ASSAYS FOR DETECTING NEUTRALIZING AUTOANTIBODIES TO BIOLOGIC THERAPY WITH TNF ALPHA

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/505,031, filed July 6, 2011, U.S. Provisional Application No. 61/528,072, filed August 26, 2011, and U.S. Provisional Application No. 61/535,884, filed September 16, 2011, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0001a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[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-inflammatory drugs (NSAIDs), as well as corticosteroids, given orally or injected directly into tissues and joints. The second line drugs promote disease remission and prevent progressive joint destruction and are also referred to as disease-modifying anti-rheumatic drugs or DMARDs. Examples of second line drugs include gold, hydrochloroquine, azulfidine and immunosuppressive agents, such as methotrexate, azathioprine, cyclophosphamide, chlorambucil and cyclosporine. Many of

these drugs, however, can have detrimental side-effects. Thus, additional therapies for rheumatoid arthritis and other autoimmune disorders have been sought.

detect the presence of autoantibodies to biologic agents such as anti-TNFα drugs in a patient sample to monitor biologic therapy and to guide treatment decisions. ~~The present invention satisfies this need and provides related advantages as well.~~

[0006a] It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

[0006b] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention provides assays for detecting and measuring the presence or level of neutralizing and non-neutralizing autoantibodies to biologics such as anti-TNFα drug therapeutics in a sample. The present invention is useful for monitoring the formation of neutralizing and/or non-neutralizing anti-drug antibodies over time while a subject is on biologic therapy (*e.g.,* anti-TNFα drug therapy). The present invention is also useful for predicting and/or determining the cross-reactivity of neutralizing anti-drug antibodies in a subject's sample with alternative biologic therapies (*e.g.,* alternative anti-TNFα therapies). As such, the present invention provides information for guiding treatment decisions for those subjects receiving therapy with a biologic agent and improves the accuracy of optimizing therapy, reducing toxicity, and/or monitoring the efficacy of therapeutic treatment to biologic therapy.

[0007a] According to a first aspect of the invention, there is provided a method for measuring the level or percent of a neutralizing form of an autoantibody to an anti-TNFα drug in a sample, the method comprising:

(a) contacting the sample with an acid to dissociate preformed complexes of the autoantibody and the anti-TNFα drug;

(b) contacting the sample with a labeled anti-TNFα drug and a labeled TNFα following dissociation of the preformed complexes; and

(c) neutralizing the acid in the sample to form:

(i) a first labeled complex of the labeled anti-TNFα drug and the autoantibody; and/or

(ii) a second labeled complex of the labeled anti-TNFα drug, the labeled TNFα, and the autoantibody;

(d) subjecting the first labeled complex and/or the second labeled complex to size exclusion chromatography to separate them from free labeled TNFα, free labeled anti-TNFα drug, and/or a complex of labeled anti-TNFa drug and labeled TNFα;

(e) measuring the level of free labeled TNFα after size exclusion chromatography; and

(f) comparing the level of free labeled TNFα measured in step (e) to a normalized level or percent of free labeled TNFα in a control sample, wherein the normalized level or percent of the free labeled TNFα in the control sample corresponds to the level or percent of a neutralizing form of the autoantibody.

[0007b] According to a second aspect of the invention, there is provided a method for detecting the presence of a neutralizing and/or non-neutralizing form of an autoantibody to an anti-TNFα drug in a sample, the method comprising:

(a) contacting the sample with an acid to dissociate preformed complexes of the autoantibody and the anti-TNFα drug;

(b) contacting the sample with a labeled anti-TNFα drug and a labeled TNFα following dissociation of the preformed complexes; and

(c) neutralizing the acid in the sample to form:

(i) a first labeled complex of the labeled anti-TNFα drug and the autoantibody; and/or

(ii) a second labeled complex of the labeled anti-TNFα drug, the labeled TNFα, and the autoantibody;

(d) subjecting the first labeled complex and/or the second labeled complex to size exclusion chromatography to separate them from free labeled TNFα, free labeled anti-TNFα drug, and/or a complex of labeled anti-TNFα drug and labeled TNFα;

(e) measuring the level of free labeled TNFα after size exclusion chromatography; and

(f) comparing the level of the free labeled TNFα measured in step (e) to the level of free labeled TNFα in a control sample, thereby detecting the presence of a neutralizing and/or non-neutralizing form of the autoantibody.

[0008] In one aspect, the present invention provides a method for detecting the presence of a neutralizing and/or non-neutralizing form of an autoantibody to a biologic in a sample, the method comprising:

(a) contacting the sample with a labeled biologic and a labeled biologic binding moiety to form:

(i) a first labeled complex (*i.e.,* immuno-complex or conjugate) of the labeled biologic and the autoantibody (*i.e.,* wherein the components of the first labeled complex are not covalently attached to each other); and/or

(ii) a second labeled complex (*i.e.,* immuno-complex or conjugate) of the labeled biologic, the labeled biologic binding moiety, and the autoantibody (*i.e.,* wherein the components of the second labeled complex are not covalently attached to each other);

(b) subjecting the first labeled complex and/or the second labeled complex to size exclusion chromatography to separate them from free (*i.e.,* unbound) labeled biologic binding moiety, free labeled biologic, and/or a complex of labeled biologic and labeled biologic binding moiety;

(c) measuring the level of free labeled biologic binding moiety after size exclusion chromatography (*e.g.,* by measuring the area under the curve (AUC)

fluorescent group is preferably selected ~~form~~from the group consisting of IRDye® 700DX, IRDye® 700, IRDye® 800RS, IRDye® 800CW, IRDye® 800, Alexa Fluor® 660, Alexa Fluor® 680, Alexa Fluor® 700, Alexa Fluor® 750, Alexa Fluor® 790, Cy5, Cy5.5, Cy7, DY 676, DY680, DY682, and DY780. In certain embodiments, the near infrared group is IRDye® 800CW, IRDye® 800, IRDye® 700DX, IRDye® 700, or Dynomic DY676.

[0157] Fluorescent labeling is accomplished using a chemically reactive derivative of a fluorophore. Common reactive groups include amine reactive isothiocyanate derivatives such as FITC and TRITC (derivatives of fluorescein and rhodamine), amine reactive succinimidyl esters such as NHS-fluorescein, and sulfhydryl reactive maleimide activated fluors such as fluorescein-5-maleimide, many of which are commercially available. Reaction of any of these reactive dyes with a biologic (*e.g.,* anti-TNFα drug) or biologic binding moiety (*e.g.,* TNFα) results in a stable covalent bond formed between a fluorophore and a biologic (*e.g.,* anti-TNFα drug) or biologic binding moiety (*e.g.,* TNFα).

[0158] In certain instances, following a fluorescent labeling reaction, it is often necessary to remove any nonreacted fluorophore from the labeled target molecule. This is often accomplished by size exclusion chromatography, taking advantage of the size difference between fluorophore and labeled protein.

[0159] Reactive fluorescent dyes are available from many sources. They can be obtained with different reactive groups for attachment to various functional groups within the target molecule. They are also available in labeling kits that contain all the components to carry out a labeling reaction. In one preferred aspect, Alexa Fluor® 647 C2 maleimide is used from Invitrogen (Cat. No. A-20347).

[0160] Specific immunological binding of a neutralizing and/or non-neutralizing anti-drug antibody (*e.g.,* NAb and/or non-NAb) to a biologic (*e.g.,* anti-TNFα drug) and/or biologic binding moiety (*e.g.,* TNFα) can be detected directly or indirectly. Direct labels include fluorescent or luminescent tags, metals, dyes, radionuclides, and the like, attached to the antibody. In certain instances, a biologic (*e.g.,* anti-TNFα drug) or biologic binding moiety (*e.g.,* TNFα) labeled with different radionuclides can be used for determining the presence or level of NAb and/or non-NAb in a sample. In other instances, a chemiluminescence assay using chemiluminescent biologic (*e.g.,* anti-TNFα drug) and biologic binding moiety (*e.g.,* TNFα) is suitable for sensitive, non-radioactive detection of the presence or level of NAb and/or non-NAb in a sample. In particular instances, a biologic (*e.g.,* anti-TNFα drug) and biologic binding moiety (*e.g.,* TNFα) labeled with different fluorochromes is suitable for

~~WHAT IS~~ CLAIM~~ED I~~S~~:~~

1. A method for measuring the level or percent of a neutralizing form of an autoantibody to an anti-TNFα drug in a sample, the method comprising:

(a) contacting the sample with an acid to dissociate preformed complexes of the autoantibody and the anti-TNFα drug;

(b) contacting the sample with a labeled anti-TNFα drug and a labeled TNFα following dissociation of the preformed complexes; and

(c) neutralizing the acid in the sample to form:

(i) a first labeled complex of the labeled anti-TNFα drug and the autoantibody; and/or

(ii) a second labeled complex of the labeled anti-TNFα drug, the labeled TNFα, and the autoantibody;

(~~b~~d) subjecting the first labeled complex and/or the second labeled complex to size exclusion chromatography to separate them from free labeled TNFα, free labeled anti-TNFα drug, and/or a complex of labeled anti-TNFα drug and labeled TNFα;

(~~e~~e) measuring the level of free labeled TNFα after size exclusion chromatography; and

(~~d~~f) comparing the level of free labeled TNFα measured in step (~~e~~e) to a normalized level or percent of free labeled TNFα in a control sample, wherein the normalized level or percent of the free labeled TNFα in the control sample corresponds to the level or percent of a neutralizing form of the autoantibody.

2. The method of claim 1, wherein the anti-TNFα drug is selected from the group consisting of ~~REMICADE™ (~~infliximab~~)~~, ~~ENBREL™ (~~etanercept~~)~~, ~~HUMIRA™ (~~adalimumab~~)~~, ~~CIMZIA®~~ (~~certolizumab pegol~~)~~, ~~SIMPONI®~~ (~~golimumab~~;~~ (CNTO 148), and combinations thereof.

3. The method of claim 1 or claim 2, wherein the autoantibody to the anti-TNFα drug is selected from the group consisting of a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), a human anti-mouse antibody (HAMA), and combinations thereof.

4. The method of any one of claims 1 to 3, wherein step (~~e~~e) comprises measuring the peak area of the free labeled TNFα after size exclusion chromatography.

5. The method of any one of claims 1 to 4, wherein the control sample is a reference sample containing only free labeled TNFα.

6. The method of ~~any one of claims 1 to~~claim 4 or claim 5, wherein the level or percent of the free labeled TNFα in the control sample is normalized by subtracting the measured peak area of a third labeled complex formed between the labeled anti-TNFα drug and the labeled TNFα from the measured peak area of the free labeled TNFα.

7. The method of any one of claims 1 to 6, wherein the difference between the normalized level or percent of the free labeled TNFα in the control sample and the level of free labeled TNFα measured in step (~~e~~e) corresponds to the level or percent of a non-neutralizing form of the autoantibody.

8. The method of any one of claims 1 to 6, wherein the sample is positive for the neutralizing form of the autoantibody when the sample has greater than or equal to about 3~~.00~~% of the neutralizing form of the autoantibody.

9. The method of any one of claims 1 to 8, wherein the sample is serum.

10. The method of any one of claims 1 to 9, wherein the sample is obtained from a subject receiving therapy with the anti-TNFα drug.

11. The method of any one of claims 1 to 10, wherein the labeled anti-TNFα drug and the labeled TNFα comprise different fluorophores or fluorescent dyes.

12. A method for detecting the presence of a neutralizing and/or non-neutralizing form of an autoantibody to an anti-TNFα drug in a sample, the method comprising:

(a) contacting the sample with an acid to dissociate preformed complexes of the autoantibody and the anti-TNFa drug;

(b) contacting the sample with a labeled anti-TNFα drug and a labeled TNFα following dissociation of the preformed complexes; and

(c) neutralizing the acid in the sample to form:

(i) a first labeled complex of the labeled anti-TNFα drug and the autoantibody; and/or

(ii) a second labeled complex of the labeled anti-TNFα drug, the labeled TNFα, and the autoantibody;

(~~b~~d) subjecting the first labeled complex and/or the second labeled complex to size exclusion chromatography to separate them from free labeled TNFα, free labeled anti-TNFα drug, and/or a complex of labeled anti-TNFα drug and labeled TNFα;

(~~c~~e) measuring the level of free labeled TNFα after size exclusion chromatography; and

(~~d~~f) comparing the level of the free labeled TNFα measured in step (~~e~~e) to the level of free labeled TNFα in a control sample, thereby detecting the presence of a neutralizing and/or non-neutralizing form of the autoantibody.

13. The method of claim 12, wherein the anti-TNFα drug is selected from the group consisting of ~~REMICADE™ (~~infliximab~~)~~, ~~ENBREL™ (~~etanercept~~)~~, ~~HUMIRA™ (~~adalimumab~~)~~, ~~CIMZIA®~~ (~~certolizumab pegol~~)~~, ~~SIMPONI®~~ (~~golimumab~~;~~ (CNTO 148), and combinations thereof.

14. The method of claim 12 or claim 13, wherein the autoantibody to the anti-TNFα drug is selected from the group consisting of a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), a human anti-mouse antibody (HAMA), and combinations thereof.

15. The method of any one of claims 12 to 14, wherein step (~~e~~e) comprises measuring the peak area of the free labeled TNFα after size exclusion chromatography.

16. The method of any one of claims 12 to 15, wherein the control sample is a reference sample containing only free labeled TNFα.

17. The method of any one of claims 12 to 16, wherein a neutralizing form of the autoantibody is detected when the level of the free labeled TNFα measured in step (~~e~~e) is the same or substantially the same as the level of the free labeled TNFα in the control sample.

18. The method of any one of claims 12 to 16, wherein a non-neutralizing form of the autoantibody is detected when the level of the free labeled TNFα measured in step (~~e~~e) is decreased or absent compared to the level of the free labeled TNFα in the control sample.

19. The method of any one of claims 12 to 18, wherein the sample is serum.

20. The method of any one of claims 12 to 19, wherein the sample is obtained from a subject receiving therapy with the anti-TNFα drug.

21. The method of any one of claims 12 to 20, wherein the labeled anti-TNFα drug and the labeled TNFα comprise different fluorophores or fluorescent dyes.

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22. A method for determining whether a neutralizing form of an autoantibody to a first anti-TNFα drug is cross-reactive with a second anti-TNFα drug, the method comprising:

(a) detecting or measuring the presence, level, or percent of a neutralizing form of the autoantibody in a sample according to a method of any one of claims 1 to 21 to determine whether the sample is positive or negative for the neutralizing form of the autoantibody; and

if the sample is positive for the neutralizing form of the autoantibody, then:

(b) contacting the sample with a labeled second anti-TNFα drug to form a labeled complex of the labeled second anti-TNFα drug and the neutralizing form of the autoantibody;

(c) subjecting the labeled complex to size exclusion chromatography to separate the labeled complex; and

(d) detecting the labeled complex, thereby determining whether a neutralizing form of an autoantibody to a first anti-TNFα drug is cross-reactive with a second anti-TNFα drug.

23. The method of claim 22, wherein the first and second anti-TNFα drugs are indepedently selected from the group consisting of ~~REMICADE™ (~~infliximab~~)~~, ~~ENBREL™ (~~etanercept~~)~~, ~~HUMIRA™ (~~adalimumab~~)~~, ~~CIMZIA® (~~certolizumab pegol~~)~~, ~~SIMPONI® (~~golimumab~~;~~ (CNTO 148), and combinations thereof.

24. The method of claim 22 or 23, wherein the autoantibody to the first anti-TNFα drug is selected from the group consisting of a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), a human anti-mouse antibody (HAMA), and combinations thereof.

25. The method of any one of claims 22 to 24, wherein the presence of the labeled complex is an indication that the neutralizing autoantibody against the first anti-TNFα drug is cross-reactive with the second anti-TNFα drug.

26. The method of any one of claims 22 to 24, wherein the absence of the labeled complex is an indication that the neutralizing autoantibody against the first anti-TNFα drug is not cross-reactive with the second anti-TNFα drug.

27. The method of any one of claims 22 to 26, wherein the sample is serum.

28. The method of any one of claims 22 to 27, wherein the sample is obtained from a subject receiving therapy with the anti-TNFα drug.

29. The method of any one of claims 22 to 28, wherein the labeled second anti-TNFα drug comprises a fluorophore or fluorescent dye.

30. A method for monitoring or optimizing therapy to an anti-TNFα drug in a subject receiving a course of therapy with the anti-TNFα drug, the method comprising:

(a) detecting or measuring the presence, level, or percent of a neutralizing form of an autoantibody to the anti-TNFα drug according to a method of any one of claims 1 to 21 at a plurality of time points over the course of therapy;

(b) detecting a change in the presence, level, or percent of the neutralizing form of the autoantibody over time; and

(c) determining a subsequent dose of the course of therapy for the subject or whether a different course of therapy should be administered to the subject based upon the change in the presence, level, or percent of the neutralizing form of the autoantibody over time.

31. The method of claim 30, wherein the anti-TNFα drug is selected from the group consisting of ~~REMICADE™ (~~infliximab~~)~~, ~~ENBREL™ (~~etanercept~~)~~, ~~HUMIRA™ (~~adalimumab~~)~~, ~~CIMZIA®~~ ~~(~~certolizumab pegol~~)~~, ~~SIMPONI®~~ ~~(~~golimumab~~;~~ (CNTO 148), and combinations thereof.

32. The method of claim 30 or 31, wherein the autoantibody to the anti-TNFα drug is selected from the group consisting of a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), a human anti-mouse antibody (HAMA), and combinations thereof.

33. The method of any one of claims 30 to 32, wherein the subsequent dose of the course of therapy is increased, decreased, or maintained based upon the change in the presence, level, or percent of the neutralizing form of the autoantibody over time.

34. The method of any one of claims 30 to 32, wherein the different course of therapy comprises a different anti-TNFα drug, the current course of therapy along with an immunosuppressive agent, or switching to a course of therapy that is not an anti-TNFα drug.

35. The method of claim 34, wherein the different course of therapy is administered when the level or percent of the neutralizing form of the autoantibody increases over time.

36. A method for optimizing therapy and/or reducing toxicity in a subject receiving a course of therapy with a first anti-TNFα drug, the method comprising:

(a) determining whether a neutralizing form of an autoantibody to the first anti-TNFα drug is cross-reactive with a second anti-TNFα drug by detecting or measuring the presence, level, or percent of a neutralizing form of the autoantibody in a sample from the subject according to a method of any one of claims 1 to 21; and

(b) determining that a different course of therapy should be administered to the subject if the neutralizing form of the autoantibody is cross-reactive with the second anti-TNFα drug.

37. The method of claim 36, wherein the first and second anti-TNFα drugs are indepedently selected from the group consisting of ~~REMICADE™ (~~infliximab~~)~~, ~~ENBREL™ (~~etanercept~~)~~, ~~HUMIRA™ (~~adalimumab~~)~~, ~~CIMZIA® (~~certolizumab pegol~~)~~, ~~SIMPONI® (~~golimumab~~;~~ (CNTO 148), and combinations thereof.

38. The method of claim 36 or claim 37, wherein the autoantibody to the first anti-TNFα drug is selected from the group consisting of a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), a human anti-mouse antibody (HAMA), and combinations thereof.

39. The method of any one of claims 36 to 38, wherein the different course of therapy comprises switching to a course of therapy that is not an anti-TNFα drug.

40. The method of claim 39, wherein the non-anti-TNFα drug is selected from the group consisting of an IL-6 receptor-inhibiting monoclonal antibody, anti-integrin molecule, JAK-2 inhibitor, tyrosine kinase inhibitor, nutritition therapy, and mixtures thereof.