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] Disclosed is 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] Disclosed is 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] Disclosed is 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.

[0013d] In one aspect, there is provided a composition comprising activated CD4+ Th1/killer cells having Th1 characteristics and direct cytolytic activity against a tumour cell line, wherein the Th1/killer cells are *ex vivo* activated CD4+ cells expressing granzyme B and perforin obtained by activating naïve CD4+ cells by cross-linking of CD3 and CD28 cell surface molecules without the addition of exogenous cytokines, freezing the activated cells and then reactivating the cells by cross-linking of CD3 and CD28 cell surface molecules.

[0013e] In one aspect, there is provided an in vitro method for destroying diseased cells comprising contacting the diseased cells with any of the disclosed composition wherein the

interaction of the diseased cells directly with the activated CD4+ Th1/killer cells of the composition leads to destruction of the diseased cells.

[0013f] In one aspect, there is provided a composition comprising any of the activated CD4+ Th1/killer cells disclosed herein for use in treating a patient, wherein the composition comprises an effective number of the Th1/killer 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

WHAT IS CLAIMED IS:

1. A composition comprising activated CD4+ Th1/killer cells having Th1 characteristics and direct cytolytic activity against a tumor cell line, wherein the Th1/killer cells are *ex vivo* activated CD4+ cells expressing granzyme B and perforin obtained by activating naïve CD4+ cells by cross-linking of CD3 and CD28 cell surface molecules without the addition of exogenous cytokines, freezing the activated cells and then reactivating the cells by cross-linking of CD3 and CD28 cell surface molecules.

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 IFN-gamma.

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

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

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

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

8. The composition of claim 1 wherein the cytolytic activity of the Th1/killer cells specifically inactivates diseased cells and not cells obtained from healthy donors, and wherein the diseased cells preferably comprise cancerous cells, infected cells or combinations thereof.

9. The composition of claim 1 wherein the tumor cell line is ARH77.

10. An *in vitro* method for destroying diseased cells comprising contacting the diseased cells with a composition according to any one of claims 1 to 9 wherein the interaction of the diseased cells directly with the activated CD4+ Th1/killer cells of the composition leads to destruction of the diseased cells.

11. The method of claim 10 wherein the diseased cells comprise cancerous cells, infected cells or a combination thereof.

12. A composition comprising activated CD4+ Th1/killer cells according to any one of claims 1 to 9 for use in treating a patient, wherein the composition comprises an effective number of the Th1/killer cells.

13. The composition for use according to claim 12 wherein the Th1/killer cells are allogeneic to the patient.