models based on tumor-specific CD4+ cells. These factors have limited translational research using CD4+ T cells and limited their clinical use.

[0013] This invention discloses a method for producing clinically-relevant numbers of CD4+ cells that also contain the cytolytic granules granzyme B and perforin and function both as Th1 cells (produce IFN-gamma and not IL-4) and have NK-like activity (recognize and kill tumor cells and not normal cells) for use in therapeutic cancer vaccine and adoptive immune cell therapy protocols.

SUMMARY

[0013a] In one aspect, there is provided a composition comprising Th1/killer cells wherein: (1) the Th1/killer cells have Th1 characteristics and cytolytic activity; or (2) the Th1/killer cells are activated naïve CD4+ cells having cytolytic activity against a tumor cell line; wherein the Th1/killer cells of the (1) are formulated with activation and cross-linking agent for CD3 and CD28 and have cross-linked CD3 and CD28 cell surface molecules, and the Th1/killer cells of the (1) are derived from normal donor peripheral blood.

[0013b] In one aspect, there is provided the use of a composition in the manufacture of a medicament for destroying diseased cells in a patient with cancer or infection, wherein the composition comprises allogeneic Th1/killer cells expressing granzyme B and perforin wherein the interaction of the diseased cells with the Th1/killer cells leads to destruction of the diseased cells, wherein the Th1/killer cells are activated naïve CD4+ cells.

[0013c] In one aspect, there is provided the use of a composition in the manufacture of a medicament for treatment of a patient with cancer or infection, wherein the composition comprises a clinically-relevant number of Th1/killer cells expressing granzyme B and perforin and cytolytic activity, wherein the Th1/killer cells are activated naïve CD4+ cells.

[0014] Cell-based therapeutic cancer vaccines (cellular immunotherapy) hold promise as a new minimally toxic approach for treatment of cancer. The promise of this approach has been demonstrated in animal models, but it has been difficult to translate this promise to the clinic (Bodey, Bodey et al. 2000). The recent FDA approval of the first cellular immunotherapy drug, Provenge® (sipuleucel-T), an autologous dendritic cell immunotherapy for the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer (Kantoff, Higano et al.), has renewed enthusiasm to develop improved cell therapy products for clinical application.

[0015] Since it has proven difficult to successfully translate the immune-mediated anti-tumor mechanisms of cellular immunotherapy strategies developed in animals, we instead concentrated on translating an anti-tumor immune mechanism already known to elicit significant clinical anti-tumor effects. Arguably, the most powerful and most effective immune-mediated anti-tumor mechanism ever described in humans is the graft vs. tumor (GVT) effect that occurs after non-myeloablative conditioning and transplant of HLA-matched stem cells (Barrett and Childs 2000; Childs 2000; Resnick, Shapira et al. 2005). Unfortunately, these GVT effects are often accompanied by chronic graft-versus-host-disease (GVHD) toxicity. Chronic GVHD is associated with significant morbidity and is the leading cause for late mortality after allogeneic hematopoietic stem cell transplantation (Lee, Klein et al. 2002), significantly limiting the clinical application of the GVT mechanism.

[0016] Separation of the beneficial GVT effects from the detrimental GVHD effects is difficult because the effects are interrelated and proportional, in that manipulations that inhibit GVHD also decrease the GVT effect (Li, Giver et al. 2009). By analyzing the known immune-mediated

mechanisms of the inter-related GVT and GVHD effects, it was hypothesized that it might be possible to develop an allogeneic cellular immunotherapy that instead of separating the GVT/GVHD effects would maintain the interrelationship of the immune mechanisms by reversing the direction of the effects from graft-to-host to host-to-graft (Har-Noy and Slavin 2008). Successful reversal of the direction of the effects would result in a host vs. tumor (HVT) effect coupled to a non-toxic host vs. graft (HVG) rejection effect.

[0017] It was hypothesized that intentionally mis-matched, activated, allogeneic Th1 cells that expressed high density CD40L and produced high amounts of interferon (IFN)-γ would be capable of eliciting coupled HVT/HVG effects. This hypothesis was tested in an animal model of chemotherapy-refractory B-cell leukemia/lymphoma (Har-Noy, Zeira et al. 2008). These studies demonstrated that significant HVT/HVG activity could be elicited by ex-vivo differentiated allogeneic Th1 cells only when the Th1 cells were activated and administered attached to anti-CD3/anti-CD28-coated microbeads. Additional studies indicated that these allogeneic CD3/CD28-crosslinked Th1 cells had both immunomodulatory effects, capable of switching systemic dominant Th2 immunity to dominant Th1 immunity; and, adjuvant effects for promoting development of Th1-specific immunity and dysregulation of tumor immunoavoidance mechanisms (Har-Noy, Zeira et al. 2009). In view of these data, human version of Th1 allografts were investigated for the possibility of translating this experimental approach to the clinic.

[0018] There were significant regulatory and logistical concerns associated with the ex vivo differentiation and expansion of allogeneic Th1 cells that were necessary to be overcome in order to use these cells for human clinical investigations. One such issue was that the mouse version of allogeneic Th1 cells required the use of exogeneous cytokines, IL-2, IL-12 and anti-IL-4 mAb to cause the ex-vivo differentiation of mouse Th1 cells. The current ~~invention~~disclosure discloses the successful development of a GMP-compliant process for the production of human allogeneic Th1 cells without the use of exogenous cytokines resulting in consistent batch-to-batch identity and functional characteristics for classification as a human biological drug. Upon characterization of the resulting allograft cells, the human production process resulted in a unexpected finding. The allogeneic Th1 cells generated by the methods described herein not only developed Th1 characteristics, but also developed NK-like characteristics. An allograft with

a combination of Th1 and NK-like activity is unique and is not described in normal blood circulation. The method of the current ~~invention~~disclosure involves the ex-vivo culture of purified, resting (CD25-), CD4+ T-cells with mixed memory and naïve phenotype (CD45RA and CD45RO, respectively). The cells are activated multiple times with immobilized anti-CD3/anti-CD28 mAbs in the absence of exogenous cytokines. The cells expand 25-100-fold after 9-days and differentiate into CD4+, CD45RO+, CD25+, $CD62^{hi}$ cells. This intermediate cell can be stored in liquid nitrogen for years. When needed for patient administration, the precursor cells are incubated for 4h with CD3/CD28 mAb coated microbeads. During this final 4h incubation, the precursor cells further differentiate to decrease expression of CD62L and increase expression of CD40L and CD25. The final CD4+, CD45RO+, $CD40L^{hi}$, $CD62L^{lo}$, CD25+ cells produce >1000pg/10^6 cells of IFN-gamma and <50pg/10^6 cells of IL-4 (Th1 phenotype) and express cytolytic granules of granzyme B and perforin and exhibit NK-like activity with the ability to lyse tumor cells and not normal cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Fig. 1A and Fig. 1B are flow cytometry plots from immunostaining of CD4 surface antigen demonstrating the cell phenotype before and after CD4+ selection, respectively.

[0020] Fig. 2A is a series of flow cytometry plots demonstrating the phenotype of source cells having as resting CD4 naïve (CD4, CD45RA) cells.

[0021] Fig. 2B is a series of flow cytometry plots demonstrating the change in phenotype after culture to activated CD4 memory cells (CD4, CD45RO and CD25).

[0022] Fig. 3 is a flow cytometry plot demonstrating the shift in CD62L expression from high to low inCAC before activation and CFB after activation.

[0023] Figs. 4A-4C are plots of the CD40L expression pattern in CD4+ cells, CAC and CFB, respectively demonstrating increased expression of CD40L in CFB.

[0024] Fig. 5 is a graph of the direct killing assay and shows CFB can kill cells from the tumor cell line, ARH77.

[0025] Fig. 6A is a plot of expression of Granzyme B by CAC.

[0026] Fig. 6B is a plot of expression of Granzyme B by CFB.

[0027] Fig. 6C is a picture of a western blot showing detection of Perforin in CFB.

[0028] Fig. 7 is a graph of perforin-Granzyme B activity with and without EGTA.

WHAT IS CLAIMED IS:

1. A composition comprising Th1/killer cells wherein:

(1) the Th1/killer cells have Th1 characteristics and cytolytic activity; or

(2) the Th1/killer cells are activated naïve CD4+ cells having cytolytic activity against a tumor cell line; wherein the Th1/killer cells of the (1) are formulated with activation and cross-linking agent for CD3 and CD28 and have cross-linked CD3 and CD28 cell surface molecules, and the Th1/killer cells of the (1) are derived from normal donor peripheral blood.

2. The composition of claim 1 wherein the cytolytic activity of (1) comprises NK characteristics.

3. The composition of claim 1 wherein the Th1/killer cells express: granzyme B and perforin; or IFN-gamma.

4. The composition of claim 1 wherein the Th1/killer cells of (1):

a) have substantially reduced or no expression of IL-4;

b) are CD4+ cells.

5. The composition of claim 1 wherein the composition of (1) comprises:

a) clinically-relevant number of the Th1/killer cells;

b) at least about 1×10^7 cells; or

c) at least about 1×10^8 cells.

6. The composition of claim 1 wherein the cytolytic activity of (1) of the Th1/killer cells inactivates diseased cells, and wherein the diseased cells preferably comprise cancerous cells, infected cells or combinations thereof.

7. The composition of claim 1 wherein the Th1/killer cells of (2):

a) comprise Th1 characteristics; or

b) do not inactivate normal cells.

8. The composition of claim 1 wherein the tumor cell line of (2) is ARH77.

9. Use of a composition in the manufacture of a medicament for destroying diseased cells in a patient with cancer or infection, wherein the composition comprises allogeneic Th1/killer cells expressing granzyme B and perforin wherein the interaction of the diseased cells with the Th1/killer cells leads to destruction of the diseased cells, wherein the Th1/killer cells are activated naïve CD4+ cells.

10. The use according to claim 9 wherein the Th1/killer cells express Th1 characteristics and cytolytic activity.

11. The use according to claim 10 wherein:

a) the cytolytic activity of the Th1/killer cells comprises expressing granzyme B-perforin;

b) the Th1 characteristics comprise expression of IFN-gamma; or

c) the Th1/killer cells lack expression of IL-4.

12. The use according to claim 9 wherein:

a) the Th1/killer cells are obtained by activation of CD4+ T-cells with cross-linked anti-CD3/anti-CD28 monoclonal antibodies;

b) the diseased cells comprise cancerous cells, infected cells or a combination thereof.

13. Use of a composition in the manufacture of a medicament for treatment of a patient with cancer or infection, wherein the composition comprises a clinically-relevant number of Th1/killer cells expressing granzyme B and perforin and cytolytic activity, wherein the Th1/killer cells are activated naïve CD4+ cells.

14. The use according to claim 13 wherein:

a) the Th1/killer cells express Th1 characteristics and cytolytic activity, wherein preferably the Th1/killer cells are activated with beads attached to anti-CD3/anti-CD28 monoclonal antibodies and crosslinked;

b) the Th1/killer cells are obtained from the peripheral blood of a normal donor;

c) the Th1/killer cells are allogeneic to the patient; or

d) the clinically-relevant number of cells is at least 1×10^8.

WHAT IS CLAIMED IS:

1. A composition comprising Th1/killer cells wherein the Th1/killer cells have Th1 characteristics and cytolytic activity.

2. The composition of claim 1 wherein the cytolytic activity comprises NK characteristics.

3. The composition of claim 1 wherein the Th1/killer cells express granzyme B and perforin.

4. The composition of claim 1 wherein the Th1/killer cells express IFN-gamma.

5. The composition of claim 1 wherein the Th1/killer cells have substantially reduced or no expression of IL-4.

6. The composition of claim 1 wherein the Th1/killer cells are CD4+ cells.

7. The composition of claim 1 wherein the Th1/killer cells are formulated with cross-linking agent for CD3 and CD28

8. The composition of claim 1 wherein the Th1/killer cells are derived from normal donor peripheral blood.

9. The composition of claim 1 wherein the composition comprises clinically-relevant number of the Th1/killer cells.

10. The composition of claim 1 wherein the composition comprises at least about 1×10^7 cells.

11. The composition of claim 1 wherein the composition comprises at least about 1×10^8 cells.

12. The composition of claim 1 wherein the cytolytic activity of the Th1/killer cells specifically inactivates diseased cells and not normal cells.

13. The composition of claim 12 wherein the diseased cells comprise cancerous cells, infected cells or combinations thereof.

14. A composition comprising Th1/killer cells wherein the Th1/killer cells are CD4+ cells having cytolytic activity against a tumor cell line.

15. The composition of claim 14 wherein the Th1/killer cells comprise Th1 characteristics.

16. The composition of claim 14 wherein the Th1/killer cells express granzyme B and perforin.

17. The composition of claim 14 wherein the Th1/killer cells express IFN-gamma.

18. The composition of claim 14 wherein the tumor cell line is ARH77.

19. The composition of claim 14 wherein the Th1/killer cells do not inactivate normal cells.

20. A method for destroying diseased cells comprising contacting the diseased cells with a composition comprising allogeneic Th1/killer cells wherein the interaction of the diseased cells with the Th1/killer cells leads to destruction of the diseased cells.

21. The method of claim 20 wherein the Th1/killer cells are CD4+ cells.

22. The method of claim 20 wherein the Th1/killer cells express Th1 characteristics and cytolytic activity.

23. The method of claim 22 wherein the cytolytic activity of the Th1/killer cells comprises expression granzyme B-perforin.

24. The method of claim 22 wherein the Th1 characteristics comprise expression of IFN-gamma.

25. The method of claim 22 wherein the Th1/killer cells lack expression of IL-4.

26. The method of claim 20 wherein the Th1/killer cells are obtained by activation of CD4+ T-cells with cross-linked anti-CD3/anti-CD28 monoclonal antibodies.

27. The method of claim 20 wherein the diseased cells comprise cancerous cells, infected cells or a combination thereof.

28. A method of treating a patient comprising administering a composition comprising a clinically-relevant number of Th1/killer cells.

29. The method of claim 28 wherein the Th1/killer cells are CD4+ cells.

30. The method of claim 28 wherein the Th1/killer cells express Th1 characteristics and cytolytic activity.

31. The method of claim 30 wherein the Th1/killer cells are activated with beads attached to anti-CD3/anti-CD28 monoclonal antibodies and crosslinked.

32. The method of claim 28 wherein the Th1/killer cells are obtained from the peripheral blood of a normal donor.

33. The method of claim 28 wherein the Th1/killer cells are allogeneic to the patient.

34. The method of claim 28 wherein the clinically-relevant number of cells is at least 1×10^8.