ASSAYS FOR THE DETECTION OF ANTI-TNF DRUGS AND AUTOANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation U.S. application Ser. No. 14/046,863, filed Oct. 4, 2013, which application is a continuation of U.S. application Ser. No. 13/441,727, filed Apr. 6, 2012, now U.S. Pat. No. 8,574,855, which is a continuation of PCT/US2010/054125, filed Oct. 26, 2010, which application claims priority to U.S. Provisional Application No. 61/255,048, filed October 26, 2009, U.S. Provisional Application No. 61/262,877, filed November 19, 2009, U.S. Provisional Application No. 61/324,635, filed April 15, 2010, U.S. Provisional Application No. 61/345,567, filed May 17, 2010, U.S. Provisional Application No. 61/351,269, filed June 3, 2010, U.S. Provisional Application No. 61/389,672, filed October 4, 2010, and U.S. Provisional Application No. 61/393,581, filed October 15, 2010, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Autoimmune disorders are a significant and widespread medical problem. For example, rheumatoid arthritis (RA) is an autoimmune disease affecting more than two million people in the United States. RA causes chronic inflammation of the joints and typically is a progressive illness that has the potential to cause joint destruction and functional disability. The cause of rheumatoid arthritis is unknown, although genetic predisposition, infectious agents and environmental factors have all been implicated in the etiology of the disease. In active RA, symptoms can include fatigue, lack of appetite, low grade fever, muscle and joint aches and stiffness. Also during disease flare ups, joints frequently become red, swollen, painful and tender, due to inflammation of the synovium. Furthermore, since RA is a systemic disease, inflammation can affect organs and areas of the body other than the joints, including glands of the eyes and mouth, the lung lining, the pericardium, and blood vessels.

[0003] Traditional treatments for the management of RA and other autoimmune disorders include fast acting “first line drugs” and slower acting “second line drugs.” The first line drugs reduce pain and inflammation. Example of such first line drugs include aspirin, naproxen, ibuprofen, etodolac and other non-steroidal anti-

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TNFR-Ig Fc fusion protein, HUMIRA™ (adalimumab), a human anti-TNFα mAb, and CIMZIA® (certolizumab pegol), a PEGylated Fab fragment, have been approved by the FDA for treatment of rheumatoid arthritis. CIMZIA® is also used for the treatment of moderate to severe Crohn's disease (CD). While such biologic therapies have demonstrated success in the treatment of rheumatoid arthritis and other autoimmune disorders such as CD, not all subjects treated respond, or respond well, to such therapy. Moreover, administration of TNFα inhibitors can induce an immune response to the drug and lead to the production of autoantibodies such as human anti-chimeric antibodies (HACA), human anti-humanized antibodies (HAHA), and human anti-mouse antibodies (HAMA). Such HACA, HAHA, or HAMA immune responses can be associated with hypersensitive reactions and dramatic changes in pharmacokinetics and biodistribution of the immunotherapeutic TNFα inhibitor that preclude further treatment with the drug. Thus, there is a need in the art for assays to detect the presence of anti-TNFα biologics and/or their autoantibodies in a patient sample to monitor TNFα inhibitor therapy and to guide treatment decisions. The present invention satisfies this need and provides related advantages as well.

BRIEF SUMMARY OF THE INVENTION

[0007] TNFα has been implicated in inflammatory diseases, autoimmune diseases, viral, bacterial and parasitic infections, malignancies, and/or neurodegenerative diseases and is a useful target for specific biological therapy in diseases, such as rheumatoid arthritis and Crohn's disease. TNFα inhibitors such as anti-TNFα antibodies are an important class of therapeutics. Assay methods are needed to detect the presence of anti-TNFα biologics and/or their autoantibodies.

[0008] As such, in one embodiment, the present invention provides a method for detecting the presence or level of an anti-TNFα drug in a sample, comprising:

(a) contacting labeled TNFα with a sample having or suspected of having an anti-TNFα drug to form a labeled complex (*i.e.*, immuno-complex or conjugate) with the anti-TNFα drug (*i.e.*, wherein the labeled TNFα and

210, 215, 220 amino acids in length. In one embodiment, the TNF-α used includes residues 77-233 of SEQ ID NO 1.

[0075] In certain instances, at least one amino acid in the soluble portion of TNF-α is labeled i.e., residues 77-233 of SEQ ID NO 1. In certain instances, an amine reactive fluorophore derivative is used. In most instances, the amine reactive group is an acylating reagent that forms carboxamides, sulfonamides or thioureas upon reaction with amines. Virtually all proteins have lysine residues, and most have a free amine at the N-terminus and thus can be used as a point of attachment to label. In a preferred embodiment, residue 77 is labeled, and the TNF-α used includes residues 77-233 of SEQ ID NO 1. In other embodiments, many primary amines are labeled and the TNF-α is multiply labeled.

[0076] In certain instances, a portion of TNF-α is not labeled, i.e., portions that are recognized by antibodies, and fragments and regions thereof. In other words, certain portions of TNF-α antigens provide a topographical or three dimensional epitope of TNF which is recognized by, and/or binds with anti-TNF activity, an antibody, and fragments, and variable regions thereof. These portions are preferably free to bind, and thus are not labeled. They include residues 136-157 and 164-185 of SEQ ID NO:1:

[0077] (SEQ ID NO: 2) Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-Cys-Pro-Ser-Thr-His-Val-Leu-Leu-Thr-His-Thr-Ile; and

[0078] (SEQ ID NO: 3) Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-Ser-Ala-Ile-Lys-Ser-Pro-Cys-Gln-Arg-Glu-Thr-Pro-Glu-Gly.

[0079] TNFα or an anti-TNFα drug can be labeled with a variety of detectable group(s). Preferably, TNFα or an anti-TNFα drug is labeled with a fluorophore or a fluorescent dye. Exemplary fluorophores suitable for use in the present invention include those listed in the Molecular Probes Catalogue, which is herein incorporated by reference (*see,* R. Haugland, *The Handbook-A Guide to Fluorescent Probes and Labeling Technologies*, 10th Edition, Molecular probes, Inc. (2005)). Such exemplary fluorophores include, but are not limited to, Alexa Fluor® dyes such as Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 430, Alexa Fluor® 488, Alexa Fluor® 514, Alexa

Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 610, Alexa Fluor® 633, Alexa Fluor® 635, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, Alexa Fluor® 700, Alexa Fluor® 750, and/or Alexa Fluor® 790, as well as other fluorophores such as, for example, Dansyl Chloride (DNS-Cl), 5-(iodoacetamida)fluoroscein (5-IAF), fluoroscein 5-isothiocyanate (FITC), tetramethylrhodamine 5- (and 6-)isothiocyanate (TRITC), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), 7-nitrobenzo-2-oxa-1,3,-diazol-4-yl chloride (NBD-Cl), ethidium bromide, Lucifer Yellow, 5-carboxyrhodamine 6G hydrochloride, Lissamine rhodamine B sulfonyl chloride, Texas Red™ sulfonyl chloride, BODIPY™, naphthalamine sulfonic acids (*e.g.*, 1-anilinonaphthalene-8-sulfonic acid (ANS), 6-(p-toluidinyl)naphthalene-2-sulfonic acid (TNS), and the like), Anthroyl fatty acid, DPH, Parinaric acid, TMA-DPH, Fluorenyl fatty acid, fluorescein-phosphatidylethanolamine, Texas Red-phosphatidylethanolamine, Pyrenyl-phophatidylcholine, Fluorenyl-phosphotidylcholine, Merocyanine 540,1-(3-sulfonatopropyl)-4-[β-[2[(di-n-butylamino)-6 naphthyl]vinyl]pyridinium betaine (Naphtyl Styryl), 3,3'dipropylthiadicarbocyanine (diS-C_3-(5)), 4-(p-dipentyl aminostyryl)-l-methylpyridinium (di-5-ASP), Cy-3 Iodo Acetamide, Cy-5-N-Hydroxysuccinimide, Cy-7-Isothiocyanate, rhodamine 800, IR-125, Thiazole Orange, Azure B, Nile Blue, Al Phthalocyanine, Oxaxine 1, 4', 6-diamidino-2-phenylindole (DAPI), Hoechst 33342, TOTO, Acridine Orange, Ethidium Homodimer, N(ethoxycarbonylmethyl)-6-methoxyquinolinium (MQAE), Fura-2, Calcium Green, Carboxy SNARF-6, BAPTA, coumarin, phytofluors, Coronene, metal-ligand complexes, IRDye® 700DX, IRDye® 700, IRDye® 800RS, IRDye® 800CW, IRDye® 800, Cy5, Cy5.5, Cy7, DY 676, DY680, DY682, DY780, and mixtures thereof. Additional suitable fluorophores include enzyme-cofactors; lanthanide, green fluorescent protein, yellow fluorescent protein, red fluorescent protein, or mutants and derivates thereof. In one embodiment of the invention, the second member of the specific binding pair has a detectable group attached thereto.

[0080] Typically, the fluorescent group is a fluorophore selected from the category of dyes comprising polymethines, pthalocyanines, cyanines, xanthenes, fluorenes, rhodamines, coumarins, fluoresceins and BODIPY™.

the labeled internal control is Biocytin-Alexa 488. In certain embodiments, the amount of the labeled internal control (*e.g.*, Biocytin-Alexa 488) is from about 1 to about 25 ng per 100 µL of sample analyzed. In certain other embodiments, the amount of the labeled internal control (*e.g.*, Biocytin-Alexa 488) is from about 5 to about 25 ng per 100 µL, from about 5 to about 20 ng per 100 µL, from about 1 to about 20 ng per 100 µL, from about 1 to about 10 ng per 100 µL, or from about 1 to about 5 ng per 100 µL of sample analyzed. In further embodiments, the amount of the labeled internal control (*e.g.*, Biocytin-Alexa 488) is about 1, 5, 10, 15, 20, or 25 ng per 100 µL of sample analyzed.

[0122] In some embodiments, the present invention provides a method for optimizing the therapeutic amount of an anti-TNFα drug in a subject receiving therapy with the anti-TNFα drug, the method comprising: determining a subsequent dose of the anti-TNFα drug for the subject based upon the comparison of the first and second ratios in accordance with the methods of the present invention, thereby optimizing the therapeutic amount of the anti-TNFα drug. In a related embodiment, the present invention further provides that the method further comprises: increasing the subsequent dose of the anti-TNFα drug when the first ratio is about 100:1 and the second ratio is less than about 95:1, thereby optimizing the therapeutic amount of the anti-TNFα drug. In another related embodiment, the present invention further provides that theanti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof. In a related embodiment, the present invention further provides that the anti-TNFα drug is infliximab (REMICADE™). In a related embodiment, the present invention further provides that the anti-TNFα drug is adalimumab (HUMIRA™). In a related embodiment, the present invention further provides that the anti-TNFα drug is etanercept (ENBREL™). In a related embodiment, the present invention further provides that the anti-TNFα drug is CIMZIA® (certolizumab pegol). In a related embodiment, the present invention further provides that the labeled TNFα is a fluorophore labeled TNFα. In a related embodiment, the

present invention further provides that the detected anti-TNFα drug is quantitated. In a related embodiment, the present invention further provides that the labeled complex is

[0174] The method according to the invention may, for example, be used for identifying patients with secondary response failure. Secondary response failures can be asymptomatic, *i.e,* the only symptoms are that the treatment has become less effective or even non-effective. In certain instances, the methods of the present invention are useful for identifying the development of secondary response failure before the patient or medical practitioner has noticed that the treatment is less effective. A higher dosage of treatment may be applied to ensure the correct *in vivo* concentration is achieved, or an alternative treatment can be selected, or a combination thereof. Where the therapeutic agent is a diagnostic agent, the development of secondary response failure can be particularly catastrophic. Radio-labeled monoclonal antibodies are routinely used in the monitoring of diseases such as cancers, and some infectious diseases, where it is important to determine the size and/or location of the disease/agent, *e.g.,* in identifying the presence and/or location of any secondary metastases. When the development of response failure (either primary or secondary) occurs unnoticed, the patient may be given the 'all clear', *i.e.,* a false negative result, which can lead to the cessation of treatment and the latter reappearance of the disease, often in a far more developed and possibly untreatable condition.

[0175] A further category of response failure is the development of (*e.g.*, secondary) response failure associated with adverse side-effects. The development of a host-immune response in a subject can be accompanied by deleterious or unpleasant side-effects. These may be caused by the development of antibodies which recognize the human or humanized therapeutic agents but which fail to distinguish between other host immunoglobulins. The methods of the present invention are therefore suitable to identify subjects who have either an innate or previously-developed immune response to the therapeutic agent and who may, for example, be vulnerable to adverse side-effects associated with response failure.

[0176] The methods of the present invention are suitable for the selection and management of treatment regimes which involve the administration of monoclonal therapeutic agents to patients. Accordingly, the methods for determining the concentration or bioavailability of a therapeutic antibody as described herein can be

measured when there is a high level of drugs in the circulation.

[0232] We have developed a proprietary non-radiolabeled, liquid-phase, mobility shift assay to measure the ADA and ADL levels in serum from patients treated with ADL which is not affected by the presence of the drug in the serum.

METHODS

[0233] Fluorophore (Fl)-labeled ADL was incubated with patient serum to form the immunocomplex. A Fl-labeled small peptide was included as an internal control in each reaction. Different amounts of anti-human IgG were used to generate a standard curve to determine the serum ADA level. Free Fl-labeled ADL was separated from the antibody bound complex based on its molecular weight by size-exclusion chromatography. The ratio of free Fl-labeled ADL to internal control from each sample was used to extrapolate the HAHA concentration from the standard curve. A similar methodology was used to measure ADL levels in patient serum samples with Fl-labeled TNF-α.

RESULTS

[0234] Figure 19 shows the separation of the anti-human IgG bound Fl-ADL complex from the free Fl-ADL due to the mobility shift of the high molecular weight complex. As seen in panels **c** to **h**, the retention time of the fluorescent peak shifted from 10.1 min to 7.3-9.5 min. The more the anti-human IgG is added in the reaction mixture, the less the free ADL remains in the chromatogram and the more the immunocomplex is formed (**h** to **c**). The retention time for the internal control is 13.5 min.

[0235] Figure 20 shows the dose-response curve of the fluorescent peak shift caused by the addition of anti-human IgG. Increasing the concentration of anti-human IgG reduces the ratio of free ADL to internal control due to the formation of the immunocomplex. The assay sensitivity is 10ng/ml of anti-human IgG. The internal control "Fl-BioCyt" corresponds to an Alexa 488-biocytin (BioCyt) which combines the green-fluorescent Alexa Fluor® 488 fluorophore with biotin and an aldehyde-fixable primary amine (lysine) (Invitrogen Corp.; Carlsbad, CA).

Equation V: [REMICADE^{TM}]/[REMICADE^{TM}•HACA$_{complex}$] = [labeled-REMICADE^{TM}]/[Labeled-REMICADE^{TM}•HACA$_{complex}$]

Equation VI: [HACA] = [REMICADE^{TM}•HACA]$_{complex}$ + [labeled-REMICADE^{TM}•HACA]$_{complex}$

Equation VII: [REMICADE^{TM}•HACA$_{complex}$] = [REMICADE^{TM}] x [labeled-REMICADE^{TM}•HACA$_{complex}$]/[labeled-REMICADE^{TM}]

Equation VIII: [labeled-REMICADE^{TM}•HACA$_{complex}$] = [labeled-REMICADE^{TM}] x [labeled-REMICADE^{TM}•HACA$_{complex}$]/[labeled-REMICADE^{TM}]

[0252]

[0253] Equation IX: [REMICADE^{TM}]$_{effective-amount}$ = [REMICADE^{TM}] – [HACA]

[0254] Determining the concentration levels of human anti-TNFα drug antibodies, *e.g.* HACA. A known concentration of Labeled-REMICADE^{TM} is added to a serum sample. HACA forms a complex with either REMICADE^{TM} or Labeled-REMICADE^{TM}**, See Equation IV**. The [REMICADE^{TM}] is determined in Step 1 above. By integrating the area-under-the-curve for the labeled-REMICADE^{TM}•HACA$_{complex}$ and dividing this number by the resultant integration for the area-under-the-curve for the free Labeled-REMICADE^{TM}, the ratio of [labeled-REMICADE^{TM}•HACA$_{complex}$] to [labeled-REMICADE^{TM}] is obtained. The ratio of [REMICADE^{TM}] to [REMICADE^{TM}•HACA$_{complex}$] is equal to the ratio of [labeled-REMICADE^{TM}] to [labeled-REMICADE^{TM}•HACA)$_{complex}$]**, See Equation V**. Because HACA equilibrates and forms a complex with both REMICADE^{TM} and Labeled-

REMICADE^{TM}, the total amount of HACA equals the sum of the amount of REMICADE^{TM}•HACA$_{complex}$ and the amount of labeled-REMICADE^{TM}•HACA$_{complex,}$

See Equation VI. Because the ratio of [REMICADE™] to [REMICADE™•$HACA_{complex}$] is equal to the ratio of [labeled-REMICADE™] to [labeled-REMICADE™•$HACA_{complex}$], both the [REMICADE™-HACA]$_{complex}$ and the [labeled-REMICADE™-$HACA_{complex}$] are determined by multiplying the ratio of the [labeled-REMICADE™•$HACA_{complex}$)]/ [labeled-REMICADE™] by, respectively, the concentration amount of REMICADE™, determined in **Step 1**, and the concentration amount of labeled-REMICADE™, known *a priori*, **See Equations VII and VIII.** Therefore, the total amount of HACA equals the sum of (1) the [REMICADE™], from step 1, multipled by [labeled-REMICADE™•HACA)$_{complex}$]/[labeled-REMICADE™], and (2) the [labeled REMICADE™], known *a priori*, multipled by [labeled-REMICADE™•HACA)$_{complex}$]/ [labeled-REMICADE™].

[0255] Determining the effective concentration levels of REMICADE™. Because HACA complexes with REMICADE™, the effective amount of REMICADE™ available in a serum sample is the amount of REMICADE™, measured from **Step 1,** minus the amount of HACA, measured from **Step 2, See Equation IX.**

[0256] Exemplary calculation. In patient JAG on V10, the [REMICADE™] was determined to be 7.5 μg/ml, See Figure 16c. This result was obtained by following **Step 1** and using **Equations I-III**. 7.5 μg/ml equals 30 ng/ 4 μL. Since 4 μL of sample was used in the measurement in **Step 2,** a total of 30.0 ng of REMICADE™ was present in the sample analyzed. The ratio of [labeled-REMICADE™•HACA]$_{complex}$/[labeled-REMICADE™] for patient JAG on V10 was 0.25, See Figure 16b. The [labeled-REMICADE™] introduced into the sample was 37.5 ng/ 100μL. Since 100μL of the labeled-REMICADE™ was used in the measurement in **Step 2**, a total of 37.5 ng of labeled-REMICADE™ was present in the sample analyzed. Using **Equation VII**, the total amount of REMICADE™•$HACA_{complex}$ was 30 ng multiplied by 0.25, which is equal to 7.5 ng labeled-REMICADE™•$HACA_{compelx}$. Using **Equation VIII**, the total amount of labeled-REMICADE™•$HACA_{complex}$ was 37.5 ng multiplied by 0.25, which is equal to 9.4 ng labeled-REMICADE™•$HACA_{compelx}$. Using **Equation VI,** the total amount of

HACA equals the sum of 9.4 ng and 7.5 ng, which equals 16.9 ng HACA. The 16.9 ng HACA was present in 4 μL of sample. The [HACA] was 16.9 ng/4μL,

HUMIRA™], known *a priori*, respectively, **See Equations XVI and XVII.** Because HAHA complexes with HUMIRA™, the effective amount of HUMIRA™ available in a serum sample is the amount of HUMIRA, measured from **Step 1,** minus the amount of HAHA, measured from **Step 2, See Equation XVIII.**

[0268] Exemplary calculation. In patient SL03246013, see Figure 25**,** the [HUMIRA™] was determined to be 16.9 μg/ml, see Figure 25. This result was obtained by following **Step 1** and using **Equations X-XII**. 16.9 μg/ml equals 67.6 ng/ 4 μL. Since 4 μL of sample was used in the measurement in **Step 2,** a total of 67.6 ng of HUMIRA™ was present in the sample analyzed. The ratio of [labeled-HUMIRA™•HAHA]$_{complex}$/[labeled-HUMIRA™] for patient SL03246013 was 0.055, see Figure 25. The [labeled-HUMIRA™] introduced into the sample was 37.5 ng/ 100μL. Since 100μL of the labeled-HUMIRA™ was used in the measurement in **Step 2**, a total of 37.5 ng of labeled-HUMIRA™ was present in the sample analyzed. Using **Equation XVI**, the total amount of HUMIRA™•HAHA$_{complex}$ was 67.6 ng multiplied by 0.055, which is equal to 3.71 ng labeled-HUMIRA™•HAHA$_{compelx}$. Using **Equation XVII**, the total amount of labeled-HUMIRA™•HAHA$_{complex}$ was 37.5 ng multiplied by 0.055, which is equal to 2.06 ng labeled-HUMIRA™•HAHA$_{compelx}$. Using **Equation XV,** the total amount of HAHA equals the sum of 3.71 ng and 2.06 ng, which equals 5.77 ng HAHA. The 5.77 ng HAHA was present in 4 μL of sample. The [HAHA] was 5.77 ng/4μL, which equals 1.44 μg/ml. Using **Equation XVIII**, the effective amount of HUMIRA™ is equal to 16.99 μg/ml HUMIRA™, determined from **Step 1**, minus 1.44 μg/ml HAHA, determined from **Step 2**. In this exemplary calculation, the effective [HUMIRA™] was equal to 15.46 μg/ml.

Example 9: Determining the Amount of a Complex of HACA or HAHA with Either REMICADE™, Labeled-REMICADE™, HUMIRA, or Labeled-HUMIRA.

[0269] This example describes a method for determining the amount of a complex of HACA or HAHA with either REMICADE™, Labeled-REMICADE™, HUMIRA, or Labeled-HUMIRA™ with reference to an internal standard.

[0270] By using an internal control, *e.g.* Biocytin-Alexa 488, serum artifacts and

WHAT IS CLAIMED IS:

1. A method for determining the presence or level of an autoantibody to a therapeutic antibody for treating an autoimmune disease in a sample, the method comprising:

(a) contacting a labeled therapeutic antibody with the sample to form a labeled complex with the autoantibody;

(b) subjecting the labeled complex to size exclusion chromatography to separate the labeled complex from free labeled therapeutic antibody and to detect an amount of the labeled complex and an amount of the free labeled therapeutic antibody; and

(c) comparing a ratio of the amount of the labeled complex and the amount of the free labeled therapeutic antibody detected in step (b) to a standard curve of known amounts of the autoantibody, thereby determining the presence or level of the autoantibody.

2. The method of claim 1, wherein the standard curve is generated by incubating the labeled therapeutic antibody with serum positive for the autoantibody.

3. The method of claim 1, wherein the sample is serum.

4. The method of claim 1, wherein the autoimmune disease is a member selected from the group consisting of rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, B-cell non-Hodgkin's lymphoma, multiple sclerosis, systemic lupus erythematosus, ankylosing spondylitis, psoriatic arthritis, juvenile idiopathic arthritis, severe psoriasis, chronic uveitis, severe sarcoidosis, and Wegener's granulomatosis.

5. The method of claim 1, wherein the therapeutic antibody is a member selected from the group consisting of an anti-cytokine antibody, an anti-chemokine antibody, an anti-cell adhesion antibody, an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD20 antibody, and an anti-CD25 antibody.

6. The method of claim 5, wherein the anti-cytokine antibody is a member selected from the group consisting of Actemra (tocilizumab) and CNTO 1275 (ustekinumab)

7. The method of claim 5, wherein the anti-cell adhesion antibody is natalizumab.

8. The method of claim 5, wherein the anti-CD3 antibody is visilizumab.

9. The method of claim 5, wherein the anti-CD4 antibody is priliximab.

10. The method of claim 5, wherein the anti-CD20 antibody is a member selected from the group consisting of rituximab, ofatumumab, and ocrelizumab.

11. The method of claim 5, wherein the anti-CD25 antibody is daclizumab.

12. The method of claim 1, wherein the autoantibody is a member selected from the group consisting of a human anti-mouse antibody (HAMA), a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), and combinations thereof.

13. The method of claim 1, wherein the size exclusion chromatography is size exclusion-high performance liquid chromatography (SE-HPLC).

14. The method of claim 1, wherein the labeled therapeutic antibody is a fluorophore-labeled therapeutic antibody.

15. The method of claim 14, wherein the fluorophore is an Alexa Fluor® dye.

16. The method of claim 1, wherein the labeled complex is eluted first, followed by the free labeled therapeutic antibody.

17. The method of claim 1, wherein the sample is obtained from a subject receiving therapy with the therapeutic antibody.

18. The method of claim 1, wherein a ratio of the free labeled therapeutic antibody to an internal control is determined and used to extrapolate the level of the autoantibody from the standard curve.