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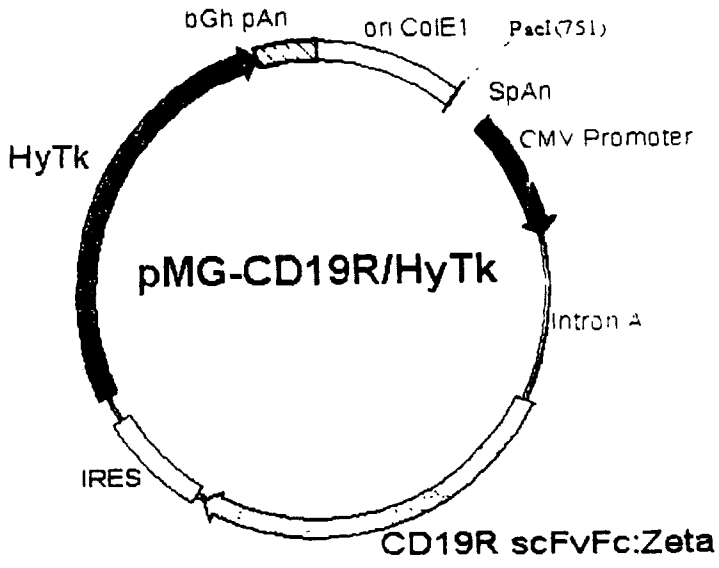
(54) Title: CD19-SPECIFIC REDIRECTED IMMUNE CELLS



(57) Abstract: Genetically engineered, CD19-specific redirected immune cells expressing a cell surface protein having an extracellular domain comprising a receptor which is specific for CD19, an intracellular signaling domain, and a transmembrane domain. Use of such cells for cellular immunotherapy of $CD19^+$ malignancies and for abrogating any untoward B cell function. In one embodiment, the immune cell is a T cell and the cell surface protein is a single chain svFvFc:ζ receptor where scFc designates the V_H and V_L chains of a single chain monoclonal antibody to CD19, Fc represents at least part of a constant region of an IgG_1, and ζ represents the intracellular signaling domain of the zeta chain of human CD3. The extracellular domain scFvFc and the intracellular domain ζ are linked by a transmembrane domain such as the transmembrane domain of CD4. A method of making a redirected T cell expressing a chimeric T cell receptor by electroportion using naked DNA encoding the receptor.

CD19-SPECIFIC REDIRECTED IMMUNE CELLS

Cross-reference to Related Application:

[0001] This application claims priority to Provisional Application Serial No. 60/246,117, filed November 7, 2000, the disclosure of which is incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] This invention relates to the field of genetically engineered, redirected immune cells and to the field of cellular immunotherapy of B-cell malignancies, B-cell lymphoproliferative syndromes and B-cell mediated autoimmune diseases.
The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice are incorporated by reference.

[0003] Approximately half of all hematopoietic stem cell transplantation (HSC) procedures performed in the United States are for the treatment of hematologic malignancy [1]. The initial obstacles for successful HSC transplantation were in large part due to inadequate treatment modalities for ameliorating regimen-related toxicities and for controlling opportunistic infections and graft-versus-host disease (GVHD) [2-5]. As supportive care measures have improved over the last decade, post-transplant disease relapse has emerged as the major impediment to improving the outcome of this patient population [6-10]. The inability of maximally intensive preparative regimens combined with immunologic graft-versus-tumor reactivity to eradicate minimal residual disease is the mechanism of treatment failure in allogeneic transplantation while, in the autologous setting, tumor contamination of the stem cell graft can also contribute to post-transplant relapse [11]. Targeting minimal residual disease early after transplantation is one strategy to consolidate the tumor cytoreduction achieved with myeloablative preparative regimens and purge, *in vivo,* malignant cells transferred with autologous stem cell grafts. The utility

of therapeutic modalities for targeting minimal residual disease shortly following stem cell rescue is dependent on both a limited spectrum of toxicity and the susceptibility of residual tumor cells to the modality's antitumor effector mechanism(s). The successful elimination of persistent minimal residual disease should not only have a major impact on the outcome of transplantation for hematologic malignancy utilizing current myeloablative preparative regimens but may also provide opportunities to decrease the intensity of these regimens and their attendant toxicities.

[0004] The prognosis for patients with bcr-abl positive Acute Lymphoblastic Leukemia (ALL) treated with chemotherapy is poor and allogeneic transplantation has offered a curative option for many patients when an appropriate donor was available. For example at the City of Hope, 76 patients with bcr-abl positive ALL were treated with allogeneic Bone Marrow Transplantation (BMT) from a HLA matched donor. Of these patients, 26 were in first remission, 35 were transplanted after first remission. The two year probability of disease free survival was 68% with a 10% relapse rate in those patients transplanted in first remission whereas for those patients transplanted after first remission, the disease-free survival and relapse rate were 36% and 38%, respectively [12]. Post-transplant Polymerase Chain Reaction (PCR) screening of blood and marrow for bcr-abl transcript is under evaluation as a molecular screening tool for identifying early those transplant recipients at high risk for later development of overt relapse [13,14]. Patients for whom detectable p190 transcript was detected following BMT had a 6.7 higher incidence of overt relapse than PCR negative patients. The median time from the development of a positive signal to morphologic relapse was 80-90 days in these patients. The identification of patients in the earliest phases of post-transplant relapse affords the opportunity for making therapeutic interventions when tumor burden is low and potentially most amenable to salvage therapy.

[0005] Recent advances in the field of immunology have elucidated many of the molecular underpinnings of immune system regulation and have provided novel opportunities for therapeutic immune system manipulation, including tumor

immunotherapy. Evidence supporting the potential of immune-mediated eradication of residual tumor cells following allogeneic transplantation can be inferred by comparing the disparate relapse rates between recipients of syngeneic and non-T cell depleted matched sibling transplants. Patients with chronic myelogenous leukemia in chronic phase (CML-CP), acute myelogenous leukemia in first complete remission (1st CR), and acute lymphoblastic leukemia in 1st CR who received a marrow transplant from a syngeneic donor had an actuarial probability of relapse at 3 years of 45%, 49%, and 41%, respectively, whereas the rates for recipients of a non-T depleted marrow transplant from an HLA identical sibling for the same diseases were 12%, 20%, and 24%, respectively [15-17]. The reduction of relapse rates following allogeneic bone marrow transplantation has been most significant in patients who develop acute and/or chronic GVHD. Currently, efforts are focused on developing strategies to selectively augment the graft-versus-leukemia (GVL) response in order to reduce post-transplant relapse rates without the attendant toxicities of augmented GVHD.

[0006] Studies in animal models have established that donor MHC-restricted $CD8^+$ and $CD4^+$ α/β^+ T cells specific for minor histocompatibility antigens encoded by polymorphic genes that differ between the donor and recipient are the principle mediators of acute GVHD and GVL [18-21]. Recently, patients with CML in chronic phase who relapse after allogeneic BMT have been identified as a patient population for whom the infusion of donor lymphocytes (DLI) successfully promotes a GVL effect [22,23]. Complete response rates of approximately 75% are achieved with DLI cell doses in the range of $0.25\text{-}12.3\text{x}10^8$ mononuclear cells/kg [24]. Although the antitumor activity of donor lymphocyte infusion underscores the potential of cellular immunotherapy for CML, the clinical benefit of DLI has not been generalizable to all forms of hematologic malignancy. Relapsed ALL is much less responsive to DLI with a reported CR rate of less than 20%; when tumor responses are observed, they are typically associated with significant GVHD morbidity and mortality [25]. In order to increase the therapeutic ratio of DLI, genetic modification of donor lymphocytes to express a suicide gene is being

evaluated as a strategy to permit the *in vivo* ablation of donor lymphocytes should toxicity from GVHD warrant this maneuver [26,27]. Alternately, efforts are underway to identify genes encoding minor histocompatibility antigens (mHA's) with restricted hematopoietic expression that elicit donor antigen-specific T cell responses. The isolation, *ex vivo* expansion, and re-infusion of donor-derived clones specific for these mHA's has the potential of selectively augmenting GVL following allogeneic bone marrow transplantation [28-30].

[0007] Non-transformed B-cells and malignant B-cells express an array of cell-surface molecules that define their lineage commitment and stage of maturation. These were identified initially by murine monoclonal antibodies and more recently by molecular genetic techniques. Expression of several of these cell-surface molecules is highly restricted to B-cells and their malignant counterparts. CD20 is a clinically useful cell-surface target for B-cell lymphoma immunotherapy with anti-CD20 monoclonal antibodies. This 33-kDa protein has structural features consistent with its ability to function as a calcium ion channel and is expressed on normal pre-B and mature B cells, but not hematopoietic stem cells nor plasma cells [31-33]. CD20 does not modulate nor does it shed from the cell surface [34]. *In vitro* studies have demonstrated that CD20 crosslinking by anti-CD20 monoclonal antibodies can trigger apoptosis of lymphoma cells [35,36]. Clinical trials evaluating the antitumor activity of chimeric anti-CD20 antibody IDEC-C2B8 (Rituximab) in patients with relapsed follicular lymphoma have documented tumor responses in nearly half the patients treated, although the clinical effect is usually transient [37-40]. Despite the prolonged ablation of normal $CD20^+$ B-cells, patients receiving Rituximab have not manifested complications attributable to B-cell lymphopenia [41]. Radioimmunotherapy with ^{131}I-conjugated and ^{90}Y-conjugated anti-CD20 antibodies also has shown promising clinical activity in patients with relapsed/refractory high-grade Non-Hodgkins Lymphoma but hematopoietic toxicities from radiation have been significant, often requiring stem cell support [42].

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