invention, the subject has COPD and the treatment suppresses in the subject one or more symptoms of COPD. In one aspect the treatment suppresses airway remodeling in the subject with COPD. In one aspect the treatment slows disease progression in the subject. In another aspect, the subject has an allergy, autoimmune disease, inflammatory condition other than allergy, an ischemia reperfusion injury, sickle cell anemia, or a vasculitic diseases, and the treatment suppresses in the subject one or more symptoms of the disease. In another aspect, the subject has received or is a candidate to receive a transplant, and the treatment suppresses one or more symptoms of transplant rejection. In another aspect, the subject has an infection, and the treatment slows the growth of, halts the growth of, or kills the involved infectious agent or suppresses in the subject one or more symptoms of the infection. In another aspect, the subject has cancer, and the treatment slows the growth of, halts the growth of, or kills the cancer or suppresses in the subject one or more symptoms of the cancer. In any of the foregoing aspects and embodiments, the subject can be human and the antibody can be a humanized antibody. In any of the forgoing aspects and embodiments, the subject can be human and the antibody can be a fully human antibody. A fully human antibody is an antibody consisting only of human amino acid sequences. In some embodiments, the fully human antibody is a composite of human amino acid sequence segments from different human antibodies.

The invention involves the use of antibodies, the nature of which is described in detail below. The antibodies in some embodiments are humanized antibodies. The antibodies in some embodiments are whole, fully human antibodies. The antibodies in some embodiments can be whole fully human, composite antibodies. In some embodiments, the antibodies can have three light-chain CDR and three heavy-chain-CDR regions, and wherein each of the three light-chain CDR and three heavy-chain-CDR regions is a composite of human sequences.

The invention in another aspect involves humanized antibodies that bind with at least a certain affinity to iNKT cells. In some embodiments, the antibody binds iNKT cells with an IC 50 that is less than 1.0 μg/ml for binding to recombinant soluble human iTCR. In some embodiments, the heavy chain variable region and the light chain variable region of the humanized antibodies consist of human sequences.

comprising SEQ ID Nos. 24, 25 and 26 and also has a leucine at position 85. In some embodiments, the antibody has a heavy chain variable region comprising SEQ ID Nos. 21, 22 and 23. In some embodiments, the antibody has a heavy chain variable region comprising SEQ ID Nos. 21, 22 and 23 and also has a valine at position 23 and/or a threonine at position 99. In some embodiments, the antibody comprises a heavy chain variable region selected from SEQ ID Nos. 7, 8, 9, 10, 11, 12, 13 or 14. In some embodiments, the antibody comprises a light chain variable region selected from SEQ ID Nos. 15, 16, 17, 18, 19 or 20. In some embodiments, the antibody comprises a light chain variable region selected from SEQ ID Nos. 15, 16, 17, 18, 19 or 20 and a heavy chain variable region selected from SEQ ID Nos. 7, 8, 9, 10, 11, 12, 13 or 14. In some embodiments, the antibody comprises SEQ ID Nos. 10 and 18. In some embodiments, the antibody comprises SEQ ID No. 27. In some embodiments, the antibody comprises SEQ ID No. 28. In some embodiments, the antibody comprises SEQ ID No. 29. In some embodiments, the antibody comprises SEQ ID Nos. 27 and 29. In some embodiments, the antibody comprises SEQ ID Nos. 28 and 29.

In some embodiments the antibody binding region is non-reactive with human peripheral blood monocytes of all human haplotypes.

In any of the foregoing embodiments, the humanized antibody may be a depleting antibody. In any of the foregoing embodiments, the humanized antibody may be an activating antibody. In any of the ~~of the~~ foregoing embodiments, the antibody may have the property of inducing [induces] no significant change in one or more serum cytokine levels after administration to the subject.

The invention in another aspect is a method. The method involves contacting iNKT cells of a subject with an isolated humanized antibody that selectively binds iNKT cells in an amount effective to block the activity of the iNKT cells or to deplete the iNKT cells. In one embodiment, the cells are contacted *in vitro*. In one embodiment, the cells are contacted *in vivo*, and the amount is an amount sufficient to suppress an immune response in the subject. In one embodiment, the antibody is a depleting antibody and the amount is sufficient to deplete iNKT cells in the subject. In these embodiments, the antibodies are any of the humanized antibodies as described above, except for activating antibodies.

The invention in another aspect is a method. The method involves contacting iNKT cells of a subject with an isolated humanized antibody that selectively binds and activates

Without wishing to be bound by any theory of the invention, it is believed that the naked blocking antibodies of the invention not only interfere with the activity of iNKT cells when administered, but rather result in a depletion of iNKT cells. It also is believed that this depletion is transient, with iNKT cell populations being restored over time. It is believed that the depletion of iNKT cells is mediated by ADCC. In this manner, surprisingly, naked antibodies can be used to suppress immune responses mediated by iNKT cells. It is believed that such immune responses include asthma (including allergic asthma), chronic obstructive, pulmonary disease (COPD), allergy, autoimmune diseases, inflammatory conditions other than allergy, ischemia reperfusion injury, vasculitic diseases, and transplant rejection.

In one embodiment, the antibodies are produced in CHO cells. It has been discovered that naked antibodies produced in CHO cells are effective in depleting iNKT cells, notwithstanding CHO cells are not considered to have an optimal glycosylation pattern for ADCC. These antibodies also are effective in depleting iNKT cells, notwithstanding the lack of any enhancement to the Fc region to increase ADCC mediated depletion. That these antibodies are so effective without such enhancements is surprising.

In some embodiments, the depleting antibody induces no significant change in one or more serum cytokine levels after administration to the subject. "No significant change" means the change in the level of one or more serum cytokines in a subject is less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% following administration of the depleting antibody. In some embodiments, the serum cytokine levels show no significant change up to 2 hours, 4 hours, 8 hours, 16 hours, or 24 hours following administration of the depleting antibody. Examples of serum cytokines include, but are not limited to those shown in Table 8. In some embodiments, the depleting antibody induces no significant change in the levels of one or more serum cytokine shown in Table 8. In some embodiments, the depleting antibody induces no significant change in the levels of all serum cytokines shown in Table 8. In some embodiments, the depleting antibody induces no significant change ~~in change~~ in the levels of any 2, 3, 4, 5, or 6 of the serum cytokines shown in Table 8. In some embodiments, the depleting antibody induces no significant change in the levels of any one or more (including all) serum IFNγ, IL-4, IL-10, IL-17, TNF-α, IL-2, IL-5, IL-8, IL-1 RA and MIP-1β.

elastase, albumin, .alpha.-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the humanized antibody (or portion thereof) coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding a humanized antibody (or portion thereof).

For example, a viral vector, such as an adeno-associated viral (AAV) vector may be operatively linked components of control elements. For example, a typical vector includes a transcriptional initiation region, a nucleotide sequence of the protein to be expressed, and a transcriptional termination region. Typically, such an operatively linked construct will be flanked at its 5 and 3 regions with AAV ITR sequences, which are viral cis elements. The control sequences can often be provided from promoters derived from viruses such as, polyoma, Adenovirus 2, cytomegalovirus, and Simian Virus 40. Viral regulatory sequences can be chosen to achieve a high level of expression in a variety of cells. Alternatively, ubiquitously expressing promoters, such as the early cytomegalovirus promoter can be utilized to accomplish expression in any cell type. A third alternative is the use of promoters that drive tissue specific expression. This approach is particularly useful where expression of the desired protein in non-target tissue may have deleterious effects. Thus, according to various embodiments, the vector contains the proximal human brain natriuretic ~~brain~~ peptide (hBNP) promoter that functions as a cardiac-specific promoter. For details on construction of such a vector see LaPointe et al., "Left Ventricular Targeting of Reporter Gene Expression In Vivo by Human BNP Promoter in an Adenoviral Vector," Am. J. Physiol. Heart Circ. Physiol., 283:H1439-45 (2002).

In various embodiments, a humanized antibody includes a heavy chain variable region having a consensus sequence. Consensus sequences were developed based on 6B11 and include all of the CDR regions of the 6B11 heavy chain, one or more framework residues, and, for the remaining amino acids, the antibody in one embodiment includes any sequence or composite of sequences from a human antibody. In one embodiment, the light chain consensus sequence comprises SEQ ID Nos. 24, 25 and 26 and also a leucine at position 85. In some embodiments, the heavy chain consensus sequence comprises SEQ ID Nos. 21, 22 and 23 and also has a valine at position 23 and/or a threonine at position 99. Additional desirable framework residues are high-lighted in Table 1.

In various embodiments, a humanized antibody includes a heavy chain variable region having an amino acid sequence of SEQ ID Nos.: 7, 8, 9, 10, 11, 12, 13, or 14, or an amino acid sequence having 90% or greater, 95% or greater, 98% or greater, or 99% or greater sequence identity to SEQ ID Nos.: 7, 8, 9, 10, 11, 12, 13 or 14, but always including the consensus sequence of SEQ ID Nos. 24, 25 and 26 and also a leucine at position 85. In some embodiments where the antibody includes a heavy chain variable region having an amino acid sequence having 90% or greater, 95% or greater, 98% or greater, or 99% or greater sequence identity to SEQ ID Nos.: 7, 8, 9, 10, 11, 12, 13 or 14, one or more or all of the amino acid differences are conservative substitutions. In some embodiments, the substitutions are only those present in a human antibody. The humanized antibody may further includes a light chain variable region amino acid sequence of SEQ ID Nos.: 15, 16, 17, 18, 19 or 20, ~~21,~~ or an amino acid sequence having 90% or greater, 95% or greater, 98% or greater, or 99% or greater sequence identity to SEQ ID Nos.: 15, 16, 17, 18, 19 or 20, but always including the consensus sequence of SEQ ID Nos. 21, 22 and 23 and also a valine at position 23 and/or a threonine at position 99. In some embodiments where the antibody includes a light chain variable region having an amino acid sequence having 90% or greater, 95% or greater, 98% or greater, or 99% or greater sequence identity to SEQ ID Nos.: 15, 16, 17, 18, 19 or 20, one or more or all of the amino acid differences are conservative substitutions, but always including the consensus sequence of SEQ ID Nos. 21, 22 and 23 and also a valine at position 23 and/or a threonine at position 99. Any combination of such heavy chain and light chain amino acid sequences are contemplated. In some embodiments, a humanized antibody includes (i) a heavy chain variable region having an amino acid sequence of any of SEQ ID Nos.: 7, 8, 9, 10, 11, 12, 13 or 14, and (ii) a light chain variable

expression is absent on K562 and the FO-1 cells, so as expected, the anti-MHC Class I control mAb did not bind to these cells in the current study.

Fc-Function Analysis of NKTT120

NKTT120 is humanized IgG1 mAb and as such, it is expected to bind to human FcγRI and C1q. As part of the characterization of its IgG-Fc properties, it was evaluated in ELISA for reactivity with FcγRI and FcγRIII, and the complement component C1q. Three control antibodies were used in the characterization studies of NKTT120: (i) NKTT103, a chimeric 6B11 mAb, which shares the same IgG-Fc domains as NKTT120 but has the parental murine V domain regions; (ii) NKTT219, a humanized mAb with the same variable region as NKTT120, but which has an antibody-dependent cell-mediated toxicity (ADCC)-enhanced humanized IgG1; and (iii) NKTT320, a humanized mAb with the same variable region as NKTT120, but which has a modified IgG4 isotype. All three antibodies (NKTT120, NKTT219 and NKTT320) bind to the same iTCR epitope with similar specificities and affinities.

NKTT120 binds to FcγRI, and FcγRIII. FcγRI, which is expressed on NK cells, is the primary mechanism by which therapeutic IgG1 mAbs mediate ADCC *in vivo*. NKTT120 also binds to C1q, which is the first subcomponent of the complement membrane attack complex, which recognizes and binds to the heavy chain of IgG, and initiates the classical complement pathway, resulting in complement-dependent cytotoxicity (CDC). The chimeric 6B11 mAb demonstrates similar Fc-binding properties as NKTT120. In subsequent BIAcore™ analysis, NKTT120 was found to bind FcγRI as well as FcγRIII, and to C1q. NKTT219, which was designed with enhanced Fc-effector function, binds to both FcγRI as well as FcγRIII, and to C1q, and thus is expected to have an improved capacity to deplete iNKT cells. In contrast, NKTT320, whose Fc-effector function was eliminated, does not recognize FcγRI, FcγRIII or C1q, and thus is not expected to cause iNKT cell depletion.

iNKT Cell Depletion in Vα24 Transgenic Mice

Mice that express the human (Vα24-Jα18) invariant TCR alpha chain transgene were used. These mice lack the murine Jα18 region, and thus are unable to produce Vα14-Jα18^{+} murine invariant NKT cells. All iNKT cells that are present in the mice are only able to express the transgenic human invariant TCR alpha chain and thus all carry the NKTT120

12. The method of claim 10, wherein the antibody binds iNKT cells with an IC 50 that is less than 1.0μg/ml for binding to recombinant soluble human iTCR.

13. The method of claim 10, where in the antibody has a light chain variable region comprising SEQ ID Nos. 24, 25 and 26 and a leucine at position 85.

14. The method of claim 10, wherein the antibody has a heavy chain variable region comprising SEQ ID Nos. 21, 22 and 23 and a valine at position 23 and/or a threonine at position 99.

15. The method of claim 1, wherein the antibody binds the epitope defined by SEQ ID No. 1.

16. The method of claim 1, wherein the antibody binds the epitope defined by SEQ ID Nos. 1 and.2.

17. The method of claim 1, wherein the antibody comprioses a light chain variable region selected from SEQ ID Nos. 15, 16, 17, 18, 19 or 20.

18. The method of claim 1, wherein the antibody comprises a heavy chain variable region selected from SEQ ID Nos. 7, 8, 9, 10, 11, 12, 13 or 14.

19. The method of claim 1, wherein the antibody comprises NKTT120.

20. The method of claim 1, wherein the antibody induces no significant change in one or more serum cytokine levels after administration to the subject.

21. A composition comprising, an isolated, humanized antibody that binds selectively iNKT cells, wherein the antibody has an IC50 of less than 0.9 μg/ml for binding to recombinant soluble, human iTCR.

22. The composition of claim 21, wherein the heavy chain variable region and the light chain variable region consist of human sequences.

23. The composition of claim 21, wherein the IC50 is less than 0.5 μg/ml.

24. The composition of claim 21, wherein the IC50 is less than 0.3 μg/ml.

25. The composition of claim 21, wherein the IC50 is between 0.1 and 0.5 μg/ml.

26. The composition of claims 21- 25, wherein the antibody binds the epitope defined by SEQ ID No. 1.

27. The composition of claims 21- 25, wherein the antibody has three light CDR regions and three heavy CDR regions, and wherein each of the three light and three heavy CDR regions is a composite of human sequences.

28. The composition of claims 21- 25, wherein the antibody has a light chain variable region comprising SEQ ID Nos. 24, 25 and 26 and a leucine at position 85.

29. The composition of claims 21- 25, wherein the antibody has a heavy chain variable region comprising SEQ ID Nos. 21, 22 and 23 and a valine at position 23 and/or a threonine at position 99.

30. The composition of claim 21, wherein the antibody has a light chain variable region having sequence comprising SEQ ID Nos. 24, 25 and 26 and a leucine at position 85 and a heavy chain variable region comprising SEQ ID Nos. 21, 22 and 23 and a valine at position 23 and/or a threonine at position 99.

31. The composition of claim 21, wherein the antibody comprises a heavy chain variable region have a sequenced selected from SEQ ID Nos. 7, 8, 9, 10, 11, 12, 13 or 14.