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(71) Applicant: MINNEAPOLIS MEDICAL RESEARCH FOUNDATION [US/US]; 701 Park Avenue, PP7.700, Minneapolis, MN 55415 (US).

(72) Inventor: PRAVETONI, Marco; Minneapolis Medical Research Foundation, 701 Park Avenue, S9.920, Minneapolis, MN 55415 (US).

(74) Agent: GRAM, Christopher, D.; Muetting, Raasch & Gebhardt, P.A., P.O. Box 581336, Minneapolis, MN 55458-1336 (US).

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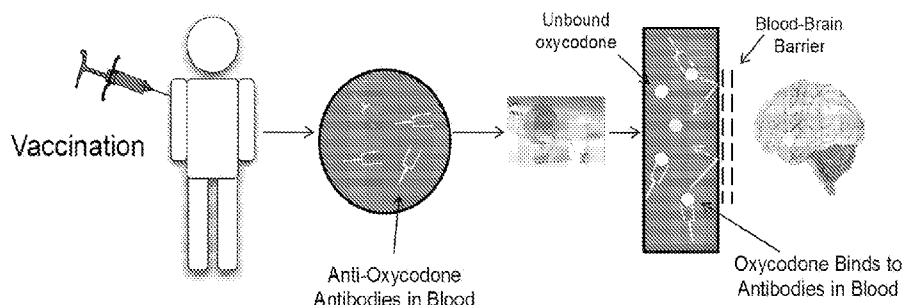
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(54) Title: CYTOKINE SIGNALING IMMUNOMODULATORS AND METHODS

FIG. 1



(57) Abstract: A method generally includes co-administering to a subject a composition that includes an antigen and a cytokine signaling immunomodulator. The method can be for treating a subject for abuse of a drug, treating a subject for toxicity from drug abuse, treating a subject for infection by a pathogen, treating a subject for a non-communicable disease, or for increasing antibody production against the antigen. The antigen can be component of a vaccine. The cytokine-signaling immunomodulator is effective to improve the subject's immune response to the antigen compared to the subject's immune response to the antigen without the cytokine-signaling immunomodulator.



CYTOKINE SIGNALING IMMUNOMODULATORS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 62/334,167, filed May 10, 2016, which is incorporated herein by reference.

GOVERNMENT FUNDING

This invention was made with government support under R01DA041730-01 awarded by the National Institute on Drug Abuse. The government has certain rights in the invention.

SUMMARY

This disclosure describes, in one aspect, a method for treating a subject for abuse of a drug or toxicity from drug abuse. Generally, the method includes co-administering to the subject a vaccine and a cytokine signaling immunomodulator. The vaccine generally includes a derivative of the drug conjugated to a carrier polypeptide in an amount effective to induce production of antibody that specifically binds to the drug. The cytokine-signaling immunomodulator is effective to improve the subject's immune response to the vaccine compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

In some embodiments, the cytokine-signaling immunomodulator can be a complex that includes a cytokine and a monoclonal antibody that binds to the cytokine. In some of these embodiments, the complex can include an IL-4:anti-IL-4 monoclonal antibody complex, an IL-6:anti-IL-6 monoclonal antibody complex, or an IL-21:anti-IL-21 monoclonal antibody complex.

In some embodiments, the cytokine-signaling immunomodulator includes a monoclonal antibody that binds to IL-4, IL-6, IL-21, or other interleukin.

In some embodiments, the cytokine-signaling immunomodulator includes an antibody that specifically binds to the IL-7 receptor α chain (anti-CD127).

In some embodiments, the cytokine-signaling immunomodulator includes an interleukin.

In some embodiments, improvement of the subject's immune response to the vaccine includes an increase in antigen-specific IgG antibody level, an increase in antigen-specific IgG antibody concentration, or an increase in antigen-specific IgG antibody titer compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

5 In some embodiments, improvement of the subject's immune response to the vaccine includes increased production of IgG_{2a} antibody subclass compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the subject's immune response to the vaccine includes increased vaccine efficacy compared to the subject's immune response to the vaccine
10 without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the subject's immune response to the vaccine includes an increase in the percentage of vaccine responders compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the subject's immune response to the vaccine
15 includes at least partial reprogramming of an adaptive immune response in response to an antigen.

In another aspect, this disclosure describes a method for treating a subject for an infectious disease or for a non-communicable disease. Generally, the method includes co-administering to the subject a vaccine and a cytokine signaling immunomodulator. The vaccine
20 generally includes a disease-specific immunogen in an amount effective to induce production of antibody that specifically binds to the immunogen. The cytokine-signaling immunomodulator is effective to improve the subject's immune response to the vaccine compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the subject's immune response includes an
25 increase in antigen-specific antibody production compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the subject's immune response includes a subclass shift of antibody from IgG₁ to IgG₂ compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

30 In another aspect, this disclosure describes a method for producing antibody against an antigen. Generally, the method includes administering to an animal a composition that includes

the antigen and a cytokine-signaling immunomodulator effective to improve the animal's immune response to the antigen compared to the animal's immune response to the antigen without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the animal's immune response includes an increase in antigen-specific antibody production compared to the animal's immune response to the antigen without the cytokine-signaling immunomodulator.

In some embodiments, improvement of the animal's immune response includes a subclass shift of antibody from IgG₁ to IgG₂ compared to the animal's immune response to the antigen without the cytokine-signaling immunomodulator.

The above summary is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1. A schematic illustrating the strategy of immunotherapy for treating drug abuse, dependence, relapse, toxicity, or overdose. In an unvaccinated individual, a drug of abuse (e.g., oxycodone, as shown) does not induce an immune response, due to its small molecular weight. The free drug crosses through the blood-brain barrier and binds to opioid receptors in the brain, causing changes in neuron signaling and brain neurochemistry. Vaccination stimulates production of antibodies against the drug. The antibodies bind to the drug and prevent it from crossing the blood-brain barrier, reducing drug-induced behavioral effects.

FIG. 2. The prescription opioids oxycodone and hydrocodone and a structurally-derived hapten. A tetraglycine linker was placed at the C6 position on the morphinan structure, and the oxycodone-based hapten (OXY) was conjugated through carbodiimide coupling chemistry to a

carrier polypeptide. Other conjugation chemistries known to those skilled in the art can also be used. In this example, the OXY hapten was conjugated to the keyhole limpet hemocyanin (KLH) carrier polypeptide. The OXY and other opioid-based haptens have been conjugated to BSA, OVA, CRM₁₉₇, TT carrier polypeptides and TT-based peptides. Haptens can be conjugated to
 5 other carriers including peptides, polypeptides, but also to biomaterials (e.g., liposomes), and synthetic carriers of various composition (e.g., nanoparticles). The hapten-carrier conjugate is administered in combination with adjuvants (e.g., alum, MPLA, or MF59), but also with other delivery platforms (e.g., gels).

FIG. 3. Co-administration of cytokine signaling immunomodulators (e.g., interleukins, mAb for interleukins or interleukin receptors, or small molecules targeting interleukin signaling)
 10 can enhance T cell-dependent B cell activation and formation of germinal centers (GC) in secondary lymphoid organs and vaccine efficacy. IL-4, IL-6, IL-13, and IL-21 promote either GC formation, isotype class switching, affinity maturation, and/or up-regulate other key pathways associated with activation of GC B cells and T follicular helper (Tfh) cells (FIG. 3,
 15 pathway #1). In contrast, IL-2 and IL-7 inhibit GC formation (FIG. 3, pathway #2) by controlling CD4⁺ T cell differentiation, and limiting formation of Tfh cells. Other less characterized signaling pathways contribute to off-target processes, which distract the adaptive immune system from GC formation (FIG. 3, pathway #3).

FIG. 4. IL-4 increased vaccine-specific GC B cells and post-vaccination GC formation.
 20 Mice were immunized subcutaneously once with the OXY-KLH vaccine adsorbed in alum adjuvant with saline or IL-4 (60,000 IU, subcutaneously). At seven days after immunization, lymph nodes and spleens were collected for analysis of antigen-specific B cell populations. Using a two-step procedure, OXY-specific B cells were first isolated by magnetic enrichment using biotinylated oxycodone-based haptens conjugated to streptavidin (SA) conjugated to the
 25 fluorescent protein phycoerythrin (PE) or a decoy reagent, and then analyzed by multi-parameter flow cytometry. (A) 6OXY-specific CD38^{neg} GL7^{high} “committed” GC B cells; (B) 6OXY-specific CD38⁺ GL7⁺ “uncommitted” GC B cells; (C) Ratio of 6OXY-specific (CD38^{neg}) to (CD38⁺) GL7⁺ B cells, a measure of GC activation and B cell commitment to GC-dependent pathways; (D) 6OXY-specific IgM^{high} naive and memory B cells. Data shown are from two
 30 independent experiments and expressed as mean±SEM (n=10). (E) Chi-square analysis of the fraction (%) of immunized subjects that showed GC formation. Statistical symbols: *p<0.05, and

**** $p < 0.001$.

FIG. 5. In vivo screening of cytokine-based immunomodulators in combination with an oxycodone vaccine in mice. Modulation of IL-4 signaling by means of IL-4:anti-IL-4 immune complexes and/or an anti-IL-4 mAb enhanced anti-oxycodone antibody titers, vaccine efficacy in blocking distribution of oxycodone to the brain, and the subset of immunized subjects that showed vaccine efficacy. Male BALB/c mice were immunized subcutaneously on days 0, 14, and 28, and challenged with 2.25 mg/kg oxycodone subcutaneously a week after the third immunization. Mice received unconjugated KLH, OXY-KLH, or OXY-KLH in combination with IL-4 (30,000 IU, low dose (L), subcutaneously), IL-4 (60,000 IU, high dose (H), subcutaneously), IL-4 plus IL-21 (60,000 IU total, subcutaneously), IL-4:anti-IL-4 mAb (subcutaneously, 30,000 IU and 0.5 mg of mAb mixed prior to injection), anti-IL-4 mAb (1.0 mg per mouse, intraperitoneally), anti-IL-2 alpha receptor mAb (anti-CD25, 1.0 mg per mouse, intraperitoneally), or anti-CD25 mAb (1.0 mg per mouse, intraperitoneally) plus IL-4 (30,000 IU, subcutaneously). The OXY-KLH and the unconjugated KLH were absorbed on alum adjuvant prior to administration. Interleukins were administered at 0 days, 14 days, and 28 in combination with each immunization. The mAb were administered two days prior and one day after the first immunization (0.5 mg each injection, total 1.0 mg per mouse). In immunized mice: (A) oxycodone-specific IgG antibody titers, (B) effect of immunization on oxycodone distribution to the brain, and (C) fraction (%) of immunized subjects that showed more than 50% reduction in oxycodone distribution to the brain compared to the mean brain concentration in the KLH control group. Data are mean $\pm$ SEM. Data shown are from at least two independent experiments (n=5-10). Percentages (%) above bars indicate reduction compared to the KLH group. In the figure, anti-IL-4 mAb and anti-CD25 mAb are described as α IL-4 and α CD25. Statistical symbols: * $p < 0.05$, ** $p < 0.001$, **** $p < 0.0001$ compared to KLH control, and brackets or # indicate significance between groups.

FIG. 6. In vivo blockage of IL-4 increased vaccine efficacy against high doses of oxycodone (5 mg/kg or 10 mg/kg challenges). Male BALB/c mice were immunized with OXY-KLH or unconjugated KLH as control subcutaneously on days 0, 14 and 28, and challenged with oxycodone one week after the third immunization. In a first experiment, mice received KLH, OXY-KLH, or OXY-KLH in combination with anti-CD25 mAb (1.0 mg, intraperitoneally), or anti-IL-4 mAb (1.0 mg, intraperitoneally) and challenged with 5.0 mg/kg oxycodone. In a

follow-up experiment, mice received KLH, OXY-KLH, or OXY-KLH in combination with anti-IL-4 mAb (1.0 mg, intraperitoneally) and challenged with 10.0 mg/kg oxycodone. After immunization: (A) oxycodone-specific IgG antibody titers, effect of immunization on oxycodone distribution to serum (B) and to the brain (C), and (D) fraction (%) of immunized subjects that showed more than 50% reduction in oxycodone distribution to the brain compared to the mean brain concentration in the KLH control group. Panels (E-H) convey the same information as the upper panel (titers, distribution to serum and brain, and % of responders), but cohort of mice received a 10 mg/kg oxycodone challenge. Data are mean $\pm$ SEM. Data shown are from at least two independent experiments (n=10). Percentages (%) above bars indicate reduction compared to the KLH group. In the figure, anti-IL-4 mAb and anti-CD25 mAb are described as α IL-4 and α CD25. Statistical symbols: *p<0.05, **p<0.001, ****p<0.0001 compared to KLH control, and brackets to indicate significance between groups.

FIG. 7. The immunogenicity and efficacy of the oxycodone vaccine combined with the anti-IL-4 mAb is conserved across clinically-relevant immunization regimens, attenuated oxycodone-induced toxicity and behavioral effects, and did not interfere with naloxone reversal of opioid-induced toxicity. Two independent cohorts of BALB/c mice were immunized with OXY-KLH or unconjugated KLH on days 0, 14 and 28, and tested for vaccine efficacy a week after the third immunization. Mice received OXY-KLH subcutaneously or intramuscularly. Anti-IL-4 mAb was administered intraperitoneally, intravenously, or intramuscularly. In a first cohort, mice were challenged with 5.0 mg/kg oxycodone: (A) oxycodone-specific IgG titers; (B) oxycodone brain concentrations; and (C) percentage of subjects that showed more than 50% reduction in oxycodone distribution to the brain compared to the KLH group. Data are mean $\pm$ SEM. Data shown are from one experiment (n=5-8). Percentages (%) above bars indicate reduction compared to the KLH group. In the figure, anti-IL-4 mAb is described as α IL-4. Statistical symbols: *p<0.05, **p<0.001, ****p<0.0001 compared to KLH control, and brackets to indicate significance between groups. In a second cohort, immunized mice were tested for opioid-induced respiratory depression, heart rate and antinociception. Oxycodone was administered subcutaneously every 15 minutes at increasing doses and the doses listed are the cumulative dose received. Naloxone 0.1 mg/kg subcutaneously was administered 15 minutes after the final oxycodone dose to reverse opioid effects. (D) Breath rate, (E) heart rate, and (F) antinociception on a hot plate. * p <0.05, ** p <0.01, *** p <0.001 for the difference between

OXY-KLH and KLH. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ for the difference between OXY-KLH plus anti-IL-4mAb and KLH. Data are mean $\pm$ SEM. Data shown are from one experiment (n=8).

FIG. 8. Depletion of IL-4 increased the quantity and quality of the post-immunization anti-oxycodone serum antibody response and activated antibody-dependent phagocytosis activities. A first study analyzed antibody subclasses in mice immunized with OXY-KLH in alum with or without the anti-IL-4 mAb treatment. Data in panels (A-F) are from the same subjects as in FIG. 5, 6, and 7A-C. IgG subclass analysis was performed on a representative sample randomly selected from each group (n=5-6 mice/group). Shown the analysis of oxycodone-specific serum IgG antibody: (A) total oxycodone-specific IgG titers; (B) IgG₁; (C) IgG_{2a}; (D) IgG₃; (E) IgG₁+IgG_{2a}+ IgG₃; and (F) IgG₁/((IgG_{2a}+ IgG₃)/2), a measure of Th2 over Th1 activation. (G) In a follow-up independent experiment, mice were immunized with either KLH or OXY-KLH (n= 5). On day 34, a group of mice immunized with OXY-KLH received liposome-encapsulated clonodrate to deplete macrophages, and 24 hours later challenged with 5.0 mg/kg oxycodone. Preventing macrophage activity reduced OXY-KLH efficacy in blocking oxycodone brain distribution and the subset (%) of immunized mice showing efficacy. In the figure, anti-IL-4 mAb is described as α IL-4. Statistical symbols: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

FIG. 9. Genetic ablation of IL-4 reproduces the effects of IL-4 depletion confirming that treatment with an anti-IL-4 mAb increases the efficacy of an oxycodone vaccine by IL-4 blockage. IL-4 deficient (IL-4^{-/-}) mice and wild-type (WT) controls were immunized with KLH or OXY-KLH in alum. After immunization: (A) oxycodone-specific IgG antibody titers, effect of immunization on oxycodone distribution to (B) serum and (C) to the brain. Antibody subclass analysis: (D) IgG₁; (E) IgG_{2a}; (F) IgG₃; (G) IgG₁/((IgG_{2a}+ IgG₃)/2). Statistical symbols: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

FIG. 10. IL-4 depletion does not impair antigen-specific plasma and germinal center B cell expansion, but significantly increases differentiation of antigen-specific T cells after immunization. BALB/c mice were immunized once with KLH, OXY-KLH in alum adjuvant, and OXY-KLH in alum adjuvant plus the anti-IL-4 mAb (KLH, n=4, OXY-KLH, n=20, and OXY-KLH plus anti-IL-4 mAb, n=20). Analysis of B cell lymphocyte populations was performed seven days after immunization. Briefly, OXY-specific B cells were first isolated by

magnetic enrichment using biotinylated oxycodone-based haptens conjugated to SA-PE and then analyzed by multi-parameter flow cytometry. Analysis of germinal center B cells shortly after immunization is a marker of germinal center formation in secondary lymphoid organs. (A) the number of OXY-specific antibody secreting B cells (ASC) and germinal center (GC) B cells was not affected by IL-4 depletion. (B-E) In a separate study, C57BL/6 mice were immunized with a model immunogen consisting of PE-SA conjugated to the biotinylated 2W peptide (PE-SA^{2W}). Groups were as follows: naive, PE-SA^{2W} and PE-SA^{2W} plus the anti-IL-4 mAb. At seven days after immunization, lymph nodes and spleens were collected for analysis of antigen-specific T cell populations. Using a two-step procedure, 2W-specific T cells were first isolated by magnetic enrichment using 2W conjugated to soluble MHCII receptors tetramerized on SA-PE and then analyzed by flow cytometry using standard surface and intracellular markers. (B) CD4⁺ Th1 cells; (C) CD4⁺ T_{FH} cells; (D) CD4⁺ GC-T_{FH} cells; (E) (T_{FH}+GC-T_{FH})/Th1. Data are mean±SEM. Statistical symbols: **p<0.01.

FIG. 11. Blockage of IL-4 signaling via anti-IL-4 mAb depletion enhances induction of antibody titers specific for tetanus toxoid and the fraction of immunized subjects showing protection against the diphtheria toxin. Mice were immunized with a commercially available tetanus toxoid, diphtheria, and acellular pertussis (TDaP) vaccine with or without the anti-IL-4 mAb. Serum TT-specific IgG, IgG₁ and IgG_{2a} antibody titers were analyzed by ELISA. (A) TT-specific IgG titer over time. (B-F) A more detailed analysis of TT-specific IgG titers was performed on day 28 after the first immunization: (B) IgG; (C) IgG₁; (D) IgG_{2a}; (E) ratio of IgG₁/IgG_{2a}; (F) percentage of immunized subjects exhibiting anti-diphtheria antibodies. Data are mean±SEM. Anti-IL-4 mAb is described as αIL-4. Statistical symbols: *p<0.05.

FIG. 12. Co-administration of vaccines and an anti-IL-4 mAb is safe. Histopathology across four treatment groups. (A-D) Images show occasionally moderate vacuolar change within the centrolobular hepatocytes that was similar in severity across four treatment groups (KLH, OXY-KLH, anti-IL-4 mAb, and OXY-KLH plus anti-IL-4 mAb). (E-H) No significant pathology was observed in the spleen. (I-L) In all treatment groups, the lungs demonstrated regions containing mild to occasionally moderately increased numbers of inflammatory cells, principally lymphocytes with fewer neutrophils, in the interstitium with concurrent congestion. Occasionally, these regions were centered on lymphocyte-rich lymphatic vessels. The severity of the lung pathology was similar across the four treatment groups. No significant pathology was

identified in the kidney, heart, thymus, stomach, small intestine, large intestine, and pancreas. In the figure, anti-IL-4 mAb is described as α IL-4.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

5 Vaccines may offer a therapeutic option for treating drug abuse and dependence, relapse to drug use after a period of abstinence, and drug-related toxicity such as overdose death. A small molecule drug such as, for example, an opioid (e.g., heroin or a prescription opioid) or a stimulant (e.g., nicotine, cocaine, amphetamine, methamphetamine), can be made immunogenic by conjugating the small molecule to a larger, foreign carrier polypeptide or other immunogenic
10 structure (e.g., nanoparticle or liposome) to elicit serum antibodies that bind drug in serum, prevent its distribution to the brain, and subsequently reduce the likelihood or extent of drug-induced behavior or drug-induced toxicity. However, eliciting high levels of drug-specific serum antibodies has remained a challenge in translating these vaccines to human use.

Clinical efficacy of therapeutic vaccines for substance use disorder depends, at least in
15 part, on achieving high levels of effective high affinity anti-drug antibodies. Antibodies are generated as a result of T cell-dependent B cell activation in germinal centers (GC) of secondary lymphoid organs where antigen-specific GC B cells, aided by T follicular helper (T_{FH}) cells, differentiate into long-lived antibody-secreting B cells (ASC) and switched immunoglobulin (swIg) memory B cells. The formation of germinal centers is a complex process modulated by
20 interleukins, co-stimulatory molecules, and by downstream signaling pathways in antigen-specific B and T cells.

Pre-clinical and clinical efficacy of vaccines for substance use disorders is achieved in the subset of immunized subjects (~30%) showing high levels of anti-drug antibodies that block drug distribution to the brain and rewarding effects. Drug-based haptens conjugated to a carrier
25 polypeptide can stimulate T cell-dependent B cell differentiation in germinal centers (GC). Post-immunization anti-drug antibody levels, anti-drug antibody efficacy in limiting drug distribution in the brain, and anti-drug antibody efficacy reducing drug-induced behavior in mice increase with an increased frequency of naïve and early-activated hapten-specific B cells, increased frequency of carrier-specific $CD4^+$ T cells, and the magnitude of GC activation. After
30 immunization, commitment of naïve B cells and T cells to germinal center formation, or GC-independent pathways, is regulated by cytokines and co-stimulatory molecules. Interleukin (IL)

receptors activate JAK/STAT signaling, while specific STAT proteins activate transcription programs leading to B cell and T cell differentiation in germinal center B cells and T_{FH} cells. The effects of interleukins on expression of STAT proteins, and activity of bcl-6 or blimp-1, have been characterized in T_{FH} cells. In contrast, less is known of the role of these pathways in germinal center B cells and their contribution to antibody generation. For instance, IL-4 and IL-21 promote germinal center formation, isotype class switching, and affinity maturation. In contrast, IL-2 and IL-7 inhibit germinal center formation by controlling CD4⁺ T cell differentiation, and limiting formation of T_{FH} cells. IL-2 receptor (IL-2R) activates STAT5, which activates blimp-1, resulting in formation of short-lived antibody-secreting B cells and Th₁ cell-based immunity.

This disclosure describes technology that involves programming T cell-dependent B cell differentiation toward GC formation by modulating cytokine signaling to increase the efficacy of vaccines for substance use disorders. Moreover, the combination of conjugate vaccines with cytokine signaling modulators had a synergistic effect (i.e., vaccine plus immunomodulator was better than vaccine alone), and this result was unexpected. Immunomodulators (e.g., IL-2, IL-2:anti-IL-2 mAb complexes, or IL-4:anti-IL-4 mAb complexes) have been previously used alone (e.g., IL-2 cancer therapy) or combined with immunogens and shown increased cellular immunity (e.g., CD8⁺ T cells) or innate immunity (e.g., macrophages). In contrast, this disclosure describes combining such immunomodulators with vaccines, thereby improving the humoral response (e.g., antigen-specific CD4⁺ T_{FH} cells and B cells, antigen-specific antibodies). Hence, the technology described herein is effective to reprogram the adaptive immune response toward an increased humoral response against the target drug.

For example, a model vaccine against the highly abused prescription opioid oxycodone was combined with IL-4, IL-6, IL-21, anti-IL-4 mAb, or an IL-4:anti-IL-4 mAb complex to promote germinal center formation, or with a mAb against the IL-2 receptor alpha chain (IL-2 α R or CD25) to block IL-2-mediated inhibition of germinal center formation. The IL-4:anti-IL-4 mAb complexes and the anti-IL-4 mAb enhanced post-immunization anti-oxycodone polyclonal antibody levels, vaccine efficacy in blocking oxycodone distribution to the brain, and the percentage of subjects showing vaccine efficacy. Specifically, blocking IL-4 with an anti-IL-4 mAb increased vaccine efficacy by inducing IgG_{2a} and IgG₃ antibody subclasses, suggesting that IgG_{2a}-mediated effector functions and/or IgG₃-mediated effector functions, such as macrophage

active removal of the target antigen, are involved. Manipulating IL-4 signaling also elicited greater post-immunization hapten-specific GC B cells and carrier-specific T_{fh} cells, but did not alter GC-independent B and T cell differentiation. For instance, blocking IL-4 with an anti-IL-4 mAb increased GC formation in response to a peptide-protein conjugate immunogen, suggesting that this approach may be extended to other vaccines. In fact, co-administration of an anti-IL-4 mAb with a tetanus-diphtheria-pertussis vaccine increased tetanus-specific antibodies, enhanced the tetanus-specific IgG_{2a} response, and resulted in greater efficacy against diphtheria. These data were supported by evidence that the immunogenicity and the efficacy of an oxycodone vaccine was enhanced in IL-4 deficient (IL-4^{-/-}) mice compared to wild-type controls. The effect of IL-4 modulation on post-immunization responses may be mediated by specific members of the STAT family and/or the master transcription factors bcl-6 and blimp-1 in antigen-specific B and T cells. These data elucidate a novel role for the anti-IL-4 mAb and IL-4:anti-IL-4 mAb complexes in programming GC-dependent T cell-dependent B cell differentiation for achieving optimal post-vaccination antibody responses and increase the subset of immunized subjects achieving clinical efficacy. Additionally, these data suggested that combining a vaccine with interleukins, interleukin:mAb complexes, anti-interleukin mAb, or anti-interleukin receptor mAb is a novel application.

Moreover, use of anti-interleukin mAb and/or anti-interleukin receptor mAb can improve vaccines that require high levels of antibody levels against pathogens, cancer, or agents of biological or chemical warfare (e.g., small molecule toxins).

Individual responses to vaccines for substance use disorder depended, at least in part, on the frequency of naïve and early-activated vaccine-specific B and T cells before and shortly after immunization. Effective individual responses correlated the magnitude of GC activation over GC-independent pathways. These data suggest that vaccines for substance use disorder can improve efficacy by incorporating immunization strategies that skew the T cell-dependent B cell differentiation toward GC formation.

This disclosure shows that interleukin signaling can be targeted to reprogram germinal center formation for enhancing efficacy of vaccines for substance use disorder. A model anti-drug vaccine (6OXY-KLH vaccine) was co-administered with IL-4, IL-6, IL-21, anti-IL-4 mAb, IL-4:anti-IL-4 mAb complexes, an anti-IL-2 α R mAb (anti-CD25), or an anti-IL-7 α R mAb (anti-CD127, not shown). FIG. 8, 9, and 11 show data indicating that combining a vaccine with an

anti-IL-4 mAb increases the production of antigen-specific IgG_{2a} and IgG₃. This approach also can be used in research setting in immunization campaigns aiming at isolation of IgG_{2a} vs. IgG₁ monoclonal antibodies. In some cases, an anti-IL-4 mAb could be administered in combination with the target vaccine to a mouse, rat, horse, llama, or other laboratory or farm animal used to produce and isolate either polyclonal antibodies (e.g., anti-venom polyclonal serum in horses), or monoclonal antibodies (e.g., single domain mAb in llamas).

Thus, this disclosure describes a method for treating drug use in a subject. The method generally includes co-administering to the subject a composition of at least two components (e.g., vaccine and a cytokine signaling immunomodulator, either with or without one or more modulators). The first component includes a vaccine that includes a derivative of the drug conjugated to a carrier polypeptide so that the conjugate is effective to induce production of antibody that specifically binds to the drug. In many cases, a small molecule drug, by itself, may be of insufficient size to induce antibody production in a subject. Conjugating the drug, or a derivative thereof, to a carrier polypeptide can induce antibody production directed against the drug moiety of the conjugate. The second component includes a cytokine signaling immunomodulator effective to increase production of the antibody.

As used herein, a “cytokine signaling immunomodulator” refers to a compound that directly induces or inhibits cytokine signaling in contrast to, for example, an adjuvant (e.g., alum, CpG, or imidoazoquinoline amines) that affect cytokine signaling only indirectly through another signaling pathway. A “cytokine signaling immunomodulator” can be a biological compound, such as, for example, interleukin, an antibody against an interleukin, an antibody against an interleukin receptor, an interleukin/monoclonal antibody complex, or a peptide ligand for an interleukin receptor. In other cases, a “cytokine signaling immunomodulator” can be a small molecule (e.g., STAT or mTOR ligand) that directly induces or inhibits cytokine signaling. For instance, the anti-IL-4 mAb inhibits exogenous or endogenous IL-4 from binding to the IL-4 receptor. In certain embodiments, a “cytokine signaling immunomodulator” can include a compound that targets the anti-IL-4 receptor (e.g., a peptide, antibody, antibody fragment, or small molecule ligand).

In another aspect, this disclosure describes a method for treating an infectious disease in a subject. Generally, the method involves co-administering a cytokine signaling immunomodulator and a vaccine that includes an immunogen effective for treating the infectious disease.

In another aspect, this disclosure describes a method for generating selected antibody responses in a research animal or farm animal. Generally, an anti-IL-4 mAb is administered in combination with a target vaccine to a mouse, rat, horse, llama, or other animal used to produce and isolate either polyclonal antibodies (e.g., anti-venom polyclonal serum in horses), or
5 monoclonal antibodies (e.g., single domain mAb in llamas) of a specific subclass.

As used herein, the term “treat” or variations thereof refer to reducing, limiting progression, ameliorating, or resolving, to any extent, the symptoms or signs related to a condition. “Symptom” refers to any subjective evidence of disease or of a patient’s condition. “Sign” or “clinical sign” refers to an objective physical finding relating to a particular condition
10 capable of being found by one other than the patient.

Also as used herein, the term “co-administered” refers to two or more components of a combination administered so that the therapeutic effects of the combination can be greater than the therapeutic effects of either component administered alone. Two components may be co-administered simultaneously or sequentially. Simultaneously co-administered components may
15 be provided in one or more pharmaceutical compositions. Sequential co-administration of two or more components includes cases in which the components are administered so that each component can be present at the treatment site at the same time. Alternatively, sequential co-administration of two components can include cases in which at least one component has been cleared from a treatment site, but at least one cellular effect of administering the component
20 (e.g., cytokine production, activation of a certain cell population, etc.) persists at the treatment site until one or more additional components are administered to the treatment site. Thus, a co-administered combination can, in certain circumstances, include components that never exist in a chemical mixture with one another. For example, in one exemplary embodiment, co-administering can refer to co-administering the two components in, for example, the same
25 syringe (e.g., OXY-KLH+IL-4) or co-administering to the subject by different routes of administration (e.g., OXY-KLH given intramuscularly plus anti-IL-4 mAb given intraperitoneally or intravenously).

As used herein, the term “antibody” refers to immunoglobulin that specifically binds to an antigen. “Antibody,” used without an article, is used collectively to refer to immunoglobulin
30 encompassing a monoclonal antibody preparation (i.e., having a single molecular species of immunoglobulin) and/or a polyclonal antibody preparation (i.e., having more than one molecular

species of immunoglobulin). As used herein, the term “specific” and variations thereof refer to having a differential or a non-general affinity, to any degree, for a particular target.

In some embodiments, the immunomodulator can include a complex formed between IL-4 and an antibody that specifically binds to IL-4. While the complex can form naturally through an affinity interaction between the antibody and IL-4, the immunomodulator can involve IL-4 and the antibody being complexed through an affinity interaction or through one or more covalent bonds. FIG. 5-7 show data indicating that administering such a composition (e.g., vaccine plus immunomodulator) to a subject increases serum IgG antibodies that specifically bind the free drug in serum and decreases the amount of drug that crosses the blood-brain barrier.

In some embodiments, the immunomodulator can include anti-IL-4 mAb. FIG. 5-7 show data indicating that administering such composition to a subject increases the serum IgG response that decreases the amount of drug reaching the brain. FIG. 7 shows data indicating that administering such a composition to a subject reduced opioid-induced toxicity including respiratory depression and bradycardia. FIG. 7 also shows data indicating that administering such composition to a subject does not interfere with pharmacological reversal of opioid-induced toxicity by the opioid antagonist naltrexone. Thus, the vaccine blocks opioid-induced toxicity that may contribute to overdose. Moreover, the combination of vaccine plus anti-IL-4 mAb enhanced vaccine efficacy against toxicity from oxycodone. FIG. 8 shows data indicating that administering such a composition to a subject increases the quantity and quality of the serum IgG response by activating various IgG subclasses, and that such a composition may activate antibody-dependent effector functions, such as phagocytosis, which removes additional free drug and thus increases vaccine efficacy. FIG. 9 shows data indicating that genetic depletion of IL-4 increases vaccine efficacy against oxycodone providing additional mechanistic evidence for this approach.

In some embodiments, the immunomodulator can include an antibody that specifically binds to IL-2 receptor α chain (IL-2 α R, also known as CD25). While described herein in the context of exemplary embodiments in which the immunomodulator includes IL-4, IL-6, IL-21, mAb anti-IL-4, and mAb anti-IL-2 α R, the immunomodulator component can include other immunomodulators such as, for example, interleukin, a small molecule that modulates interleukin signaling (e.g., JAK/STAT ligand), a mAb that specifically binds an interleukin, and/or a mAb that specifically binds an interleukin receptor (e.g., mAb anti-IL-7 α R).

While described herein in the context of exemplary embodiments in which the immunotherapy is directed against a small molecule drug for treating drug use, the immunomodulatory component may be co-administered with existing vaccines directed against, for example, one or more antigens associated with an infectious disease or other small molecules or peptides associated with non-communicable diseases (e.g., cancer, Alzheimer's disease, or cardiovascular disease). FIG. 10A shows data indicating that treatment with an anti-IL-4 mAb does not impair GC formation in response to small molecule haptens, suggesting that hapten-specific B cell responses may be partially IL-4 independent. In contrast, FIG. 10B-E show that treatment with anti-IL-4 mAb, administered with a peptide-protein conjugate immunogen, increases peptide-specific CD4⁺ T cell differentiation into peptide-specific GC-T_{fh} cells, a T cell subset responsible for B cell help. FIG. 11 shows data indicating that an anti-IL-4 monoclonal antibody, administered with a tetanus diphtheria acellular pertussis (TDaP) vaccine increases serum IgG titer compared to administering the vaccine alone, and that inhibiting IL-4 increases IgG_{2a} subclass responses, which increased antibody production against diphtheria. The same approach could be used to increase the post-immunization antibody response against a small antigen (e.g., a carbohydrate, a small molecule, or a peptide) or purified subunits associated with other infectious diseases or non-communicable chronic diseases. Pre-clinical toxicology data (Table 1) and histopathology data (FIG. 12) supporting the safety for this approach.

Table 1

Treatment	Total protein (g/dl)	Total bilirubin (mg/dl)	ALT (U/L)	AST (U/L)	Urea nitrogen (mg/dl)	Cholesterol (mg/dl)	Body weight (g)
KLH	5.7 ± 0.32	0.2 ± 0.10	71.6 ± 3.2	626 ± 24.7	20 ± 1.5	161 ± 10.4	26.5 ± 0.39
OXY-KLH	5.4 ± 0.17	0.3 ± 0.11	74 ± 4.7	709 ± 51.6	20 ± 1.0	154 ± 11.0	27.4 ± 0.75
Anti-IL-4 mAb	5.8 ± 0.06	0.46 ± 0.03	104 ± 9.5	605 ± 253	27 ± 2.8	163 ± 0.3	27.7 ± 0.72
OXY-KLH + Anti-IL-4 mAb	5.5 ± 0.26	0.27 ± 0.09	101 ± 32.3	840 ± 286	24 ± 1.0	158 ± 13.0	27.1 ± 0.24

Data are expressed as mean ± SEM.

Serum biochemistry parameters in BALB/C mice treated with KLH, OXY-KLH, anti-IL-4 mAb, or anti-IL-4 mAb plus OXY-KLH. Protein, bilirubin, ALT (alanine aminotransferase), urea nitrogen, and total cholesterol were in the normal range. The values for AST (aspartate aminotransferase) were higher among groups, perhaps as a result of muscle injury due from the injections.

While described herein in the context of an exemplary embodiment using recombinant mouse interleukins with rat anti-mouse anti-interleukin mAb to enhance vaccine efficacy in mice, the methods described herein can be practiced using any suitable combination of interleukin from any source and any anti-interleukin antibody from any source. Thus, exemplary
5 alternative embodiments include the use of, for example, recombinant human interleukins and an anti-human anti-interleukin mAb of any suitable origin (e.g., a mouse anti-human mAb, a llama anti-human mAb, a chimeric mAb, or a humanized mAb). In this context, the mAb can include the full monoclonal antibody or a fragment thereof such as, for example, a single domain antibody fragment.

10 The compositions described herein may be formulated with a pharmaceutically acceptable carrier. As used herein, a “pharmaceutically acceptable carrier” includes any solvent, dispersion medium, vehicle, coating, diluent, antibacterial, and/or antifungal agent, isotonic agent, absorption delaying agent, buffer, carrier solution, suspension, colloid, polymers, nanoparticles, liposomes, and the like. The use of such media and/or agents for pharmaceutical
15 active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions. As used herein, “pharmaceutically acceptable” refers to a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with an active agent
20 without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

The vaccine and the immunomodulator may therefore be formulated into a pharmaceutical composition or, as described above, multiple pharmaceutical compositions. A pharmaceutical composition may be formulated in a variety of forms adapted to a preferred route
25 of administration. Thus, a composition can be administered via known routes including, for example, oral, parenteral (e.g., intradermal, transcutaneous, subcutaneous, intramuscular, intravenous, intraperitoneal, etc.), or topical (e.g., intranasal, intrapulmonary, intramammary, intravaginal, intrauterine, intradermal, transcutaneous, rectally, etc.). A pharmaceutical composition can be administered to a mucosal surface, such as by administration to, for example,
30 the nasal or respiratory mucosa (e.g., by spray, aerosol, or gel). A composition also can be administered via a sustained or delayed release. In embodiments in which the vaccine and the

immunomodulator are formulated into separate formulations, each component may be administered by a route independent of the other component.

Thus, vaccine and/or immunomodulator may be provided in any suitable form including but not limited to a solution, a suspension, an emulsion, a spray, an aerosol, or any form of mixture. The composition may be delivered in formulation with any pharmaceutically acceptable excipient, carrier, or vehicle. A formulation may be conveniently presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. Methods of preparing a composition with a pharmaceutically acceptable carrier include the step of bringing the active agent—i.e., the vaccine and/or immunomodulator—into association with a pharmaceutically acceptable carrier that constitutes one or more accessory ingredients. In some embodiments, for example, a formulation may be prepared by uniformly and/or intimately bringing the active compound into association with a liquid pharmaceutically acceptable carrier, a finely divided solid pharmaceutically acceptable carrier, or both, and then, if necessary, shaping the product into the desired formulations.

The amount of vaccine and immunomodulator that is administered can vary depending on various factors including, but not limited to, the specific vaccine being administered, the specific immunomodulator being administered, the weight, physical condition, and/or age of the subject, and/or the route of administration. Thus, the absolute weight of active agents included in a given unit dosage form can vary widely, and depends upon factors such as the species, age, weight and physical condition of the subject, and/or the method of administration. Accordingly, it is not practical to set forth generally the amount that constitutes an amount of active agents effective for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

For example, certain vaccines and/or certain immunomodulators may be administered at the same dose and frequency for which the vaccine and/or immunomodulator has received regulatory approval. In other cases, certain active agents may be administered at the same dose and frequency at which the drug is being evaluated in clinical or preclinical studies. One can alter the dosages and/or frequency as needed to achieve a desired level of antibody production. Thus, one can use standard/known dosing regimens and/or customize dosing as needed.

In some embodiments, the method can include administering sufficient immunomodulator to provide a dose of, for example, from about 100 ng/kg to about 50 mg/kg to

the subject, although in some embodiments the methods may be performed by administering the immunomodulator in a dose outside this range. In some of these embodiments, the method includes administering sufficient immunomodulator to provide a dose of from about 10 µg/kg to about 5 mg/kg to the subject, for example, a dose of from about 100 µg/kg to about 1 mg/kg. In preliminary studies using a mouse model, the use of mAb doses within or below the human range (0.1-10 mg/kg) supported the efficacy and cost-effectiveness of this approach.

In some embodiments, the vaccine/immunomodulator composition may be administered, for example, from a single dose to multiple doses per week, although in some embodiments the method can be performed by administering the vaccine/immunomodulator composition at a frequency outside this range. In certain embodiments, the vaccine/immunomodulator composition may be administered at least once per month. In clinical trials of nicotine and cocaine vaccines, immunogens were administered once a month for 5-6 months followed by boosts at 3-6 months.

In the preceding description and following claims, the term “and/or” means one or all of the listed elements or a combination of any two or more of the listed elements; the terms “comprises,” “comprising,” and variations thereof are to be construed as open ended—i.e., additional elements or steps are optional and may or may not be present; unless otherwise specified, “a,” “an,” “the,” and “at least one” are used interchangeably and mean one or more than one; and the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

In the preceding description, particular embodiments may be described in isolation for clarity. Unless otherwise expressly specified that the features of a particular embodiment are incompatible with the features of another embodiment, certain embodiments can include a combination of compatible features described herein in connection with one or more embodiments.

For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

EXAMPLES

Example 1

IL-4 increased vaccine-specific germinal center (GC) B cells and the fraction of subjects that showed post-vaccination GC formation. Mice were immunized subcutaneously once with OXY-KLH in alum adjuvant in combination with IL-4 (60,000 IU, Bio-Techne Corp., Minneapolis, MN). At seven days after immunization, lymph nodes and spleens were collected and analysis of OXY-specific B cells performed by magnetic enrichment paired with flow cytometry as previously described (Laudenbach et al., 2015. *J Immunol* 194(12):5926-5936). Results are shown in FIG. 4.

Example 2

Male BALB/c mice were immunized subcutaneously on days 0, 14, and 28, and challenged with 2.25 mg/kg oxycodone subcutaneously a week after the third immunization. Mice received unconjugated KLH, OXY-KLH, or OXY-KLH in combination with IL-4 (30,000 IU and 60,000 IU, subcutaneously), IL-4 plus IL-21 (60,000 IU total, subcutaneously), IL-4:anti-IL-4 mAb (subcutaneously, 30,000 IU and 0.5 mg of mAb mixed prior to injection), anti-IL-4 mAb (1.0 mg per mouse, intraperitoneally), anti-IL-2 α receptor mAb (anti-CD25, 1.0 mg per mouse, intraperitoneally), or anti-CD25 mAb (1.0 mg per mouse, intraperitoneally) plus IL-4 (30,000 IU, subcutaneously). The OXY-KLH and the unconjugated KLH were absorbed on alum adjuvant prior to administration. Interleukins were administered at 0, 14 and 28 in combination with each immunization. Monoclonal antibodies were administered two days prior and one day after the first immunization. Recombinant mouse IL-4 and IL-21 were obtained from Bio-Techne Corp., Minneapolis, MN. The mAb anti-IL-4 (rat anti-mouse IgG₁, clone 11B11) and mAb anti-IL-CD25 (rat anti-mouse IgG₁, clone 7D4) were obtained from Bio X Cell, West Lebanon, NH. Results are shown in FIG. 5.

Example 3

Male BALB/c mice were immunized with OXY-KLH or unconjugated KLH as control subcutaneously on days 0, 14 and 28, and challenged with oxycodone a week after the third immunization. In a first experiment, mice received KLH, OXY-KLH, OXY-KLH in combination with anti-CD25 mAb (1.0 mg, intraperitoneally), or anti-IL-4 mAb (1.0 mg, intraperitoneally) and challenged with 5.0 mg/kg oxycodone. In a subsequent experiment, mice received KLH, OXY-KLH, and OXY-KLH in combination with anti-IL-4 mAb (1.0 mg, intraperitoneally) and challenged with 10.0 mg/kg oxycodone. Reagents were obtained as EXAMPLE 2. Results are shown in FIG. 6.

Example 4

Male BALB/c mice were immunized with OXY-KLH or unconjugated KLH as control on days 0, 14 and 28, and challenged with 5.0 mg/kg oxycodone a week after the third immunization. Mice received OXY-KLH subcutaneously or intramuscularly. The anti-IL-4 mAb was administered either intraperitoneally, intravenously, or intramuscularly. Results are shown in FIG. 7A-C.

Example 5

Male BALB/c mice immunized with either KLH, OXY-KLH, or OXY-KLH plus the anti-IL-4 mAb, as in EXAMPLE 4, were tested for opioid-induced respiratory depression, heart rate, and antinociception. Oxycodone was administered subcutaneously every 15 minutes at increasing doses and the doses listed are the cumulative dose received. Naloxone 0.1 mg/kg was administered subcutaneously 15 minutes after the final oxycodone dose to reverse opioid effects. Results are shown in FIG. 7D-F.

Example 6

Male BALB/c mice from EXAMPLES 2-4 were randomly selected for IgG subclass analysis (n=5-6 mice/group). ELISA plates were coated with 5 ng/well of OVA conjugate or unconjugated protein control in carbonate buffer at pH 9.6 and blocked with 1% gelatin. Primary antibodies were incubated with goat-anti-mouse IgG1, IgG2a, or IgG3 conjugated to horseradish

peroxidase (Alpha Diagnostic International, Inc., San Antonio, TX) to measure oxycodone-specific serum IgG antibody subclass titers. Results are shown in FIG. 8.

Example 7

IL-4 deficient (IL-4^{-/-}) mice and wild-type (WT) controls (The Jackson Laboratory, Bar Harbor, ME, stock no. 002496 and 000651, respectively) were immunized subcutaneously with unconjugated KLH or OXY-KLH in alum on days 0, 14 and 28, and challenged with 5.0 mg/kg oxycodone a week after the third immunization. Analysis of OXY-specific IgG antibody titers was performed as EXAMPLE 5. Results are shown in FIG. 9.

Example 8

BALB/c mice were immunized once with OXY-KLH in alum adjuvant subcutaneously with or without the anti-IL-4 mAb (KLH, n=4, OXY-KLH, n=20, and OXY-KLH plus anti-IL-4 mAb, n=20) as described in EXAMPLE 2. Antibodies were given intraperitoneally in two separate 0.5 mg doses, three days prior to immunization and again one day after immunization. Analysis of OXY-specific B cells was performed by magnetic enrichment paired with flow cytometry as previously described (Laudenbach et al., 2015. *J Immunol* 194(12):5926-5936). Results are shown in FIG. 10A.

Example 9

C57BL/6 mice were immunized with the model immunogen pycoerythrin (PE) conjugated to streptavidin (SA) and the PE-SA immunogen conjugated with a model T cell peptide (2W). C57BL/6 mice were immunized subcutaneously with 25 µg of a model immunogen consisting of PE-SA (ProZyme, Inc., Hayward, CA, PJRS25) conjugated the 2W peptide (PE-SA^{2W}), and adsorbed on alum adjuvant prior to injection. The biotinylated peptide was conjugated to streptavidin at a mole ratio of 4.5:1 in 0.01 M PBS for 30 minutes at a final concentration of 1 mg/ml, and purified by filtration (AMICON, 100Kda cutoff, EMD Millipore Corp., Billerica, MA) and centrifugation at 4000 × g for 15 minutes at 4°C. Analysis of 2W-specific T cells was performed by tetramer-based magnetic enrichment and flow cytometry as described (Laudenbach et al., 2015. *J Immunol* 194(12):5926-5936). Antigen-specific T_{FH} and GC-T_{FH} were further characterized as described (Tubo et al., 2013. *Cell* 153(4):785-96). The

biotinylated 2W peptide and the 2Wp:MHCII tetramers were a generous gift from Dr. Marc Jenkins at the University of Minnesota Center for Immunology. Results are shown in FIG. 10A.

Example 10

5 Male BALB/c mice were immunized subcutaneously on days 0, 14 and 28 with a commercially available tetanus toxoid, diphtheria, and acellular pertussis (TDaP) vaccine (BOOSTRIX, GlaxoSmithKline plc, Isleworth, London, England). Mice received 25 μ l of TDaP diluted in sterile 0.01 M phosphate-buffered saline to a final volume of 100 μ l, with or without the anti-IL-4 mAb. For ELISA, 96-well plates were coated with 50 ng/well of tetanus toxoid or
10 unconjugated OVA control in carbonate buffer and blocked with 1% gelatin. Serum from immunized animals was incubated with goat anti-mouse IgG to detect TT-specific IgG antibodies. For IgG₁ and IgG_{2a}, serum was incubated with anti-mouse IgG₁ or IgG_{2a} antibodies (Alpha Diagnostic International, Inc., San Antonio, TX). TT-specific IgG titers were analyzed over time (FIG. 11A) or on day 28 after the first immunization (FIG. 11 B-E). Finally,
15 diphtheria-protecting serum antibodies were analyzed in vitro using a standard diphtheria toxin protection assay using Vero cells as substrate in the laboratory of Dr. Sunil A. David at the University of Minnesota, Department of Medicinal Chemistry. Results are shown in FIG. 11.

Example 11

20 Male BALB/c mice were immunized with KLH, OXY-KLH, anti-IL-4 mAb, or OXY-KLH plus anti-IL-4 mAb. The OXY-KLH and the unconjugated KLH control were administered subcutaneously on days 0, 14 and 28, while the anti-IL-4 mAb was administered intraperitoneally as in EXAMPLE 2. A week after the third immunization, mice were euthanized with CO₂ to perform histopathology and clinical pathology by serum chemistry. Organs were
25 harvested and preserved in 10% formaldehyde (methanol free, ultrapure EM grade, Polysciences, Inc., Warminster, PA), and sent to the University of Minnesota School of Veterinary Medicine for histology analysis in the laboratory of Dr. Davis Seelig. Serum was collected by centrifugation at 7500 rpm for three minutes at 4°C and collected in lithium heparin (VACUTAINER, Becton, Dickinson and Co., Franklin Lakes, NJ). Frozen samples were sent to
30 Marshfield Labs (Marshfield, WI) for the panel tests analysis.

The complete disclosure of all patents, patent applications, and publications, and electronically available material cited herein are incorporated by reference in their entirety. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

What is claimed is:

1. A method for treating a subject for abuse of a drug or toxicity from drug abuse, the method comprising co-administering to the subject:
 - a vaccine comprising a derivative of the drug conjugated to a carrier polypeptide, wherein
 - 5 the conjugate is effective to induce production of antibody that specifically binds to the drug; and
 - a cytokine-signaling immunomodulator effective to improve the subject's immune response to the vaccine compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.
- 10 2. The method of claim 1 wherein the cytokine-signaling immunomodulator comprises a complex comprising a cytokine and a monoclonal antibody that binds to the cytokine.
3. The method of claim 2 wherein the complex comprises IL-4:anti-IL-4 monoclonal antibody, IL-6:anti-IL-6 monoclonal antibody, or IL-21:anti-IL-21 monoclonal antibody.
- 15 4. The method of claim 1 wherein the cytokine-signaling immunomodulator comprises a monoclonal antibody that binds to IL-4, IL-6, IL-21, or other interleukin.
5. The method of claim 1 wherein the cytokine-signaling immunomodulator comprises an
- 20 antibody that specifically binds to the IL-7 receptor α chain (anti-CD127).
6. The method of claim 1 wherein the cytokine-signaling immunomodulator comprises an interleukin.
- 25 7. The method of claim 6 wherein the interleukin comprises IL-4, IL-6, or IL-21.
8. The method of claim 1 wherein the cytokine-signaling immunomodulator comprises a small molecule that activates cytokine signaling.
- 30 9. The method of claim 8 wherein the small molecule comprises a STAT ligand, an mTOR ligand, or ligand for other downstream signaling pathway.

10. The method of claim 9 wherein the small molecule comprises a JAK/STAT ligand.

11. The method of claim 1 wherein the cytokine-signaling immunomodulator comprises a
5 small-molecule that inhibits cytokine signaling.

12. The method of claim 11 wherein the small molecule comprises a STAT inhibitor.

13. The method of any preceding claim wherein improvement of the subject's immune response
10 to the vaccine comprises an increase in antigen-specific IgG antibody level, an increase in
antigen-specific IgG antibody concentration, or an increase in antigen-specific IgG antibody titer
compared to the subject's immune response to the vaccine without the cytokine-signaling
immunomodulator.

14. The method of any preceding claim wherein improvement of the subject's immune
15 response to the vaccine comprises increased production of IgG_{2a} antibody subclass compared to
the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

15. The method of any preceding claim wherein improvement of the subject's immune
20 response to the vaccine comprises increased vaccine efficacy compared to the subject's immune
response to the vaccine without the cytokine-signaling immunomodulator.

16. The method of any preceding claim wherein improvement of the subject's immune
25 response to the vaccine comprises an increase in the percentage of vaccine responders compared
to the subject's immune response to the vaccine without the cytokine-signaling
immunomodulator.

17. The method of any preceding claim wherein improvement of the subject's immune
30 response to the vaccine comprises at least partial reprogramming of an adaptive immune
response in response to an antigen.

18. The method of any preceding claim wherein the cytokine signaling immunomodulator comprises an inhibitor of an interleukin receptor.

19. The method of claim 18 wherein the inhibitor of an interleukin receptor is an inhibitor of
5 IL-4 receptor signaling.

20. The method of claim 18 or claim 19 wherein the inhibitor of an interleukin receptor is an anti-IL-4 antibody or a fragment thereof.

10 21. A method for treating a subject for an infectious disease or for a non-communicable disease, the method comprising co-administering to the subject:

a vaccine comprising a disease-specific immunogen, wherein the vaccine is effective to induce production of antibody that specifically binds to the immunogen; and

15 a cytokine-signaling immunomodulator effective to improve the subject's immune response to the vaccine compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

22. The method of claim 21 wherein improvement of the subject's immune response comprises an increase in antigen-specific antibody production compared to the subject's immune
20 response to the vaccine without the cytokine-signaling immunomodulator.

23. The method of claim 21 or claim 22 wherein improvement of the subject's immune response comprises a subclass shift of antibody from IgG₁ to IgG₂ compared to the subject's immune response to the vaccine without the cytokine-signaling immunomodulator.

25 24. A method for producing antibody against an antigen, the method comprising:
administering to an animal a composition comprising:

an antigen in an amount to induce production of antibody that specifically binds the antigen; and

a cytokine-signaling immunomodulator effective to improve the animal's immune response to the antigen compared to the animal's immune response to the antigen without the cytokine-signaling immunomodulator.

5 25. The method of claim 24 wherein improvement of the animal's immune response comprises an increase in antigen-specific antibody production compared to the animal's immune response to the antigen without the cytokine-signaling immunomodulator.

10 26. The method of claim 24 or claim 25 wherein improvement of the animal's immune response comprises a subclass shift of antibody from IgG₁ to IgG₂ compared to the animal's immune response to the antigen without the cytokine-signaling immunomodulator.

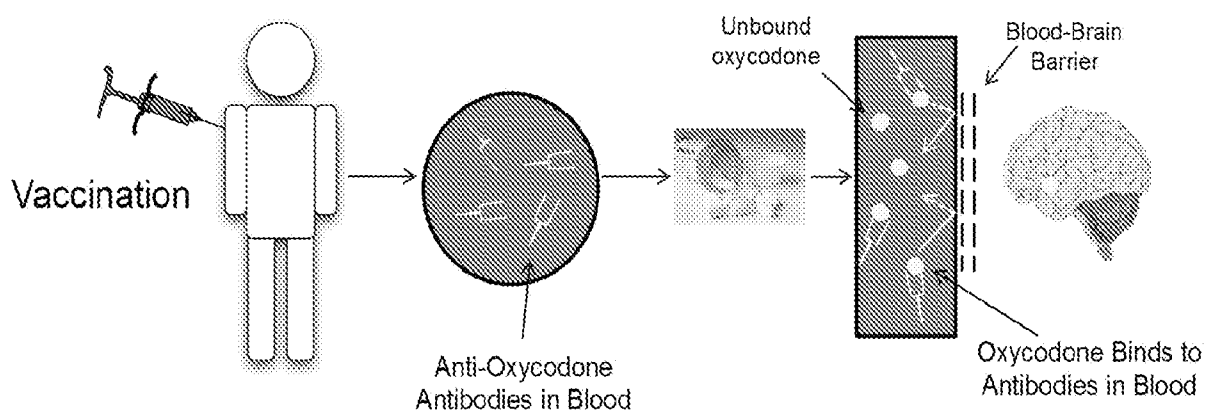
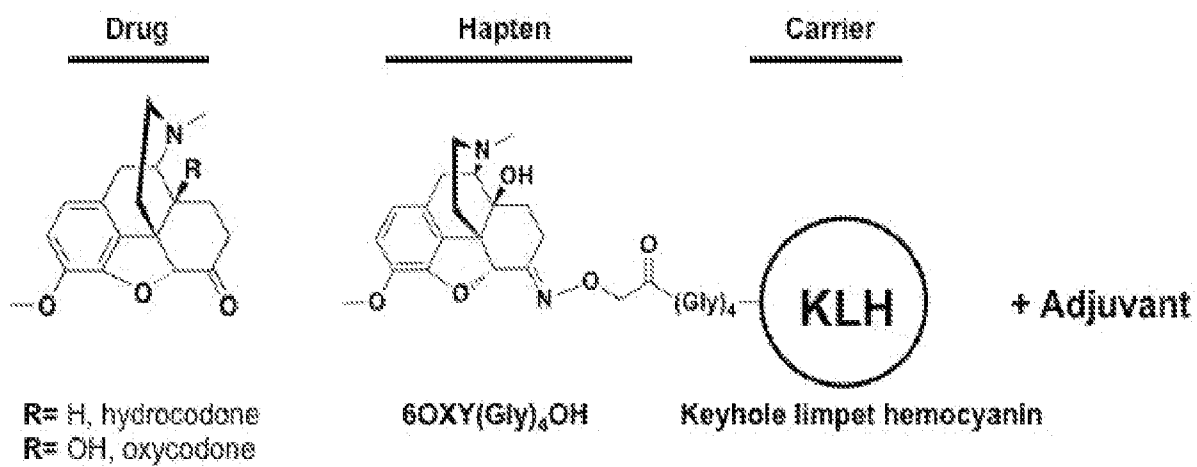
FIG. 1*FIG. 2*

FIG. 3

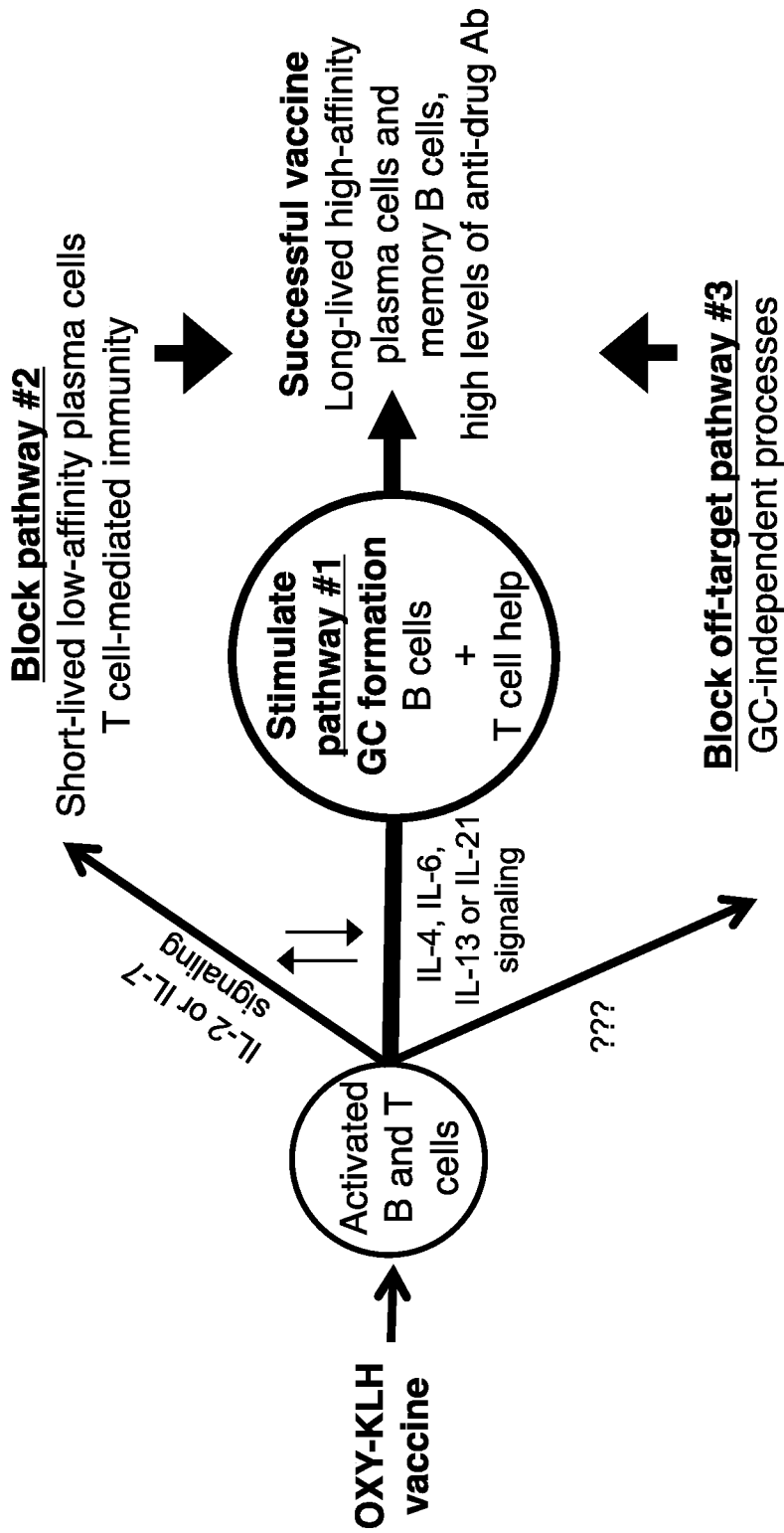


FIG. 4

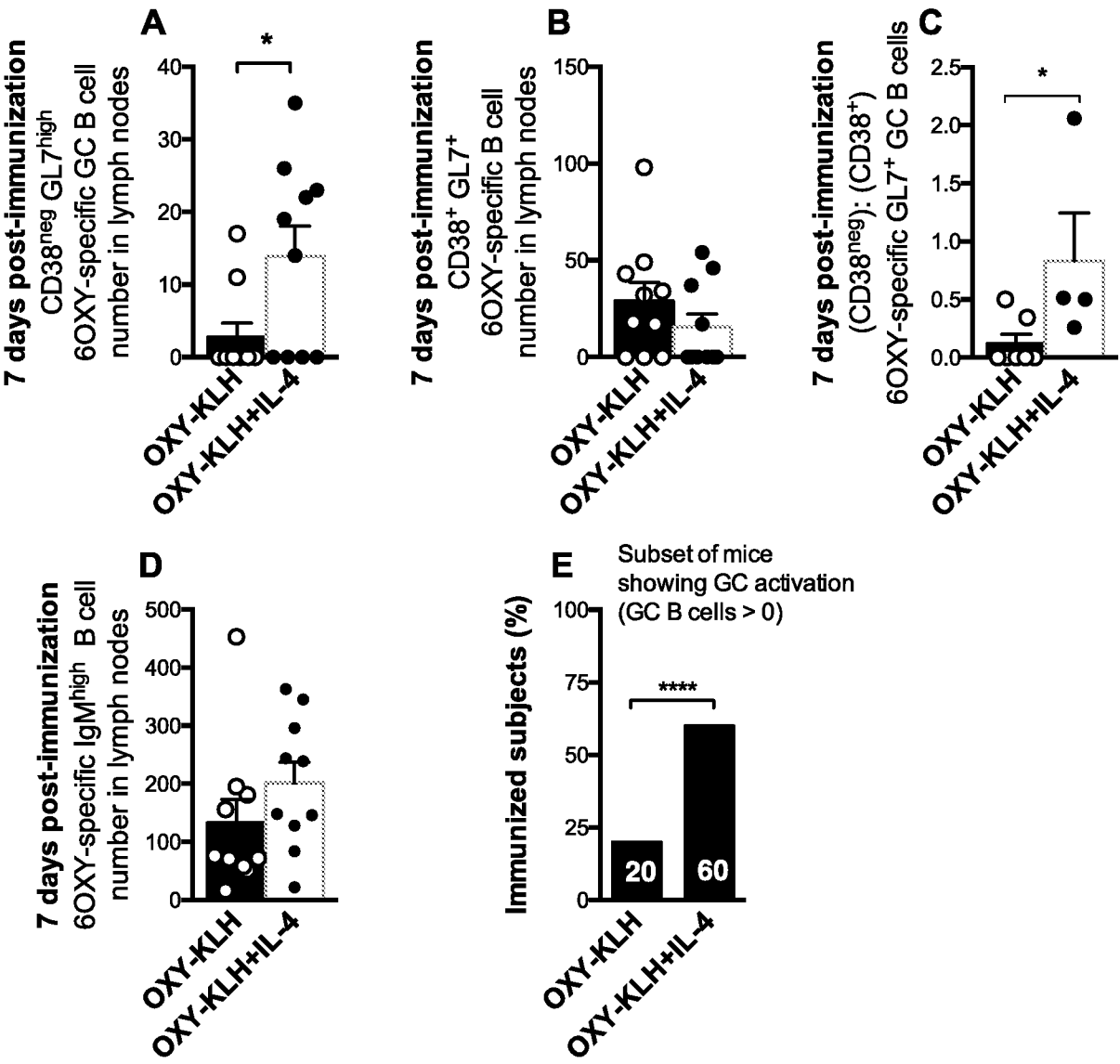


FIG. 5

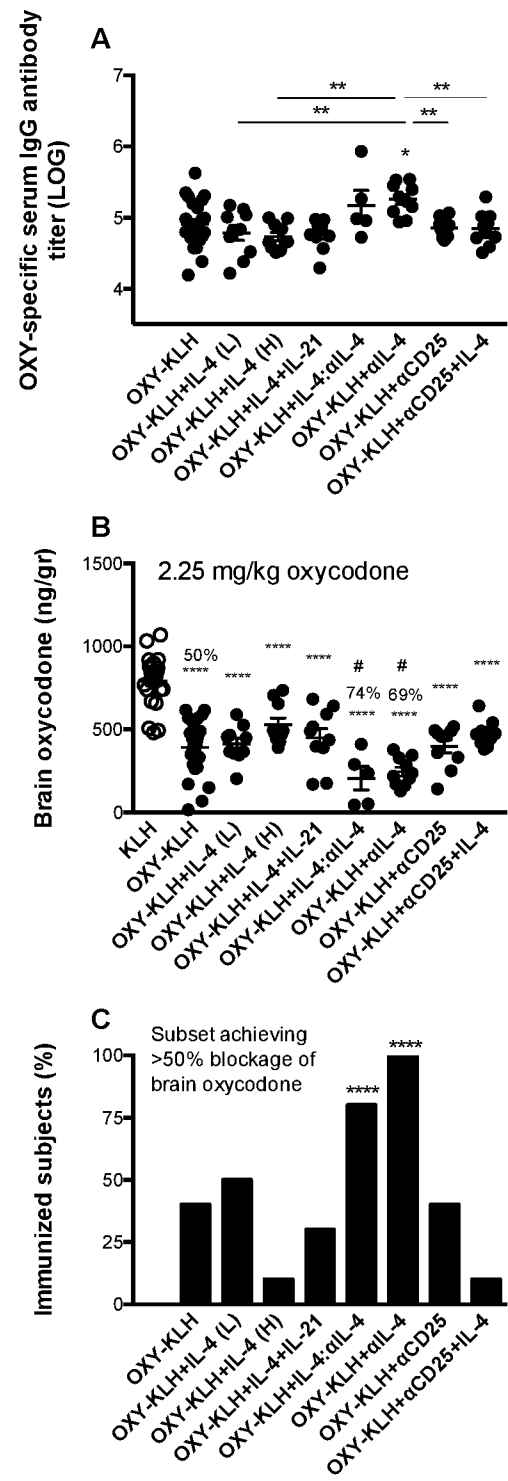


FIG. 6

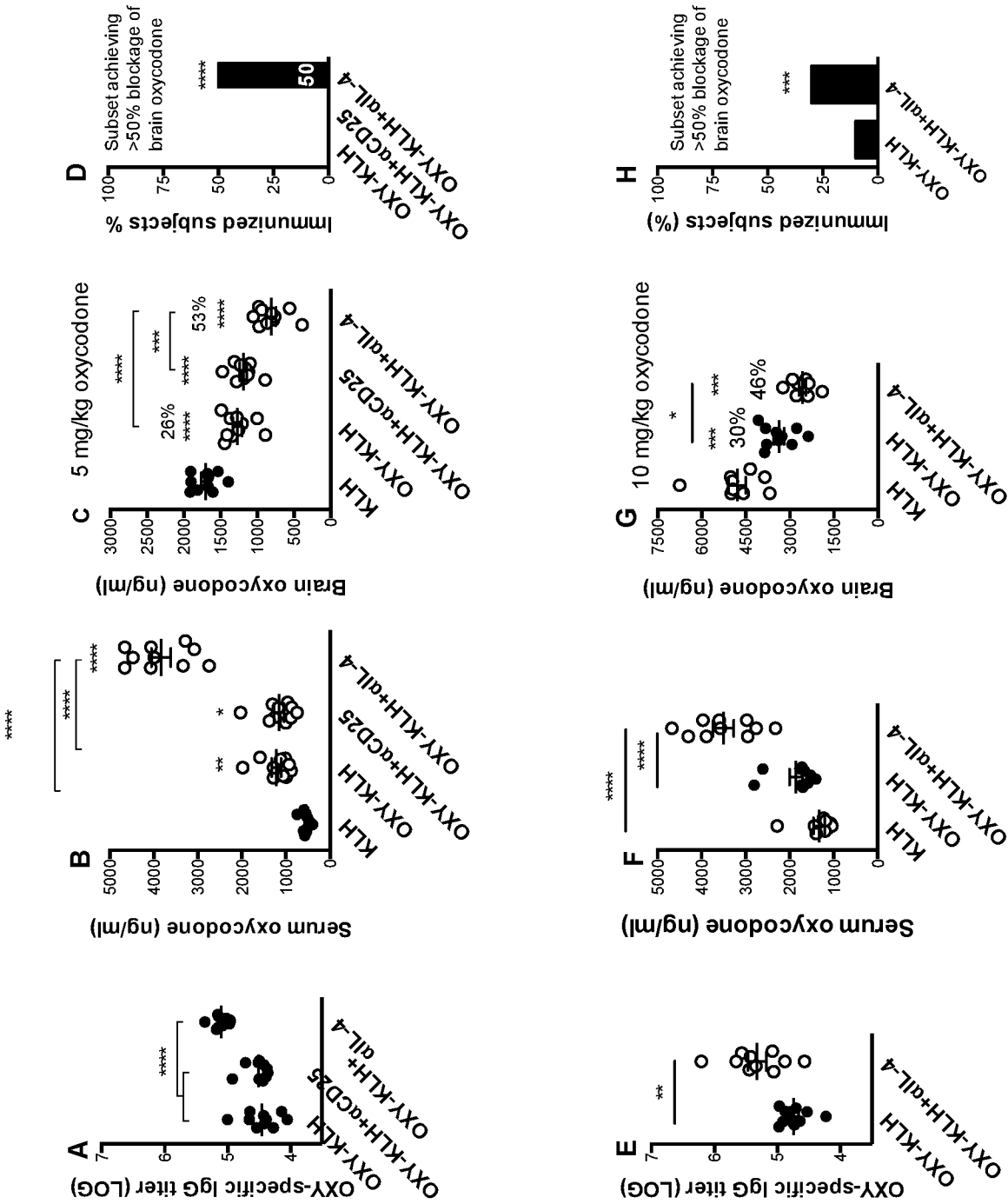


FIG. 7

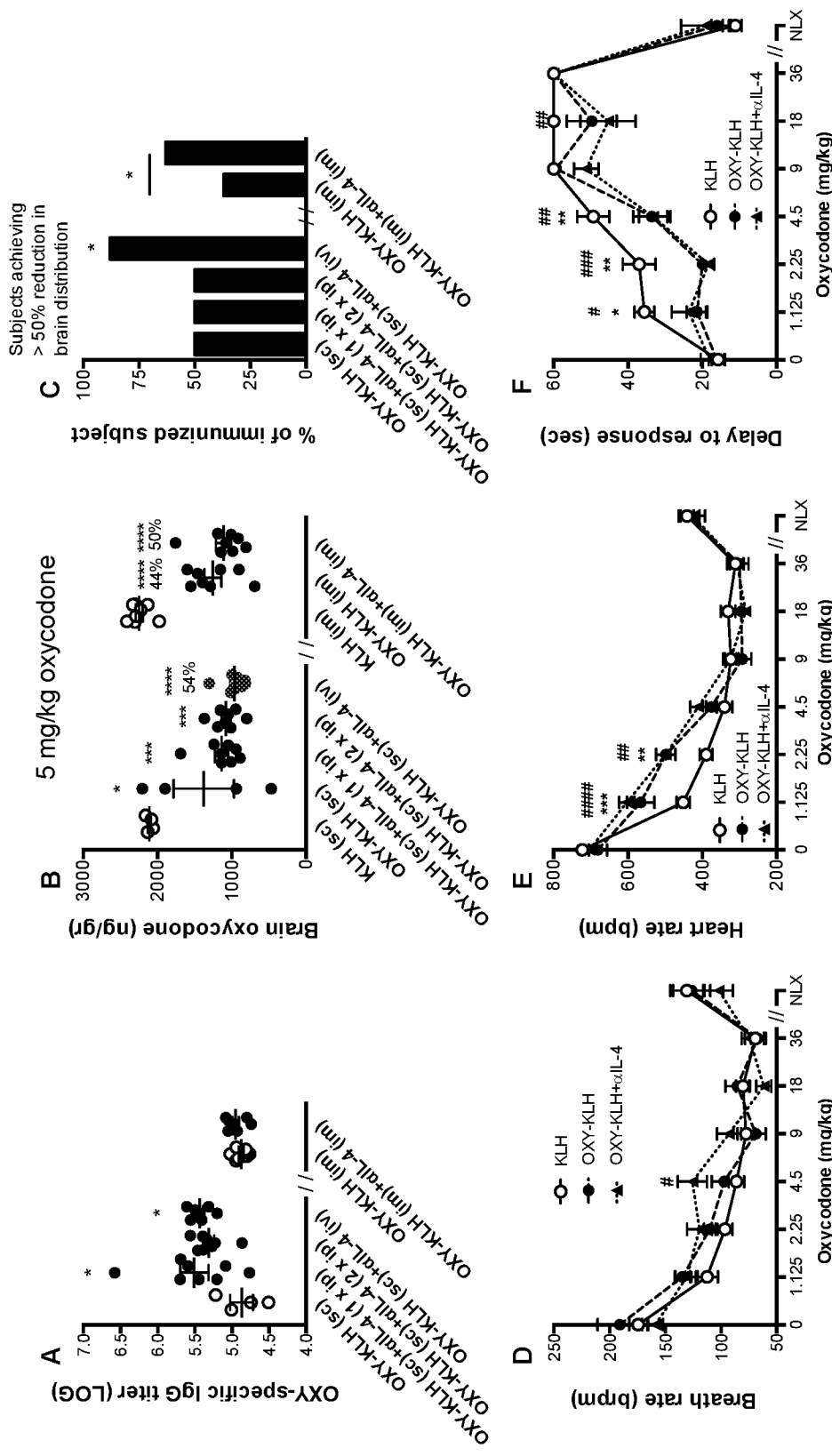


Figure 2 consists of seven panels (A-G) showing the results of immunization with OX42-KLH and OX43-KLH, and the effect of clodronate on brain oxytocin levels.

- Panel A:** Scatter plot of OX42-specific IgG titer (LOG) for OX42-KLH (sc), OX42-KLH (ip), OX42-KLH (im)+adL-4 (ip), and OX42-KLH (im)+adL-4 (im). The y-axis ranges from 3 to 7. The x-axis labels are OX42-KLH (sc), OX42-KLH (ip), OX42-KLH (im)+adL-4 (ip), and OX42-KLH (im)+adL-4 (im). A significant difference (****) is shown between the first two groups.
- Panel B:** Scatter plot of OX42-specific IgG₁ titer (LOG) for the same groups as in A. The y-axis ranges from 3 to 7. A significant difference (****) is shown between the first two groups.
- Panel C:** Scatter plot of OX42-specific IgG_{2a} titer (LOG) for the same groups as in A. The y-axis ranges from 2 to 7. Significant differences (**** and **) are shown between the first two groups.
- Panel D:** Scatter plot of OX43-specific IgG₃ titer (LOG) for the same groups as in A. The y-axis ranges from 2 to 7. A significant difference (**) is shown between the first two groups.
- Panel E:** Scatter plot of OX42-specific Ig titer (LOG) for the same groups as in A. The y-axis ranges from 3 to 7. A significant difference (****) is shown between the first two groups.
- Panel F:** Scatter plot of the ratio of OX42-specific Ig titer to (IgG_{2a}+IgG₃)/2 for the same groups as in A. The y-axis ranges from 0 to 2500. A significant difference (*) is shown between the first two groups.
- Panel G:** Bar graph showing the percentage of immunized subjects achieving >50% blockage of brain oxytocin for OX42-KLH and OX42-KLH+clodronate groups. The y-axis ranges from 0 to 100. The x-axis labels are OX42-KLH and OX42-KLH+clodronate. A significant difference (**) is shown between the two groups.

FIG. 9

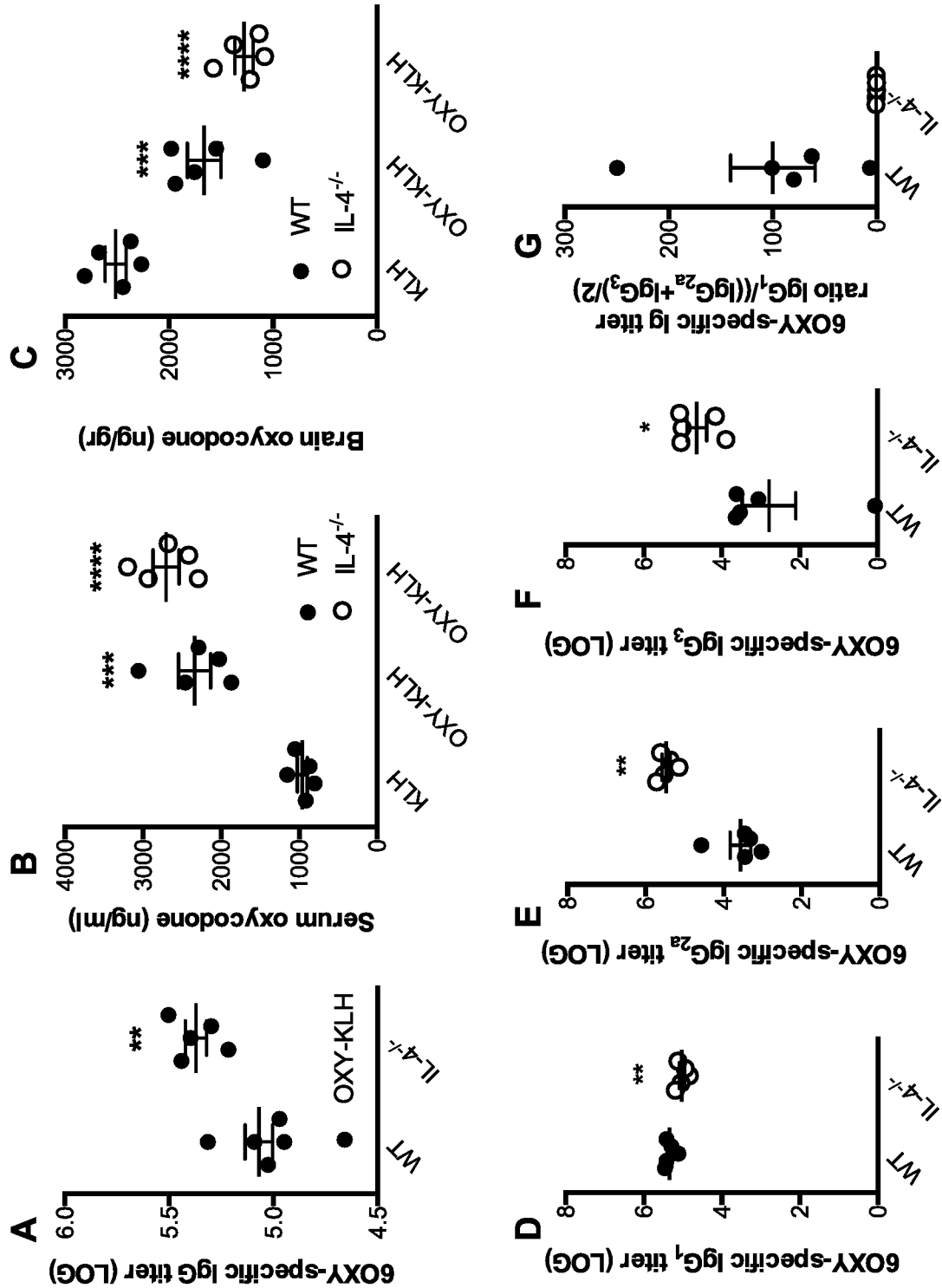


FIG. 10

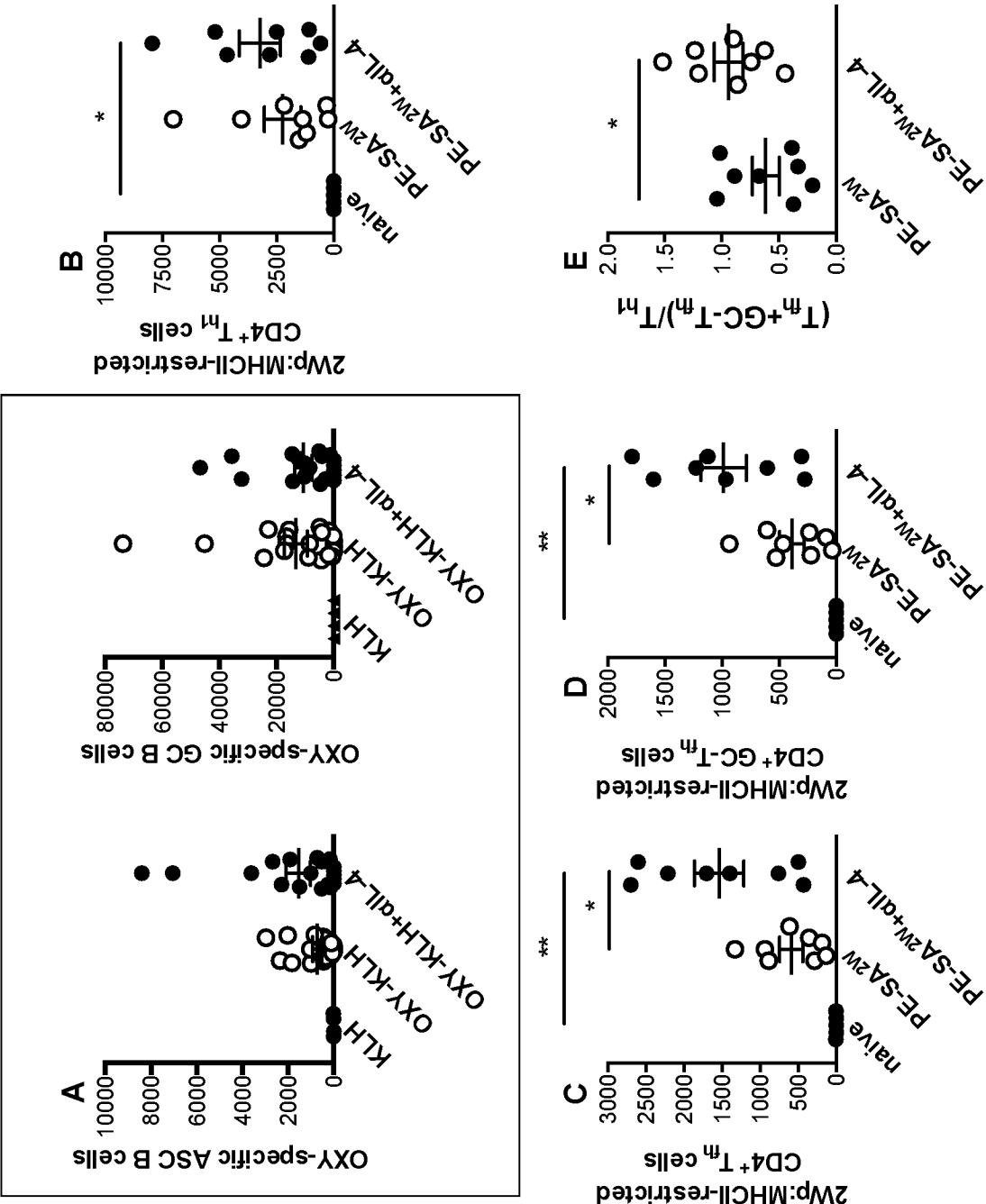


FIG. 11

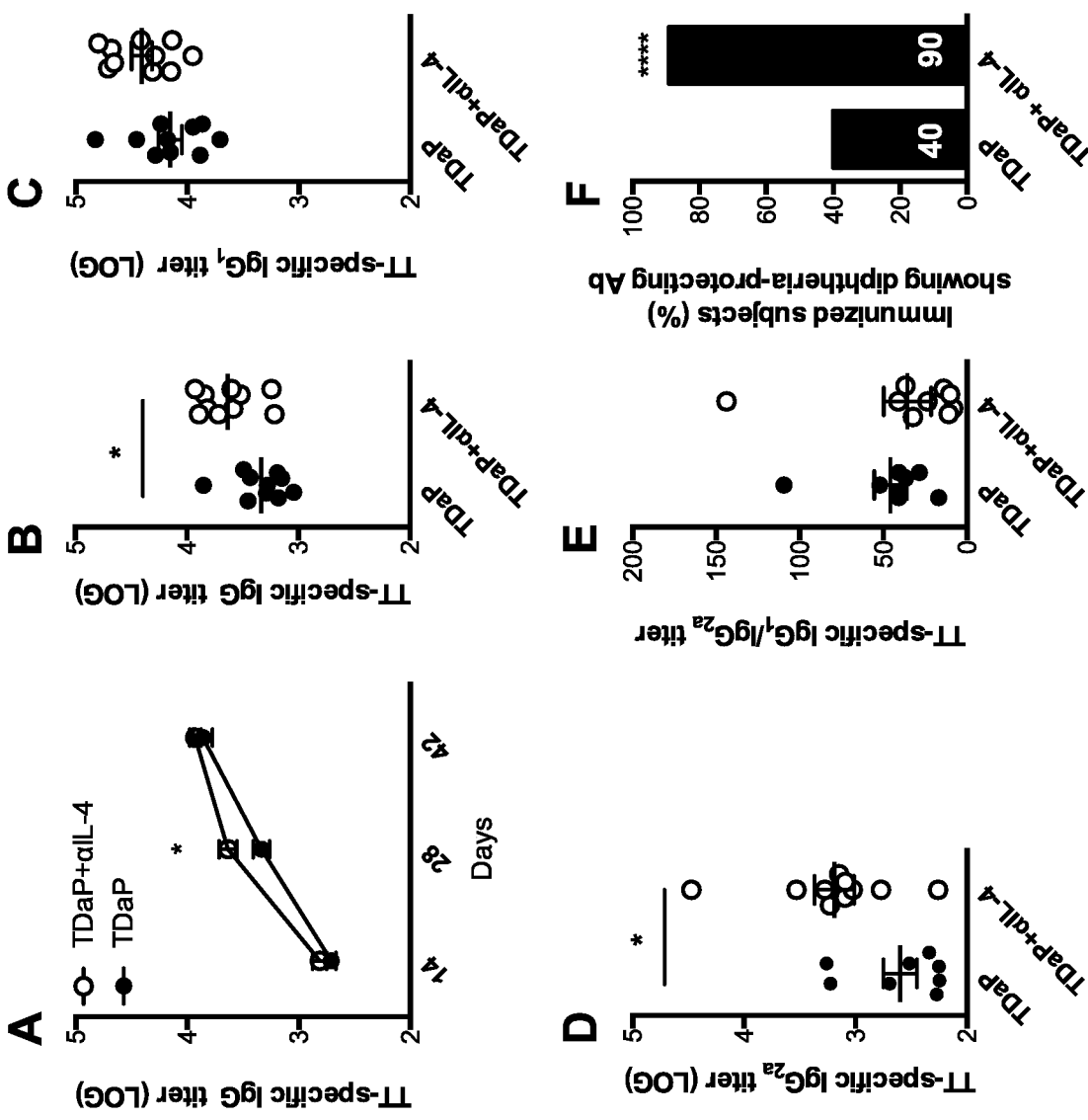
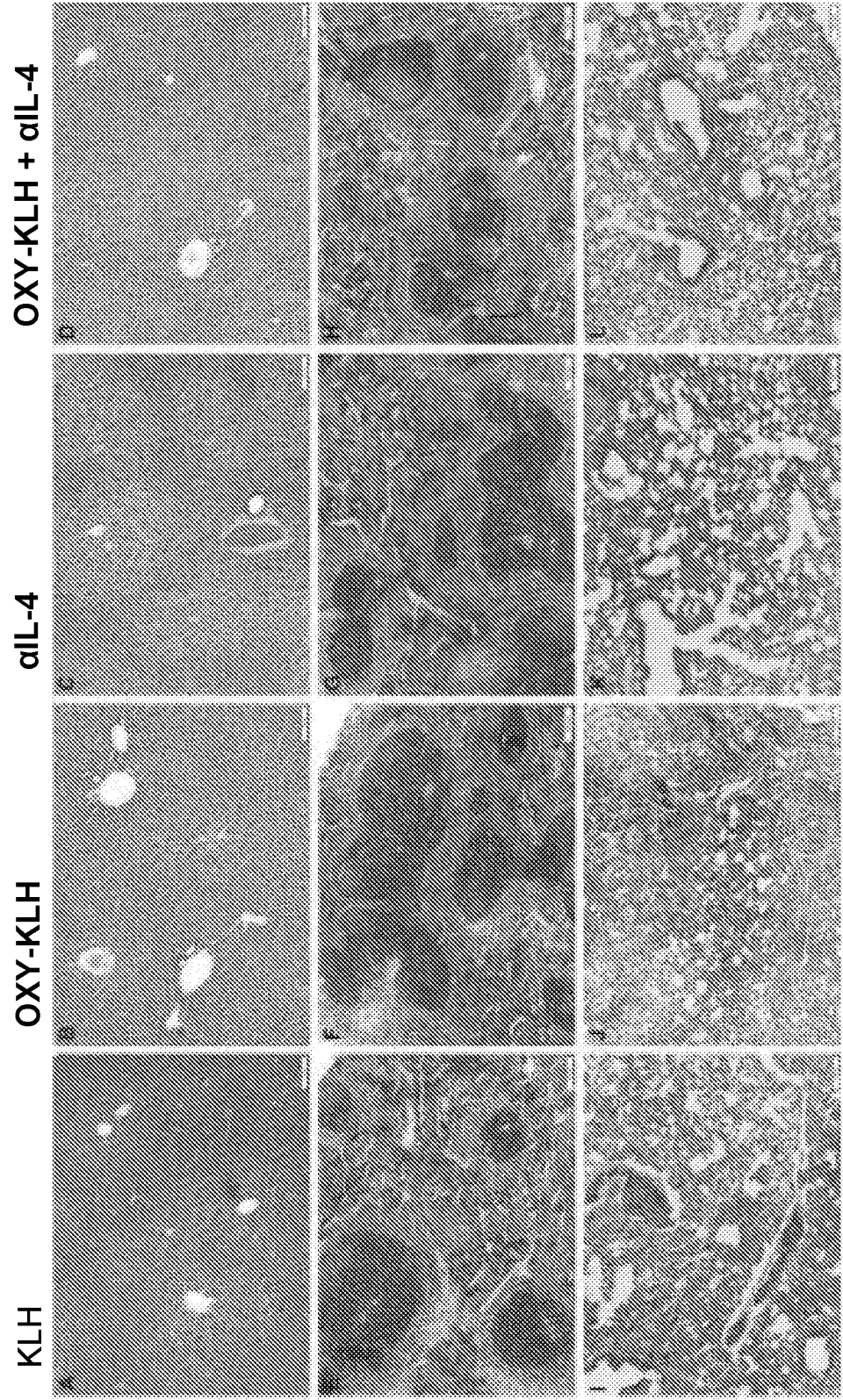


FIG. 12



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/31907

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/02, 38/20, 39/39, 39/385; C07K 16/06, 16/24 (2017.01)

CPC - C07K 16/247, 16/248, 16/06; A61K 39/0013, 38/02, 38/2026, 38/204, 39/39, 39/385

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 9,308,280 B2 (Massachusetts Institute of Technology) 12 April 2016; column 2, line 66; column 3, lines 1, 7, 21; column 4, line 15; column 8, line 44; column 16, lines 5, 6, 8, 19, 21, 37; column 17, lines 36, 38; column 33, line 52; column 34, line 16; column 35, line 35; column 37, line 38; column 55, line 25, column 56, line 1; column 68, line 14; column 102, lines 34, 66; column 103, line 11; column 118, line 54; column 119, line 25; Figure 36	1-2, 6, 8-9, 13/1-2, 13/6, 13/8-9, 21-22, 24-25 ----- 3-4, 7, 10-12, 13/3-5, 13/7, 13/10-12, 23/21-22, 26/24-25
Y	(SUNDBERG, T et al.) Small-molecule control of cytokine function: new opportunities for treating immune disorders. Current Opinion in Chemical Biology. 15 September 2014, Vol. 23; pages 23-30; page 24, 2nd column, 1st paragraph; Table 1; Figure 1B; DOI: 10.1016/j.cbpa.2014.08.013	9, 10-12, 13/9, 13/10-12
Y	(VERDEIL G, et al.) Adjuvants targeting innate and adaptive immunity synergize to enhance tumor immunotherapy. PNAS. 28 October 2008, Vol. 105, No. 43; pages 16683-16688; page 16683, 1st column, 3rd paragraph; page 16687, 2nd column, 2nd paragraph; DOI: 10.1073/pnas.0805054105	3-4, 7, 13/3-4, 13/7
Y	(YAMAZAKI M, et al.) Mucosal T cells expressing high levels of IL-7 receptor are potential targets for treatment of chronic colitis. The Journal of Immunology. 1 August 2003, Vol. 171, No. 3; pages 1556-1563; abstract; page 1556, 2nd column, 3rd paragraph; page 1557, 2nd column, 2nd paragraph; page 1560, 1st column, 1st paragraph; DOI: 10.4049/jimmunol.171.3.1556	5, 13/5

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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)

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

29 July 2017 (29.07.2017)

Date of mailing of the international search report

22 AUG 2017

Name and mailing address of the ISA/

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P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/31907

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
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:
3. ☒ Claims Nos.: 14-20
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:

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 claims 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 claims Nos.:

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/US17/31907

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(WACK, A et al.) Combination adjuvants for the induction of potent, long-lasting antibody and T-cell responses to influenza vaccine in mice. Vaccine. 24 January 2008, Vol. 26, No. 4; pages 552-561; page 553, 2nd column, 3rd paragraph; page 558, 1st column, 1st paragraph; Figure 3A; DOI: 10.1016/j.vaccine.2007.11.054	23/21-22, 26/24-25