Claims

1. A method of determining profiles of viable cells, comprising:

providing a plurality of microwells each dimensioned to hold a subnanoliter volume of liquid, wherein the microwells contain cells from a subject and, on average, contain no more than one single cell per microwell;

performing a first contacting step when cells in a first portion of the microwells experience a first treatment condition, which first contacting step involves contacting the first portion of the microwells with a first substrate, wherein the first substrate is pretreated with one or more detection agents that bind to one or more secreted products produced by at least one of the cells, to yield a first printed array;

performing a second contacting step when cells in a second portion of the microwells experience a second treatment condition, which second contacting step involves contacting the second portion of the microwells with a second substrate, wherein the second substrate is pretreated with the detection agents of the first substrate, to yield a second printed array;

quantifying levels of the secreted products bound to the printed arrays, thereby determining the profiles of the single cells, so that differences in the profiles associated with differences between the first and second treatment conditions are determined.

2. The method of claim 1, wherein the profile is a Th_1 profile, a Th_2 profile, a Th_9 profile, a Th_{17} profile, a Th profile, or an antibody isotype level.

3. The method of claim 1, wherein the detection agents detect a T cell panel of secreted products.

4. The method of claim 1, wherein the detection agents detect a plurality of cytokines, the cytokines being selected from the group consisting of IL-17, IL-10, IL-4, and IFN-γ.

5. The method of claim 1, wherein the detection agents detect a T-helper 2 (Th_2) panel of secreted products.

6. The method of claim 1, wherein the detection agents detect a plurality of cytokines, said cytokines being selected from the group consisting of IL-4, IL-5, IL-13, and IL-9.

7. The method of claim 1, wherein the detection agents detect a cytotoxic T lymphocyte (CTL) cell or Th_1 panel of secreted products.

8. The method of claim 1, wherein the detection agents detect a plurality of CTL or Th_1 panel products, said products being selected from the group consisting of IFNγ, MIP-1β, TNFα, perforin, and IL-2.

9. The method of claim 1, wherein the detection agents detect an antibody isotype selected from the group consisting of IgE, IgG_1, IgG_4 and IgA.

10. The method of claim 1, wherein the detection agents detect antibody isotype selected from the group consisting of IgG_3, IgG_1, IgM, and IgA.

11. The method of claim 1, wherein the cells are whole blood cells from said subject.

12. The method of claim 1, wherein the cells are peripheral blood mononuclear cells (PBMC).

13. The method of claim 1, wherein the cells are stimulated prior to depositing cells on the plurality of microwells.

14. The method of claim 1, wherein the cells are stimulated with a suspected or known allergen.

15. The method of claim 14, wherein said allergen is a food product.

16. The method of claim 15, wherein said food product is milk, egg, peanut, tree nut, fish, shellfish, soy, or wheat, an egg products, a legumes, seafood or shellfish.

17. The method of claim 14, wherein said allergen is a drug.

18. The method of claim 17, wherein said drug is amoxicillin, penicillin, a sulfa drug, a barbiturate, anticonvulsants, insulin, of iodine.

19. The method of claim 14, wherein said allergen is dust, pollen, pet dander, latex, or chlorine, or venom associated with an insect bite.

20. The method of claim 14, wherein said insect bite is from a wasp, fire ant or bee sting.

21. The method of claim 1, wherein the step of determining the profiles comprises determining a rate of secretion of said secreted product from said cell.

22. The method of claim 1, further comprising determining the phenotype or lineage of said cell.

23. The method of claim 22, further matching said phenotype or lineage with said level of secreted products.

24. The method of claim 1, wherein the profile is an immune profile.

25. The method of claim 1, wherein the step of quantifying comprising imaging said printed array to yield a dataset; filtering said dataset to identify locations on said array consisting of a single cell; and matching said location with levels of secreted products detected from said single cell locations, thereby determining the profile of the single cells.

26. The method of claim 1, wherein the detection agents identify an antibody isotype.

27. The method of claim 26, wherein an increase in a level of IgE isotype compared to other antibody isotype indicates that subject is allergic or is at risk of developing an allergy to said allergen.

28. The method of claim 26, wherein an increase in a level of IgE isotype compared to a level of IgG_4 isotype indicates that subject is allergic or is at risk of developing an allergy to said

allergen.

29. The method of claim 1, wherein the detection agents identify a cytokine.

30. The method of claim 29, wherein an increase in the level of a Th2 cytokine compared to a level of a Th1 cytokine indicates that subject is allergic or is at risk of developing an allergy to said allergen.

31. The method of claim 30, wherein the Th2 cytokine comprises IL-4.

32. The method of claim 30, wherein the Th1 cytokine comprises IFNγ.

33. The method of claim 29, further comprising establishing a cytokine profile indicative of an autoimmune disease or infectious disease.

34. The method of claim 33, wherein the cytokine profile indicative of an autoimmune disease or infectious disease comprises an increase in a level of IFNγ or IL-2 compared to a normal IFNγ or IL-2.

Claims:

1. A method of determining a secreted cytokine profile in a subject, the method comprising:

providing a suspension of live peripheral blood mononuclear cells from a subject deposited onto a moldable slab containing at least one microwell in a microwell array, wherein at least one microwell in said microwell array contains a single cell in a subnanoliter volume;

contacting the microwell array with a substrate, wherein the substrate is pretreated with at least two detection agents and wherein each said detection agent binds to a secreted cytokine of said cell;

measuring a level of each said secreted cytokine on said substrate, said level corresponding to an amount of said secreted cytokine of said single cell; and

determining a rate of secretion of each said secreted cytokine from said cell;

thereby determining said secreted cytokine profile;

wherein the method further comprises measuring a dynamic change in said secreted cytokine profile.

2. A method of determining a profile of viable T cells, comprising:

(a) performing a first contacting step at a first time point, wherein the first contacting step comprises contacting a slab with a first substrate,

wherein the slab contains an array of microwells, each microwell dimensioned to hold a subnanoliter volume of liquid, wherein microwells in the array of microwells contain viable T cells and products secreted from said T cells, and, on average, contain no more than one single T cell or a few T cells per microwell;

wherein the first substrate is pretreated with a plurality of first detection agents, wherein said plurality of first detection agents comprises first detection agents that respectively bind to different cytokines, whereby cytokines secreted by said T cells that are present in said microwells and are binding partners of a first detection agent are bound to the

first detection agent in regions on the first substrate contacted by contents of the microwells, to yield a first printed array;

(b) after step (a), performing a second contacting step at a second time point, which second contacting step comprises contacting the slab with a second substrate,

wherein the second substrate is pretreated with a plurality of second detection agents, wherein said plurality of second detection agents comprises second detection agents that are identical to said first detection agents that respectively bind to different cytokines, whereby cytokines secreted by said T cells that are present in said microwells and are binding partners of a second detection agent are bound to the second detection agent in regions on the second substrate contacted by contents of the microwells, to yield a second printed array;

(c) quantifying levels of said different cytokines bound to the first printed array and levels of said different cytokines bound to the second printed array, and

(d) determining rates of secretion of the different cytokines bound to the first printed array and rates of secretion of the different cytokines bound to the second printed array, thereby determining the profile of the T cells.

3. The method of claim 1, wherein said secreted cytokines are Th_1, Th_2, Th_9, Th_{17}, or Th cytokines.

4. The method of claim 1 or claim 3, wherein the substrate is pretreated with at least three detection agents.

5. The method of claim 4, wherein the substrate is pretreated with at least four detection agents.

6. The method of any one of claims 1, 3, 4, and 5, wherein said secreted cytokines are selected from the group consisting of IL-17, IL-10, IL-4, and IFN-γ.

7. The method of any one of claims 1, 3, 4, and 5, wherein said secreted cytokines are Th_2 cytokines.

8. The method of any one of claims 1, 3, 4, and 5, wherein said secreted cytokines comprise IL-4, IL-5, IL-13, and IL-9.

9. The method of any one of claims 1, 3, 4, and 5, wherein said secreted cytokines are cytotoxic T lymphocyte (CTL) or Th_1 cytokines.

10. The method of any one of claims 1, 3, 4, and 5, wherein said secreted cytokines are selected from the group consisting of IFNγ, MIP-1β, TNFα, perforin, and IL-2.

11. The method of claim 2, wherein said different cytokines are Th_1, Th_2, Th_9, Th_{17}, or Th cytokines.

12. The method of claim 2 or claim 11, wherein said different cytokines are selected from the group consisting of IL-17, IL-10, IL-4, and IFN-γ.

13. The method of claim 2 or claim 11, wherein said different cytokines are Th_2 cytokines.

14. The method of claim 2 or claim 11, wherein said different cytokines comprise IL-4, IL-5, IL-13, and IL-9.

15. The method of claim 2 or claim 11, wherein said different cytokines are cytotoxic T lymphocyte (CTL) or Th_1 cytokines.

16. The method of claim 2 or claim 11, wherein said different cytokines are selected from the group consisting of IFNγ, MIP-1β, TNFα, perforin, and IL-2.

17. The method of any one of claims 1 to 16, which comprises an initial step of stimulating the cells.

18. The method of any one of claims 1 to 17, wherein the cells are stimulated with a suspected or known allergen.

19. The method of any one of claims 1 to 18, further comprising a step of determining correlation coefficients for co-secretion of cytokines within the multiple cytokines.

20. The method of claim 2, wherein the first and second time points are within a temporal regime during which the amount of captured cytokine approximates the total amount of that cytokine secreted by the relevant T cell.

21. The method of claim 2, wherein the first and second time points are within 30 minutes to 20 hours of deposition of the T cells into the microwells.

22. The method of claim 2 or claim 20, further comprising a step of determining the frequency of T cells in the array of microwells that secrete individual cytokines in the plurality of cytokines.

23. The method of any one of claims 1 to 22, wherein the cells are from a human donor.

24. The method of claim 2, wherein the cells are from a human donor, and wherein the cells are stimulated with a first stimulus, and the method further comprises repeating steps (a)-(d), wherein the cells are stimulated with a second stimulus.

25. The method of claim 2, further comprising identifying the phenotype or lineage of the T cells.

26. The method of claim 25, further matching the phenotype or lineage with the levels of said different cytokines bound to the first printed array and the levels of said different cytokines bound to the second printed array.

27. The method of claim 2, wherein the step of quantifying comprises imaging each of the first printed array and the second printed array to yield a dataset; filtering the dataset to identify locations on the array of microwells in which the microwells contain a single cell; and matching the locations of the microwells with the corresponding locations on the first printed array and the second printed array and with levels of said different cytokines detected from the locations.

28. The method of claim 18, wherein the T cells are from a subject, and an increase in the level of a Th_2 cytokine compared to a level of a Th_1 cytokine indicates that the subject is allergic or is at risk of developing an allergy to the allergen.

29. The method of claim 28, wherein the Th_2 cytokine comprises IL-4.

30. The method of claim 28, wherein the Th_1 cytokine comprises IFNγ.

31. The method of claim 2, wherein the T cells are from a subject, and the profile of the T cells is indicative of an autoimmune disease or infectious disease in the subject.

32. The method of claim 2, wherein said first detection agents that respectively bind to different cytokines are capture antibodies that respectively bind to said different cytokines, and said quantifying step comprises contacting the first printed array and second printed array with detection antibodies that respectively bind to said different cytokines.

33. The method of claim 32, wherein the detection antibodies that respectively bind to said different cytokines are labeled with different fluorescent labels.

34. The method of claim 2, wherein said different cytokines are up to four different cytokines.

35. The method of claim 2, wherein the different cytokines comprise IFNγ, IL-2, and TNFα.

36. The method of claim 2, further comprising one or more additional contacting steps after step (b), wherein each additional contacting step: (i) is at a different time point, and (ii) comprises contacting the slab with an additional substrate, wherein the additional substrate is pretreated with a plurality of additional detection agents that comprise additional detection agents identical to said first detection agents that respectively bind to different cytokines, whereby cytokines secreted by said T cells that are present in said microwells and are binding partners of an additional detection agent are bound to the additional detection agent in regions on the additional substrate contacted by contents of the microwells, to yield an additional printed array;

and wherein said quantifying step further comprises quantifying levels of said different cytokines bound to each said additional printed array, and determining rates of secretion of the different cytokines bound to each said additional printed array.

37. The method of claim 33, wherein the T cells are human T cells, the different cytokines comprise IFNγ, IL-2, and TNFα, and the method further comprises one or more additional contacting steps after step (b), wherein each additional contacting step: (i) is at a different time point, and (ii) comprises contacting the slab with an additional substrate, wherein the additional substrate is pretreated with a plurality of additional detection agents that comprise additional detection agents identical to said first detection agents that respectively bind to different cytokines, whereby cytokines secreted by said T cells that are present in said microwells and are binding partners of an additional detection agent are bound

to the additional detection agent in regions on the additional substrate contacted by contents of the microwells, to yield an additional printed array;

and wherein said quantifying step further comprises quantifying levels of said different cytokines bound to each said additional printed array, and determining rates of secretion of the different cytokines bound to each said additional printed array.

38. The method of claim 2, which further comprises, for a plurality of locations in the array of microwells that contain only a single cell, matching each location with data extracted from the first printed array and data extracted from the second printed array in a region contacted by contents of the microwell at the location, wherein the data represents the level bound in said region of a selected cytokine included within said different cytokines; and repeating said matching for each cytokine included within said different cytokines.

39. The method of claim 33, which further comprises, for a plurality of locations in the array of microwells that contain only a single cell, matching each location with data extracted from the first printed array and data extracted from the second printed array in a region contacted by contents of the microwell at the location, wherein the data represents the level bound in said region of a selected cytokine included within said different cytokines; and repeating said matching for each cytokine included within said different cytokines.

40. The method of claim 35, which further comprises, for a plurality of locations in the array of microwells that contain only a single cell, matching each location with data extracted from the first printed array and data extracted from the second printed array in a region contacted by contents of the microwell at the location, wherein the data represents the level bound in said region of a selected cytokine included within said different cytokines; and repeating said matching for each cytokine included within said different cytokines.

41. The method of claim 36, which further comprises, for a plurality of locations in the array of microwells that contain only a single cell, matching each location with data extracted from the first printed array, data extracted from the second printed array, and data extracted from each said additional printed array, in a region contacted by contents of the microwell at the location, wherein the data represents the level bound in said region of a selected cytokine included within said different cytokines; and repeating said matching for each cytokine included within said different cytokines.