In one embodiment there is provided use of the binding protein as described herein in the manufacture of a medicament for reducing human TNF-α activity in a human subject suffering from a disorder in which TNF-α activity is detrimental.

In one embodiment there is provided use binding protein as described herein in the manufacture of a medicament for treating a patient suffering from a disorder in which TNF-α is detrimental wherein said medicament is to be administered either before, concurrent or after administration to the patient of a second agent, wherein the second agent is selected from the group consisting of an antibody, or fragment thereof, capable of binding human IL-12; PGEA; LPA; NGF, CGRP, SubP, RAGE; histamine; a histamine receptor blocker; bradykinin; IL-1aplha, IL-1beta, VEGF; PLGF; methotrexate; a corticosteroid, a glucocorticoid receptor modular; cyclosporine, rapamycin, FK506, and a non-steroidal anti-inflammatory agent.

In one embodiment there is provided a binding protein that specifically binds human TNFα, the binding protein comprising at least one heavy chain variable region (VH region) comprising three complementarity determining regions (CDRs) found within the amino acid sequence of SEQ ID NO: 1077, wherein the CDRs are CDR-H1, CDR-H2, and CDR-H3, wherein each amino acid in the CDR-H1, the CDR-H2 and the CDR-H3 is represented by a one letter code, wherein

the CDR-H1 has an amino acid sequence N-Y-G-M-N (residues 31-35 of SEQ ID NO:1077) or a modified amino acid sequence X1-Y-G-X2-X3, wherein

X1 is selected from the group consisting of: N, D, H, T, S, Q, and K;

X2 is selected from the group consisting of: M and I; and

X3 is selected from the group consisting of: N, I, T, Q, S, G, A, and V;

the CDR-H2 has an amino acid sequence W-I-N-T-Y-T-G-E-P-T-Y-A-D-D-F-K-G (residues 50-66 of SEQ ID NO:1077) or a modified amino acid sequence W-I-N-T-Y-X4-G- X5-P-X6-Y-A-X7-X8-X9- X10-G, wherein

X4 is selected from the group consisting of: T, N, and S;

X5 is selected from the group consisting of: E, K, V, Q, R, L, S, N, I, D, and A;

X6 is selected from the group consisting of: T, S, H, N, M, K, A, R, Q, and D;

X7 is selected from the group consisting of: D and Q;

X8 is selected from the group consisting of: D, G, and K;

X9 is selected from the group consisting of: F and L; and

X10 is selected from the group consisting of: K, T, and Q;

the CDR-H3 has an amino acid sequence K-F-L-T-T-V-V-V-T-D-Y-A-M-D-Y (residues 95-113 of SEQ ID NO:1077) or a modified amino acid sequence X11-X12-X13-X14-X15-X16 -X17-X18-X19-X20-X21-X22-M-D-Y, wherein

X11 is selected from the group consisting of: K, R, and N;

X12 is selected from the group consisting of: F, L, Y, I, V, and C;

X13 is selected from the group consisting of: L, F, M, R, S, W, Q, V, N, G, and C;

X14 is selected from the group consisting of: T, N, K, S, R, L, P, F, D, M, and A;

X15 is selected from the group consisting of: T, P, V, S, and A;

X16 is selected from the group consisting of: V, M, E, A, R, I, Q, G, and D;

X17 is selected from the group consisting of: V, D, A, E, S M, I, and P;

X18 is selected from the group consisting of: V, A, E, M, D, G, I, Q, P, N, L, and H;

X19 is selected from the group consisting of: T, S, M, and N;

X20 is selected from the group consisting of: D and E;

X21 is selected from the group consisting of: Y, N, S, C, H, D, I, F, V, L, and A; and

X22 is selected from the group consisting of: A, T, R, and C.

In one embodiment there is provided a binding protein that specifically binds human TNFα, the binding protein comprising at least one light chain variable regions (VL region) comprising three complementarity determining regions (CDRs) found within the amino acid sequence of SEQ ID NO:1078, wherein the CDRs are CDR-L1, CDR-L2, and CDR-L3, wherein each amino acid in the CDR-L1, the CDR-L2 and the CDR-L3 is represented by a one letter code, wherein

the CDR-L1 has an amino acid sequence R-A-S-Q-D-I-S-N-Y-L-N (residues 24-34 of SEQ ID NO:1078) or a modified amino acid sequence R-A-S-Q-X1-I-X2-X3-X4-L-N, wherein

X1 is selected from the group consisting of: D, N, E, H, G, V, R, and A;

X2 is selected from the group consisting of: S, Y, E, A, T, W, G, N, F, and C;

X3 is selected from the group consisting of: N, Q, S, K, T, H, D, and R; and

X4 is selected from the group consisting of: Y, V, F, and T;

the CDR-L2 has an amino acid sequence Y-T-S-R-L-Q-S (residues 50-56 of SEQ ID NO:1078) or a modified amino acid sequence X5- X6 -S- X7-L-Q-S, wherein

X5 is selected from the group consisting of: Y, F, and N;

X6 is selected from the group consisting of: T and A; and

X7 is selected from the group consisting of: R, L, K, and G; and

the CDR-L3 has an amino acid sequence Q-Q-G-N-T-L-P-P-T (residues 89-97 of SEQ ID NO:1078) or a modified amino acid sequence Q-Q-G- X8- X9-X10 -P- X11-T, wherein

X8 is selected from the group consisting of: N and I;

X9 is selected from the group consisting of: T, S, M, I, A, and R;

X10 is selected from the group consisting of: L, W, Q, P, M, R, F, G, V, Y, and A; and

X11 is selected from the group consisting of: P, T, S, and A.

In one embodiment there is provided a binding protein that specifically binds human TNFα, the binding protein comprising at least one heavy chain variable region (VH region) and at least one light chain variable region (VL region), wherein the VH region comprises three complementarity determining regions (CDRs) found within the amino acid sequence of SEQ ID NO: 1077, wherein the CDRs for the VH region are CDR-H1, CDR-H2, and CDR-H3, wherein each amino acid in the CDR-H1, the CDR-H2 and the CDR-H3 is represented by a one letter code, wherein

the CDR-H1 has an amino acid sequence N-Y-G-M-N (residues 31-35 of SEQ ID NO:1077) or a modified amino acid sequence X1-Y-G-X2-X3, wherein

X1 is selected from the group consisting of: N, D, H, T, S, Q, and K;

X2 is selected from the group consisting of: M and I; and

X3 is selected from the group consisting of: N, I, T, Q, S, G, A, and V;

the CDR-H2 has an amino acid sequence W-I-N-T-Y-T-G-E-P-T-Y-A-D-D-F-K-G (residues 50-66 of SEQ ID NO:1077) or a modified amino acid sequence W-I-N-T-Y-X4-G- X5-P-X6-Y-A-X7-X8-X9- X10-G, wherein

X4 is selected from the group consisting of: T, N, and S;

X5 is selected from the group consisting of: E, K, V, Q, R, L, S, N, I, D and A;

X6 is selected from the group consisting of: T, S, H, N, M, K, A, R, Q, and D;

X7 is selected from the group consisting of: D and Q;

X8 is selected from the group consisting of: D, G, and K;

X9 is selected from the group consisting of: F and L; and

X10 is selected from the group consisting of: K, T, and Q;

the CDR-H3 has an amino acid sequence K-F-L-T-T-V-V-V-T-D-Y-A-M-D-Y (residues 95-113 of SEQ ID NO:1077) or a modified amino acid sequence X11-X12-X13-X14-X15-X16 - X17-X18-X19-X20-X21-X22-M-D-Y, wherein

X11 is selected from the group consisting of: K, R and N;

X12 is selected from the group consisting of: F, L, Y, I, V, and C;

X13 is selected from the group consisting of: L F, M, R, S, W, Q, V, N, G, and C;

X14 is selected from the group consisting of: T, N, K, S, R, L, P, F, D, M, and A;

X15 is selected from the group consisting of: T, P, V, S, and A;

X16 is selected from the group consisting of: V, M, E, A, R, I, Q, G, and D;

X17 is selected from the group consisting of: V, D, A, E, S M, I, and P;

X18 is selected from the group consisting of: V, A, E, M, D, G, I, Q, P, N, L, and H;

X19 is selected from the group consisting of: T, S, M, and N;

X20 is selected from the group consisting of: D and E;

X21 is selected from the group consisting of: Y, N, S, C, H, D, I, F, V, L, and A; and

X22 is selected from the group consisting of: A, T, R, and C; and

the VL region comprises:

the amino acid sequences of the three CDRs from SEQ ID NO: 1078, wherein the CDRs for the VL region comprise CDR-L1, CDR-L2, and CDR-L3, wherein each amino acid in the CDR-L1, the CDR-L2 and the CDR-L3 is represented by the one letter code, wherein

the CDR-L1 has an amino acid sequence R-A-S-Q-D-I-S-N-Y-L-N (residues 24-34 of SEQ ID NO: 1078) or a modified amino acid sequence R-A-S-Q-X23-I-X24-X25-X26-L-N, wherein

X23 is selected from the group consisting of: D, N, E, H, G, V, R, and A;

X24 is selected from the group consisting of: S, Y, E, A, T, W, G, N, F, and C;

X25 is selected from the group consisting of: N, Q, S, K, T, H, D and R;

X26 is selected from the group consisting of: Y, V, F, and T; and

the CDR-L2 has an amino acid sequence Y-T-S-R-L-Q-S (residues 50-56 of SEQ ID NO: 1078) or a modified amino acid sequence X27- X28 -S- X29-L-Q-S, wherein

X27 is selected from the group consisting of: Y, F, and N;

X28 is selected from the group consisting of: T and A; and

X29 is selected from the group consisting of: R, L, K, and G; and

the CDR-L3 has an amino acid sequence Q-Q-G-N-T-L-P-P-T (residues 89-97 of SEQ ID NO: 1078) or a modified amino acid sequence Q-Q-G-X30- X31-X32 -P- X33-T, wherein

X30 is selected from the group consisting of: N and I;

X31 is selected from the group consisting of: T, S, M, I, A, and R;

X32 is selected from the group consisting of:L, W, Q, P, M, R, F, G, V, Y, and A; and

X33 is selected from the group consisting of: P, T, S, and A.

In one embodiment there is provided an isolated nucleic acid encoding a binding protein comprising the amino acid sequence as disclosed herein.

In one embodiment there is provided a vector comprising the isolated nucleic acid as disclosed herein.

In one embodiment there is provided a host cell comprising the vector as disclosed herein.

In one embodiment there is provided a method for producing a protein that binds TNF-α, the method comprising the steps of culturing the host cell as disclosed herein in a culture medium under conditions sufficient to produce a binding protein that binds TNF-α.

In one embodiment there is provided a pharmaceutical composition comprising the binding protein as disclosed herein, and a pharmaceutically acceptable carrier.

In one embodiment there is provided a method for determining the presence of TNF-α or a fragment thereof in a test sample by an immunoassay, wherein the immunoassay comprises contacting the test sample with at least one binding protein or fragment thereof as disclosed herein, and at least one detectable label.

Detailed Description of the Invention

Provided are TNF-α binding proteins, or antigen-binding portions thereof, that bind TNF-α, pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such binding proteins and fragments. Also provided are methods of using the binding proteins disclosed herein to detect human TNF-α, to inhibit human TNF-α either in vitro or in vivo, and to regulate gene expression or TNF-α related functions.

Claims

We claim:

1. A binding protein comprising at least one heavy chain variable region (VH region) comprising:

(a) three complementarity determining regions (CDRs) from any one of SEQ ID NOS: 22, 24, 26, 28, 30, 32, 34-58, 74-83, 94-266, 478-486, 496-675, 738-762, 778-956, 1053-1062, 1073, 1075, and 1077; or

(b) any one of SEQ ID NOS: 22, 24, 26, 28, 30, 32, 34-58, 74-83, 94-266, 478-486, 496-675, 738-762, 778-956, 1053-1062, 1073, 1075, and 1077.

2. A binding protein comprising at least one light chain variable regions (VL region) comprising:

(a) three complementarity determining regions (CDRs) from any one of SEQ ID NOS: 23, 25, 27, 29, 31, 33, 59-73, 84-93, 267-477, 487-495, 676-737, 763-777, 957-1052, 1063-1072, 1074, 1076, and 1078; or

(b) any one of SEQ ID NOS: 23, 25, 27, 29, 31, 33, 59-73, 84-93, 267-477, 487-495, 676-737, 763-777, 957-1052, 1063-1072, 1074, 1076, 1078.

3. A binding protein comprising at least one heavy chain variable region (VH region) and at least one light chain variable region (VL region), wherein the VH region comprises:

(a) three complementarity determining regions (CDRs) from any one of SEQ ID NOS: 22, 24, 26, 28, 30, 32, 34-58, 74-83, 94-266, 478-486, 496-675, 738-762, 778-956, 1053-1062, 1073, 1075, and 1077; or

(b) any one of SEQ ID NOS: 22, 24, 26, 28, 30, 32, 34-58, 74-83, 94-266, 478-486, 496-675, 738-762, 778-956, 1053-1062, 1073, 1075, and 1077; and

the VL region comprises:

(c) three CDRs from any one of SEQ ID NOS: 23, 25, 27, 29, 31, 33, 59-73, 84-93, 267-477, 487-495, 676-737, 763-777, 957-1052, 1063-1072, 1074, 1076, and 1078; or

(d) any one of SEQ ID NOS: 23, 25, 27, 29, 31, 33, 59-73, 84-93 , 267-477, 487-495, 676-737, 763-777, 957-1052, 1063-1072, 1074, 1076, 1078.

4. The binding protein of claim 3, wherein the binding protein comprises two VH regions and two VL regions.

5. The binding protein of claim 3, wherein the binding protein comprises at least one VH region and at least one VL region comprising a set of amino acid sequences selected from the

group consisting of SEQ ID NOS: 22 and 23; 24 and 25; 26 and 27; 28 and 29; 30 and 31; and 32 and 33.

6. The binding protein of claim 4, wherein the binding protein:

(a) modulates a biological function of TNF-α;

(b) neutralizes TNF-α;

(c) diminishes the ability of TNF-α to bind to its receptor;

(d) diminishes the ability of pro-human TNF-α, mature-human TNF-α, or truncated-human TNF-α to bind to its receptor; and/or

(e) reduces one or more of TNF-dependent cytokine production, TNF-dependent cell killing, TNF-dependent inflammation, TNF-dependent bone erosion, and TNF-dependent cartilage damage.

7. The binding protein of claim 4, wherein the binding protein has an on rate constant (Kon) of at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; or at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

8. The binding protein of claim 4, wherein the binding protein has an off rate constant (Koff) of at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about 10^{-5}s-1; or at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

9. The binding protein of claim 4, wherein the binding protein has a dissociation constant (KD) of at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; or at most 10^{-13} M.

10. A binding protein capable of binding human TNF-α, the binding protein comprising:

(a) a heavy chain constant region comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO: 3;

(b) a light chain constant region comprising an amino acid sequence of SEQ ID NO:4 or SEQ ID NO: 5;

(c) a heavy chain variable region (VH region) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 22, 24, 26, 28, 30, 32, 34-58, 74-83, 94-266, 478-486, 496-675, 738-762, 778-956, 1053-1062, 1073, 1075, and 1077; and

(d) a light chain variable region (VL region) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 23, 25, 27, 29, 31, 33, 59-73, 84-93, 267-477, 487-495, 676-737, 763-777, 957-1052, 1063-1072, 1074, 1076, and 1078.

11. The binding protein of claim 3, wherein the binding protein comprises an immunoglobulin molecule, an Fv, a disulfide linked Fv, a monoclonal antibody, an scFv, a chimeric binding protein, a single domain binding protein, a CDR-grafted binding protein, a diabody, a humanized binding protein, a multispecific binding protein, an Fab, a dual specific binding protein, an Fab' fragment, a bispecific binding protein, an F(ab')2 fragment, a DVD-Ig™, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, a Fd fragment consisting of the VH and CH1 domains, a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), or a single chain binding protein.

12. The binding protein of claim 4, wherein the binding protein is conjugated to an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

13. The binding protein of claim 4, wherein the binding protein further comprises a therapeutic or cytotoxic agent selected from the group consisting of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, an apoptotic agent, and at least one additional therapeutic agent for treating a disorder in which TNF-α activity is detrimental.

14. An isolated nucleic acid encoding a binding protein comprising the amino acid sequence of claim 3.

15. A vector comprising the isolated nucleic acid of claim 14.

16. A host cell comprising the vector of claim 15.

17. A method for producing a protein that binds TNF-α, the method comprising the steps of culturing the host cell of claim 16 in a culture medium under conditions sufficient to produce a binding protein that binds TNF-α.

18. A pharmaceutical composition comprising the binding protein of claim 3, and a pharmaceutically acceptable carrier.

19. Use of an effective amount of the pharmaceutical composition of claim 18, in the manufacture of a medicament for treating a mammal.

20. Use of the binding protein of claim 3 in the manufacture of a medicament for reducing human TNF-α activity.

21. Use of the binding protein of claim 3 in the manufacture of a medicament for reducing human TNF-α activity in a human subject suffering from a disorder in which TNF-α activity is detrimental

22. Use of the binding protein of claim 3in the manufacture of a medicament for treating a patient suffering from a disorder in which TNF-α is detrimental, wherein said medicament is to be administered either before, concurrent, or after the administration to the patient of a second agent, wherein the second agent is selected from the group consisting of an antibody, or fragment thereof, capable of binding human IL-12; PGE2; LPA; NGF; CGRP; SubP; RAGE; histamine; a histamine receptor blocker; bradykinin; IL-1alpha; IL-1beta; VEGF; PLGF; methotrexate; a corticosteroid, a glucocorticoid receptor modulator; cyclosporin, rapamycin, FK506, and a non-steroidal anti-inflammatory agent.

23. The use of claim 21 or 22, wherein the disorder is an autoimmune and/or inflammatory disorder.

24. The use of claims 23, wherein the disorder is selected from the group consisting of Crohn's disease, psoriasis, plaque psoriasis, arthritis, rheumatoid arthritis, psoratic arthritis, osteoarthritis, or juvenile idiopathic arthritis, multiple sclerosis, and ankylosing spondylitis.

25. The use of claim 23, wherein the disorder is selected from the group consisting of a respiratory disorder; asthma; allergic and nonallergic asthma; asthma due to infection; asthma

due to infection with respiratory syncytial virus (RSV); chronic obstructive pulmonary disease (COPD); a condition involving airway inflammation; eosinophilia; fibrosis and excess mucus production; cystic fibrosis; pulmonary fibrosis; an atopic disorder; atopic dermatitis; urticaria; eczema; allergic rhinitis; allergic enterogastritis; an inflammatory and/or autoimmune condition of the skin; an inflammatory and/or autoimmune condition of gastrointestinal organs; inflammatory bowel diseases (IBD); ulcerative colitis; Crohn's disease; an inflammatory and/or autoimmune condition of the liver; liver cirrhosis; liver fibrosis; liver fibrosis caused by hepatitis B and/or C virus; scleroderma; tumors or cancers; hepatocellular carcinoma;

glioblastoma; lymphoma; Hodgkin's lymphoma; a viral infection; a bacterial infection; a parasitic infection; HTLV-1 infection; suppression of expression of protective type 1 immune responses, and suppression of expression of a protective type 1 immune response during vaccination.

26. A method for determining the presence of TNF-α or a fragment thereof in a test sample by an immunoassay, wherein the immunoassay comprises contacting the test sample with at least one binding protein or fragment thereof according to claim 3, and at least one detectable label.

27. The method of claim 26, wherein the method further comprises:
(i) contacting the test sample with the at least one binding protein or fragment thereof, wherein the binding protein binds to an epitope on the TNF-α or fragment thereof so as to form a first complex;
(ii) contacting the complex with the at least one detectable label, wherein the detectable label binds to an epitope on the first complex, or on the TNF-α or fragment thereof, that is not bound by the binding protein or fragment thereof, to form a second complex; and
(iii) detecting the presence of the TNF-α or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence of the TNF-α or fragment thereof is directly correlated with the signal generated by the detectable label.

28. The method of claim 26, wherein the method further comprises:
(i) contacting the test sample with the at least one binding protein or fragment thereof, wherein the binding protein or fragment thereof binds to an epitope on the TNF-α or fragment thereof so as to form a first complex;
(ii) contacting the complex with the at least one detectable label, wherein the detectable label competes with the TNF-α or fragment thereof for binding to the binding protein or fragment thereof so as to form a second complex; and
(iii) detecting the presence of the TNF-α or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence of the TNF-α or fragment thereof is indirectly correlated with the signal generated by the detectable label.

29. The method of claim 26, wherein the method optionally further comprises diagnosing, prognosticating, or assessing the efficiency of therapeutic/prophylactic treatment of the patient.

CLAIMS

1. A binding protein that specifically binds human TNFα, the binding protein comprising at least one heavy chain variable region (VH region) comprising three complementarity determining regions (CDRs) found within the amino acid sequence of SEQ ID NO: 1077, wherein the CDRs are CDR-H1, CDR-H2, and CDR-H3, wherein each amino acid in the CDR-H1, the CDR-H2 and the CDR-H3 is represented by a one letter code, wherein

the CDR-H1 has an amino acid sequence N-Y-G-M-N (residues 31-35 of SEQ ID NO:1077) or a modified amino acid sequence X1-Y-G-X2-X3, wherein

X1 is selected from the group consisting of: N, D, H, T, S, Q, and K;

X2 is selected from the group consisting of: M and I; and

X3 is selected from the group consisting of: N, I, T, Q, S, G, A, and V;

the CDR-H2 has an amino acid sequence W-I-N-T-Y-T-G-E-P-T-Y-A-D-D-F-K-G (residues 50-66 of SEQ ID NO:1077) or a modified amino acid sequence W-I-N-T-Y-X4-G- X5-P-X6-Y-A-X7-X8-X9- X10-G, wherein

X4 is selected from the group consisting of: T, N, and S;

X5 is selected from the group consisting of: E, K, V, Q, R, L, S, N, I, D, and A;

X6 is selected from the group consisting of: T, S, H, N, M, K, A, R, Q, and D;

X7 is selected from the group consisting of: D and Q;

X8 is selected from the group consisting of: D, G, and K;

X9 is selected from the group consisting of: F and L; and

X10 is selected from the group consisting of: K, T, and Q;

the CDR-H3 has an amino acid sequence K-F-L-T-T-V-V-V-T-D-Y-A-M-D-Y (residues 95-113 of SEQ ID NO:1077) or a modified amino acid sequence X11-X12-X13-X14-X15-X16 -X17-X18-X19-X20-X21-X22-M-D-Y, wherein

X11 is selected from the group consisting of: K, R, and N;

X12 is selected from the group consisting of: F, L, Y, I, V, and C;

X13 is selected from the group consisting of: L, F, M, R, S, W, Q, V, N, G, and C;

X14 is selected from the group consisting of: T, N, K, S, R, L, P, F, D, M, and A;

X15 is selected from the group consisting of: T, P, V, S, and A;

X16 is selected from the group consisting of: V, M, E, A, R, I, Q, G, and D;

X17 is selected from the group consisting of: V, D, A, E, S M, I, and P;

X18 is selected from the group consisting of: V, A, E, M, D, G, I, Q, P, N, L, and H;

X19 is selected from the group consisting of: T, S, M, and N;

X20 is selected from the group consisting of: D and E;

X21 is selected from the group consisting of: Y, N, S, C, H, D, I, F, V, L, and A; and

X22 is selected from the group consisting of: A, T, R, and C.

2. A binding protein that specifically binds human TNFα, the binding protein comprising at least one light chain variable regions (VL region) comprising three complementarity determining regions (CDRs) found within the amino acid sequence of SEQ ID NO:1078, wherein the CDRs are CDR-L1, CDR-L2, and CDR-L3, wherein each amino acid in the CDR-L1, the CDR-L2 and the CDR-L3 is represented by a one letter code, wherein

the CDR-L1 has an amino acid sequence R-A-S-Q-D-I-S-N-Y-L-N (residues 24-34 of SEQ ID NO:1078) or a modified amino acid sequence R-A-S-Q-X1-I-X2-X3-X4-L-N, wherein

X1 is selected from the group consisting of: D, N, E, H, G, V, R, and A;

X2 is selected from the group consisting of: S, Y, E, A, T, W, G, N, F, and C;

X3 is selected from the group consisting of: N, Q, S, K, T, H, D, and R; and

X4 is selected from the group consisting of: Y, V, F, and T;

the CDR-L2 has an amino acid sequence Y-T-S-R-L-Q-S (residues 50-56 of SEQ ID NO:1078) or a modified amino acid sequence X5- X6 -S- X7-L-Q-S, wherein

X5 is selected from the group consisting of: Y, F, and N;

X6 is selected from the group consisting of: T and A; and

X7 is selected from the group consisting of: R, L, K, and G; and

the CDR-L3 has an amino acid sequence Q-Q-G-N-T-L-P-P-T (residues 89-97 of SEQ ID NO:1078) or a modified amino acid sequence Q-Q-G- X8- X9-X10 -P- X11-T, wherein

X8 is selected from the group consisting of: N and I;

X9 is selected from the group consisting of: T, S, M, I, A, and R;

X10 is selected from the group consisting of: L, W, Q, P, M, R, F, G, V, Y, and A; and

X11 is selected from the group consisting of: P, T, S, and A.

3. A binding protein that specifically binds human TNFα, the binding protein comprising at least one heavy chain variable region (VH region) and at least one light chain variable region (VL region), wherein the VH region comprises three complementarity determining regions (CDRs) found within the amino acid sequence of SEQ ID NO: 1077, wherein the CDRs for the VH region are CDR-H1, CDR-H2, and CDR-H3, wherein each amino acid in the CDR-H1, the CDR-H2 and the CDR-H3 is represented by a one letter code, wherein

the CDR-H1 has an amino acid sequence N-Y-G-M-N (residues 31-35 of SEQ ID NO:1077) or a modified amino acid sequence X1-Y-G-X2-X3, wherein

X1 is selected from the group consisting of: N, D, H, T, S, Q, and K;

X2 is selected from the group consisting of: M and I; and

X3 is selected from the group consisting of: N, I, T, Q, S, G, A, and V;

the CDR-H2 has an amino acid sequence W-I-N-T-Y-T-G-E-P-T-Y-A-D-D-F-K-G (residues 50-66 of SEQ ID NO:1077) or a modified amino acid sequence W-I-N-T-Y-X4-G- X5-P-X6-Y-A-X7-X8-X9- X10-G, wherein

X4 is selected from the group consisting of: T, N, and S;

X5 is selected from the group consisting of: E, K, V, Q, R, L, S, N, I, D and A;

X6 is selected from the group consisting of: T, S, H, N, M, K, A, R, Q, and D;

X7 is selected from the group consisting of: D and Q;

X8 is selected from the group consisting of: D, G, and K;

X9 is selected from the group consisting of: F and L; and

X10 is selected from the group consisting of: K, T, and Q;

the CDR-H3 has an amino acid sequence K-F-L-T-T-V-V-V-T-D-Y-A-M-D-Y (residues 95-113 of SEQ ID NO:1077) or a modified amino acid sequence X11-X12-X13-X14-X15-X16 -X17-X18-X19-X20-X21-X22-M-D-Y, wherein

X11 is selected from the group consisting of: K, R and N;

X12 is selected from the group consisting of: F, L, Y, I, V, and C;

X13 is selected from the group consisting of: L F, M, R, S, W, Q, V, N, G, and C;

X14 is selected from the group consisting of: T, N, K, S, R, L, P, F, D, M, and A;

X15 is selected from the group consisting of: T, P, V, S, and A;

X16 is selected from the group consisting of: V, M, E, A, R, I, Q, G, and D;

X17 is selected from the group consisting of: V, D, A, E, S M, I, and P;

X18 is selected from the group consisting of: V, A, E, M, D, G, I, Q, P, N, L, and H;

X19 is selected from the group consisting of: T, S, M, and N;

X20 is selected from the group consisting of: D and E;

X21 is selected from the group consisting of: Y, N, S, C, H, D, I, F, V, L, and A; and

X22 is selected from the group consisting of: A, T, R, and C; and

the VL region comprises:

the amino acid sequences of the three CDRs from SEQ ID NO: 1078, wherein the CDRs for the VL region comprise CDR-L1, CDR-L2, and CDR-L3, wherein each amino acid in the CDR-L1, the CDR-L2 and the CDR-L3 is represented by the one letter code, wherein

the CDR-L1 has an amino acid sequence R-A-S-Q-D-I-S-N-Y-L-N (residues 24-34 of SEQ ID NO: 1078) or a modified amino acid sequence R-A-S-Q-X23-I-X24-X25-X26-L-N, wherein

X23 is selected from the group consisting of: D, N, E, H, G, V, R, and A;

X24 is selected from the group consisting of: S, Y, E, A, T, W, G, N, F, and C;

X25 is selected from the group consisting of: N, Q, S, K, T, H, D and R;

X26 is selected from the group consisting of: Y, V, F, and T; and

the CDR-L2 has an amino acid sequence Y-T-S-R-L-Q-S (residues 50-56 of SEQ ID NO: 1078) or a modified amino acid sequence X27- X28 -S- X29-L-Q-S, wherein

X27 is selected from the group consisting of: Y, F, and N;

X28 is selected from the group consisting of: T and A; and

X29 is selected from the group consisting of: R, L, K, and G; and

the CDR-L3 has an amino acid sequence Q-Q-G-N-T-L-P-P-T (residues 89-97 of SEQ ID NO: 1078) or a modified amino acid sequence Q-Q-G-X30- X31-X32 -P- X33-T, wherein

X30 is selected from the group consisting of: N and I;

X31 is selected from the group consisting of: T, S, M, I, A, and R;

X32 is selected from the group consisting of:L, W, Q, P, M, R, F, G, V, Y, and A; and

X33 is selected from the group consisting of: P, T, S, and A.

4. The binding protein of claim 3, wherein the binding protein comprises two VH regions and two VL regions.

5. The binding protein of claim 4, wherein the binding protein:

(a) modulates a biological function of TNF-α;

(b) neutralizes TNF-α;

(c) diminishes the ability of TNF-α to bind to its receptor;

(d) diminishes the ability of pro-human TNF-α, mature-human TNF-α, or truncated-human TNF-α to bind to its receptor; and/or

(e) reduces one or more of TNF-dependent cytokine production, TNF-dependent cell killing, TNF-dependent inflammation, TNF-dependent bone erosion, and TNF-dependent cartilage damage.

6. The binding protein of claim 4, wherein the binding protein has an on rate constant (Kon) of at least about $10^2 M^{-1}s^{-1}$; at least about $10^3 M^{-1}s^{-1}$; at least about $10^4 M^{-1}s^{-1}$; at least about $10^5 M^{-1}s^{-1}$; or at least about $10^6 M^{-1}s^{-1}$, as measured by surface plasmon resonance.

7. The binding protein of claim 4, wherein the binding protein has an off rate constant (Koff) of at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about 10^{-5}s-1; or at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

8. The binding protein of claim 4, wherein the binding protein has a dissociation constant (KD) of at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; or at most 10^{-13} M.

9. The binding protein of claim 3, wherein the binding protein further comprising:

(a) a heavy chain constant region comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO: 3; and

(b) a light chain constant region comprising an amino acid sequence of SEQ ID NO:4 or SEQ ID NO: 5.

10. The binding protein of claim 1, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from any one of SEQ ID NOS: 74-83.

11. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 74.

12. The binding protein of claim 10, wherein the least one heavy chain VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 75.

13. The binding protein of claim 10, wherein the at least one heavy chain VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 76.

14. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 77.

15. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 78.

16. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 79.

17. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 80.

18. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 81.

19. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 82.

20. The binding protein of claim 10, wherein the at least one VH region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 83.

21. The binding protein of claim 2, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from any one of SEQ ID NOS: 84-93.

22. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 84.

23. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 85.

24. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 86.

25. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 87.

26. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 88.

27. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 89.

28. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 90.

29. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 91.

30. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 92.

31. The binding protein of claim 21, wherein the at least one VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 93.

32. The binding protein of claim 3, wherein

the VH region comprises the amino acid sequences of the three complementarity determining regions (CDRs) from any one of SEQ ID NOS: 74-83, and 778-956; and

the VL region comprises the amino acid sequences of the three CDRs from any one of SEQ ID NOS: 84-93 and 957-1052.

33. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 74 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 84.

34. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 75 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 85.

35. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 76 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 86.

36. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 77 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 87.

37. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 78 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 88.

38. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 79 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 89.

39. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 80 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 90.

40. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 81 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 91.

41. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 82 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 92.

42. The binding protein of claim 32, wherein the VH region comprises the amino acid sequences of the three CDRs of SEQ ID NO: 83 and the VL region comprises the amino acid sequences of the three CDRs from SEQ ID NO: 93.

43. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 74 and the VL region comprises the amino acid sequence of SEQ ID NO: 84.

44. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 75 and the VL region comprises the amino acid sequences of three CDRs from SEQ ID NO: 85

45. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 76 and the VL region comprises the amino acid sequence of SEQ ID NO: 86.

46. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 77 and the VL region comprises the amino acid sequence of SEQ ID NO: 87.

47. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 78 and the VL region comprises the amino acid sequence of SEQ ID NO: 88.

48. The binding protein of claim 32, wherein the VH region comprises the sequence of SEQ ID NO: 79 and the VL region comprises the amino acid sequence of SEQ ID NO: 89.

49. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 80 and the VL region comprises the amino acid sequence of SEQ ID NO: 90.

50. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 81 and the VL region comprises the amino acid sequence of SEQ ID NO: 91.

51. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 82 and the VL region comprises the amino acid sequence of SEQ ID NO: 92.

52. The binding protein of claim 32, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 83 and the VL region comprises the amino acid sequence of SEQ ID NO: 93.

53. The binding protein of claim 1, wherein the binding protein comprises the amino acid sequence of SEQ ID NO:1077.

54. The binding protein of claim 2, wherein the binding protein comprises the amino acid sequence of SEQ ID NO:1078.

55. The binding protein of claim 3, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 1077 and the VL region comprises the amino acid sequence of SEQ ID NO:1078.

56. The binding protein of claim 1, wherein the at least one VH region comprises the amino acid sequence of any one of SEQ ID NOS: 74-83 and 778-956.

57. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 74.

58. The binding protein of claim 56, wherein the least one VH region comprises the amino acid sequence of SEQ ID NO: 75.

59. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 76.

60. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 77.

61. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 78.

62. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 79.

63. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 80.

64. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 81.

65. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 82.

66. The binding protein of claim 56, wherein the at least one VH region comprises the amino acid sequence of SEQ ID NO: 83.

67. The binding protein of claim 2, wherein the at least one VL region comprises the amino acid sequence any one of SEQ ID NOS: 84-93 and 957-1052.

68. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 84.

69. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 85.

70. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 86.

71. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 87.

72. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 88.

73. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 89.

74. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 90.

75. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 91.

76. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 92.

77. The binding protein of claim 67, wherein the at least one VL region comprises the amino acid sequence of SEQ ID NO: 93.

78. The binding protein of claim 67, wherein the VH region comprises the amino acid sequence of any one of SEQ ID NOS: 74-83 and 778-956; and the VL region comprises the amino acid sequence of any one of SEQ ID NOS: 84-93 and 957-1052.

79. The binding protein of any of claims 3-9 and 32-52, wherein the binding protein comprises an immunoglobulin molecule, an Fv, a disulfide linked Fv, a monoclonal antibody, an scFv, a chimeric binding protein, a single domain binding protein, a CDR-grafted binding protein, a diabody, a humanized binding protein, a multispecific binding protein, an Fab, a dual specific binding protein, an Fab' fragment, a bispecific binding protein, an F(ab')2 fragment, a DVD-Ig™, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, a Fd fragment consisting of the VH and CH1 domains, a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), or a single chain binding protein.

80. The binding protein of any of claims 3-9 and 32-52, wherein the binding protein is conjugated to an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

81. The binding protein of any of claims 3-9 and 32-52, wherein the binding protein further comprises a therapeutic or cytotoxic agent selected from the group consisting of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, an apoptotic agent, and at least one additional therapeutic agent for treating an autoimmune or inflammatory disorder in which TNF-α activity is detrimental.

82. An isolated nucleic acid encoding a binding protein comprising the amino acid sequence of any of claims 3-9 and 32-52.

83. A vector comprising the isolated nucleic acid of claim 82.

84. A host cell comprising the vector of claim 83.

85. A method for producing a protein that binds TNF-α, the method comprising the steps of culturing the host cell of claim 84 in a culture medium under conditions sufficient to produce a binding protein that binds TNF-α.

86. A pharmaceutical composition comprising the binding protein of any of claims 3-9 and 32-52, and a pharmaceutically acceptable carrier.

87. Use of an effective amount of the pharmaceutical composition of claim 86, in the manufacture of a medicament for treating a mammal.

88. Use of the binding protein of any of claims 3-9 and 32-52 in the manufacture of a medicament for reducing TNF-α activity.

89. Use of the binding protein of any of claims 3-9 and 32-52 in the manufacture of a medicament for reducing human TNF-α activity in a human subject suffering from an autoimmune or inflammatory disorder in which TNF-α activity is detrimental.

90. Use of the binding protein of any of claims 3-9 and 32-52 in the manufacture of a medicament for treating a patient suffering from an autoimmune or inflammatory disorder in which TNF-α is detrimental, wherein said medicament is to be administered either before, concurrent, or after the administration to the patient of a second agent, wherein the second agent is selected from the group consisting of an antibody, or fragment thereof, capable of binding human IL-12; PGE2; LPA; NGF; CGRP; SubP; RAGE; histamine; a histamine receptor blocker; bradykinin; IL-1alpha; IL-1beta; VEGF; PLGF; methotrexate; a corticosteroid, a glucocorticoid receptor modulator; cyclosporin, rapamycin, FK506, and a non-steroidal anti-inflammatory agent.

91. The use of claim 89 or 90, wherein the disorder is an autoimmune and/or inflammatory disorder.

92. The use of claim 91, wherein the disorder is selected from the group consisting of Crohn's disease, psoriasis, plaque psoriasis, arthritis, rheumatoid arthritis, psoratic arthritis, osteoarthritis, or juvenile idiopathic arthritis, multiple sclerosis, and ankylosing spondylitis.

93. The use of claim 91, wherein the disorder is selected from the group consisting of a respiratory disorder; asthma; allergic and nonallergic asthma; asthma due to infection; asthma due to infection with respiratory syncytial virus (RSV); chronic obstructive pulmonary disease (COPD); a condition involving airway inflammation; eosinophilia; fibrosis and excess mucus production; cystic fibrosis; pulmonary fibrosis; an atopic disorder; atopic dermatitis; urticaria; eczema; allergic rhinitis; allergic enterogastritis; an inflammatory and/or autoimmune condition of the skin; an inflammatory and/or autoimmune condition of gastrointestinal organs; inflammatory bowel diseases (IBD);

ulcerative colitis; Crohn's disease; an inflammatory and/or autoimmune condition of the liver; liver cirrhosis; liver fibrosis; liver fibrosis caused by hepatitis B and/or C virus; scleroderma; tumors or cancers; hepatocellular carcinoma; glioblastoma; lymphoma; Hodgkin's lymphoma; a viral infection; a bacterial infection; a parasitic infection; HTLV-1 infection; suppression of expression of protective type 1 immune responses; and suppression of expression of a protective type 1 immune response during vaccination.

94. A method for determining the presence of TNF-α or a fragment thereof in a test sample by an immunoassay, wherein the immunoassay comprises contacting the test sample with at least one binding protein or fragment thereof according to any of claims 3-9 and 32-52, and at least one detectable label.

95. The method of claim 94, wherein the method further comprises:
(i) contacting the test sample with the at least one binding protein or fragment thereof, wherein the binding protein binds to an epitope on the TNF-α or fragment thereof so as to form a first complex;
(ii) contacting the complex with the at least one detectable label, wherein the detectable label binds to an epitope on the first complex, or on the TNF-α or fragment thereof, that is not bound by the binding protein or fragment thereof, to form a second complex; and
(iii) detecting the presence of the TNF-α or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence of the TNF-α or fragment thereof is directly correlated with the signal generated by the detectable label.

96. The method of claim 94, wherein the method further comprises:
(i) contacting the test sample with the at least one binding protein or fragment thereof, wherein the binding protein or fragment thereof binds to an epitope on the TNF-α or fragment thereof so as to form a first complex;
(ii) contacting the complex with the at least one detectable label, wherein the detectable label competes with the TNF-α or fragment thereof for binding to the binding protein or fragment thereof so as to form a second complex; and
(iii) detecting the presence of the TNF-α or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence of the TNF-α or fragment thereof is indirectly correlated with the signal generated by the detectable label.

97. The method of claim 96, wherein the method further comprises diagnosing, prognosticating, or assessing the efficiency of therapeutic/prophylactic treatment of the patient.