency of a vaccine.

Group K - Claim 51, 52, and 69-72 (all wholly) featuring a method for decreasing or increasing nuclear expression of IκBβ.

The claims must be limited to one invention as set out in Rule 13 of the PCT.

Documents cited:

D1: MARTELIN, J.A.F. et al. "Gfi1 ubiquitination and proteasomal degradation is inhibited by the ubiquitin ligase Triad1" BLOOD. 1 November 2007 (01-11-2007), V.110(9), p. 3128-3135. ISSN 0006-4971.

D2: US20060179497 A1 (LI, HAO) 10 August 2006 (10-08-2006).

Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features (those technical features that define a contribution which each of the inventions makes over the prior art). The common technical feature of Groups A-K is the Arih2 protein or polynucleotide. An *a posteriori* analysis has concluded that D1 discloses the Triad1 protein or polynucleotide (where the *ARIH2* gene encodes Triad1 and "Arih2" is an alternative name for Triad1). D1 also discloses a cell comprising a recombinant Triad1 protein or polynucleotide, corresponding to Group H (see Materials and Methods, page 3129, right column, second paragraph, and Figure 4). Further, with regard to the common technical feature of Groups A-G, J and K (therapeutic or diagnostic methods, kits or uses), an *a posteriori* analysis has concluded that D2 discloses that the *ariadne 2* gene (human homolog NM_006321) is a modifier of the insulin signalling pathway (see Table 5). D2 discloses methods to treat, prevent or ameliorate conditions associated with dysregulation of the insulin signalling pathway, including diabetes, comprising administering a modulator of a protein produced by said gene (see claims 1 and 2 of D2), corresponding to Group E. Finally, with regard to Group I, an *a posteriori* analysis has concluded that an agent that decreases the biological activity of Triad1 is disclosed in D1 (the down-regulation of endogenous Triad1 by siRNAs; see abstract). Therefore, the claims do not relate to one or more of the same or corresponding special technical features that define a contribution over the prior art, and the claims are directed to a plurality of inventions.

Continued from page 4:

A61P 31/04 (2006.01) , *A61P 35/00* (2006.01) , *A61P 37/06* (2006.01) , *G01N 33/48* (2006.01)