COMPOSITIONS AND METHODS FOR ASSESSING CYTOTOXICITY OF SINGLE CELLS

FIELD OF THE INVENTION

The invention provides a method of analyzing interactions between pairs of target and effector cells utilizing high-throughput screenings methods for profiling large numbers of single cells in microarrays.

BACKGROUND OF THE INVENTION

Despite more than twenty five years of research on the interactions between humans and the human immunodeficiency virus type 1 (HIV-1), HIV/AIDS remains one of the most prevalent threats to global health. Current estimates suggest it will become the third leading cause of mortality worldwide over the next twenty years behind cancer and cardiovascular disease. A vaccine that would either prevent infection or elicit natural mechanisms to control the disease has not yet been developed. Existing analytical tools simply are not adequate to define the critical characteristics associated with cells of the immune system that provide effective protective immunity to the virus. Technologies such as flow cytometry and immunosorbant assays (ELISpot, ELISA) can evaluate populations of cells, but have poor sensitivity for rare events. Other important functions, such as cytoxicity and proliferation, can only be measured in bulk presently. Together, these limitations make it difficult, if not impossible, to evaluate the human immune response to HIV with sufficient clarity to determine correlates of protection. As such, there is a pressing need for new strategies for analyzing protective immunity to viruses such as HIV.

WO 2007/035633 discloses methods, apparatus, and kits for identifying antibodies and other secreted products. For example, a single or a few hybridomas are placed in a microwell of a moldable slab, and secreted monoclonal antibodies are immobilized on a substrate and exposed to known antigen(s) to determine which monoclonal antibodies bind the antigen.

Bradshaw et al., 2008, Clinical Immunology 129:10-18 discloses the concurrent detection of secreted products from single human lymphocytes by microengraving.

Yamamura et al., 2005, Anal. Chem. 77:8050-8056 discloses a single-cell microarray to monitor Ca^{2+} mobilization in B cells.

Snyder et al. (2003, Nature Medicine 9(2):231-235 discloses a Lysispot assay for measuring the frequency of cytotoxic T cells in a population.

Saez-Cirion et al.(2007, Proc. Natl. Acad. Sci USA 104(16):6776-6781) discloses that $CD8^+$ T cells from HIV controllers eliminated infected $CD4^+$T cells.

SUMMARY OF THE INVENTION

The invention provides methods for identifying CD8+ cells able to lyse CD4+ HIV-infected cells in a subject by providing a suspension of effector CD8+ cells and target cells from a subject deposited onto a moldable slab containing at least one micro well in a microwell array, wherein at least one microwell in the microwell array has a single effector cell; culturing the suspension under conditions allowing for lysis of the target cell by the CD8+ cells; detecting lysis of the target cell by the effector cells, and identifying CD8+ cells able to lyse CD4+ HIV-infected cells. Optionally, the effector cells that lyse the target cell are recovered. Preferably, the recovered effector cells that have lysed the target cell are cultured. In one aspect, the effector cells and target cells are mixed prior to depositing cells

What is claimed is:

1. A method of identifying CD8+ cells able to lyse CD4+ HIV-infected cells in a subject, the method comprising:

providing a suspension of effector CD8+ cells and CD4+ HIV-infected target cells from a subject and deposited onto a moldable slab containing at least one microwell in a microwell array, wherein at least one microwell in said microwell array has a single effector CD8+ cell;

culturing said suspension under conditions allowing for lysis of said target cells by said CD8+ cells;

detecting lysis of said target cells by said effector CD8+ cells, and

identifying effector CD8+ cells able to lyse CD4+ HIV-infected cells.

2. The method of claim 1, further comprising recovering effector CD8+ cells that lyse said target cell.

3. The method of claim 2, further comprising culturing effector CD8+ cells that have lysed said target cell.

4. The method of claim 1, wherein said effector CD8+ cells and target cells are mixed prior to depositing cells into said microwell.

5. The method of claim 1, wherein said effector cells and target cells are mixed after depositing cells into said microwell.

6. The method of claim 1, wherein lysis is detected by monitoring change in fluorescence of a labeled cell.

7. The method of claim 1, wherein lysis is detected by monitoring changes in intracellular calcium levels of said target cell.

8. The method of claim 7, wherein said calcium is detected with a calcium sensitive fluorescent dye.

9. The method of claim 8, wherein said dye is Fura 2AM (Invitrogen).

10. The method of claim 1, further comprising

contacting the microwell array with a substrate, wherein the substrate is pretreated with at least one agent that can specifically detect a product of said effector CD8+ cell; and detecting said product.

11. The method of claim 10, wherein said product is an antibody, cytokine, or soluble mediator of lysis.

12. The method of claim 10, wherein said product is a cytokine.

13. The method of claim 12, wherein said cytokine is TNF-α or IFN-γ.

14. The method of claim 8, wherein said product is a soluble mediator of lysis.

15. The method of claim 14, wherein said soluble mediator of lysis is granzyme B (GzB) or perforin.

16. The method of claim 1, wherein said method further comprises labeling an effector CD8+ cell with CD69.

17. A method of using arrays of microwells to study interactions between cells, comprising monitoring killing of infected target cells by single cytotoxic T lymphocytes in microwells while simultaneously profiling activation markers or secreted soluble mediators.

18. The method of claim 17, wherein the depth and size of the microwells are less than 100 μm.

19. The method of claim 17 or 18, wherein a single cytotoxic T lymphocyte is deposited on average into each well.

20. The method of any one of claims 17-19, wherein the profiling is by holding the microwells loaded with the target cells and single cytotoxic T lymphocytes in physical contact with a glass slide pre-functionalized with the appropriate reagents to capture the activation markers or secreted soluble mediators.

21. The method of any one of claims 17-20, wherein dyes are used to highlight both the target cells and single cytotoxic T lymphocytes.

22. The method of any one of claims 17-21, wherein the target cells are HIV-infected.

23. The method of any one of claims 17-22, further comprising using software to recognize where killing took place and to locate those microwells for a micromanipulator.

24. A method of generating a functional profile for an effector cell responsive to an HIV infection in a subject, the method comprising:

providing a population of effector cells selected from the group consisting of cytotoxic T cells ($CD8^+$), natural killer (NK) cells ($CD16^+$), NK T cells ($CD1d^+$,$V\alpha24^+$), and $\gamma\delta$ T cells ($V\gamma9^+$,$V\delta2^+$), wherein said effector cells are obtained from a subject and deposited onto a moldable slab containing at least one microwell in a microwell array, wherein at least one microwell in said microwell array has a single effector cell, wherein said population of effector cells is co-loaded with a cognate target cell population;

visualizing said effector cells;

assessing cytotoxicity of said effector cells;

contacting the microwell array with a first substrate, wherein the substrate is pretreated with an agent that specifically detects one or more of IL-2, IL-4, IL-10, TNF-α, and IFN-γ; and

determining whether said effector cells in said micro well binds said one or more agents.

25. The method of claim 24, wherein cytotoxicity is assessed by detecting release of Calcein AM.

26. The method of claim 24, further comprising labeling the effector cells for one or more specific surface marker proteins.

27. The method of claim 26, wherein surface marker protein is CD62L, CXCR3, CCR4, or CCR7.

28. The method of claim 24, further comprising recovering an effector cell from one or more microwell.

29. The method of claim 28, further comprising culturing the recovered cell to obtain a clonal amplification of said recovered cell.

30. The method of claim 28, further comprising characterizing expression of one or more genes in said recovered cell.

31. The method of claim 24, wherein said subject is at an acute stage of infection, a highly active antiretroviral therapy (HAART) subject, or an elite controller.

32. A method of assessing clonal diversity in a population of NK effector cells, the method comprising

providing a suspension of NK effector cells and target 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 has a single effector cell;

culturing said suspension under conditions allowing for lysis of said target cell by said NK effector cells;

detecting lysis of said target cell by said NK effector cells; and

identifying said NK effector cells,

thereby assessing clonal diversity in said population of NK effector cells.

33. The method of claim 32, further comprising recovering NK effector cells that lyse said target cell.

34. The method of claim 33, further comprising culturing said recovered cells.

35. The method of claim 32, further comprising culturing NK effector cells that have lysed said target cell.

36. The method of claim 32, wherein said NK effector cells and target cells are mixed prior to depositing cells into said microwell.

37. The method of claim 32, wherein said NK effector cells and target cells are mixed after depositing cells into said microwell.

38. The method of claim 32, wherein lysis of target cells is determined by monitoring a change in fluourescence of a labeled cell.

39. The method of claim 32, further comprising contacting the microwell array with a substrate, wherein the substrate is pretreated with at least one agent that can specifically detect a product of said NK effector cell; and detecting said product.

40. The method of claim 39, wherein said product is an antibody, cytokine, or soluble mediator of lysis.

41. The method of claim 40, wherein said cytokine is TNF-α or IFN-γ.

METHODS FOR ASSESSING CYTOTOXICITY OF SINGLE CELLS

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culturing said suspension under conditions allowing for lysis of said target cells by said CD8+ cells;

detecting lysis of said target cells by said effector CD8+ cells, and

identifying effector CD8+ cells able to lyse CD4+ HIV-infected cells.

2. The method of claim 1, further comprising recovering effector CD8+ cells that lyse said target cell.

3. The method of claim 2, further comprising culturing effector CD8+ cells that have lysed said target cell.

4. The method of claim 1, wherein said effector CD8+ cells and target cells are mixed prior to depositing cells into said microwell.

5. The method of claim 1, wherein said effector cells and target cells are mixed after depositing cells into said microwell.

6. The method of claim 1, wherein lysis is detected by monitoring change in fluorescence of a labeled cell.

7. The method of claim 1, wherein lysis is detected by monitoring changes in intracellular calcium levels of said target cell.

8. The method of claim 7, wherein said calcium is detected with a calcium sensitive fluorescent dye.

9. The method of claim 8, wherein said dye is Fura 2AM (Invitrogen).

10. The method of claim 1, further comprising

contacting the microwell array with a substrate, wherein the substrate is pretreated with at least one agent that can specifically detect a product of said effector CD8+ cell; and detecting said product.

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