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Gene-Marked Autologous Hematopoietic Stem Cell Transplantation of Autoimmune Disease

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In phase I (safety) trials, we have demonstrated the feasibility of autologous hematopoietic stem cell transplantation (HSCT) for patients with autoimmune diseases. Although this review comments on results of our phase I trials, the focus is on phase II (efficacy) trials using gene-marked autologous stem cells.

KEY WORDS: Hematopoietic stem cell transplantation; gene-marked autologous stem cells; autoimmune disease.

RATIONALE

Stem cells within the bone marrow give rise to circulating blood cells including immune cells, i.e., white blood cells (WBC). In patients with life- or organ-threatening autoimmune disease, both marrow and blood cells may be destroyed by nonspecific high-dose chemotherapy or chemoradiotherapy. Subsequent infusion of hematopoietic stem cells (HSC) will then facilitate regeneration of new marrow and blood cells including new immune cells.

HSC may have be harvested from the patient (autologous cells) prior to treatment or obtained from a normal healthy individual (allogeneic cells). Since autologous stem cells may be genetically predisposed to generation of particular autoimmune phenotypes, allogeneic stem cells are thought to be more likely to prevent disease relapse. Allogeneic stem cell transplantation is complicated by significant morbidity and mortality from graft versus host disease (GVHD). For this reason, stem cell therapy for autoimmune disease is being initiated by our group and others using autologous hematopoietic stem cells (HSC) (Table I) (1–12). Early results suggest autologous hematopoietic stem cell transplantation (HSCT) is able to induce remission in otherwise treatment refractory disease, although durability of remission is as yet unknown.

The mechanism of remission following an autologous hematopoietic stem cell transplant is unknown. The immune system may be fundamentally unaltered and an autologous transplant may be nothing more than dose-intense immunosuppression. At the other extreme, the disease-mediating effector cells may be entirely destroyed. Alternatively, even if pretransplant disease-causing cells persist, an autologous transplant may shift the balance between immunity and tolerance through as yet undefined mechanisms. Theoretically, this may include clonal exhaustion, veto cells, suppresser cells, other autoregulatory cells, immune indifference, idiotypic T or B cell networks, cytokine alterations, changes in receptor avidity, or changes in T or B cell repertoire or function.

If relapse occurs it is unclear whether disease is reinitiated by lymphocytes surviving the immune-intense conditioning regimen or from the stem cell compartment. Scenarios can be imaged where lymphocytes which survived the conditioning regimen expand in the periphery and display abnormalities consist with an autoimmune disease, while lymphocytes that arise from the gene-marked stem cell compartment do not display these abnormalities or are actually autoregulatory and inhibit the autoimmune phenotype. The phenotype and charac-

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Table I. Results of Autologous Hematopoietic Stem Cell Transplantation (HSCT) for Autoimmune Disease

Author (Ref. No.)	Disease (number of patients)	Outcome
Fassas (1)	MS (15)	Improved mean Kurtzke disability score
Burt (2)	MS (3)	No progression
Burt (6)	MS, SLE, RA (10)	All patients stabilized or improved
Joske (3)	RA (1)	Improved from wheelchair bound to ambulating with ease
Tyndall (5)	Scleroderma (1)	Improved
Burt (4)	SLE (1)	Improved; no evidence of active disease after transplant
Brooks (9)	RA (8)	At 100 mg/kg of cyclophosphamide 4/4 relapsed, at 200 mg/kg 3/4 improved (2 at 6 months)
McSweeney (7)	Scleroderma (5)	No progression, improved skin scores
Wulffraat (8)	JRA (4)	All in remission
Huhn (12)	ITP (4)	1 relapse, 3 responding
Burt (10)	SLE, RA (6)	All 4 SLE in remission, 1 RA relapsed, 1 RA improved beyond 1 year
Burt (11)	MS, SLE, RA (17)	2 patients with RA relapsed, all others improved

teristics of lymphocytes that arise from reinfused stem cells may be determined by stem cell gene marking.

ORIGIN OF POST TRANSPLANT LYMPHOCYTES BY TRANSDUCTION OF INFUSED CD34 HEMATOPOIETIC PROGENITOR CELLS WITH A RETROVIRAL VECTOR

Background and Significance

A number of studies have confirmed that exposure of human hematopoietic stem cells to retroviral vectors will result in limited but discernible gene transfer and *in vivo* expression (13–18). Demonstration of long-term gene transfer into repopulating progenitor cells and subsequent expression in T and B lymphocytes allow monitoring of lymphocytes arising from the stem cell compartment.

Desirability and Feasibility of Transducing Stem Cells

We could gene-mark immune cells such as T lymphocytes but the goal of our therapy is to ablate the immune system, not to reinfuse potential disease-causing lymphocytes. Marking T cells will not answer the question as to whether the posttransplant immune system arises from memory lymphocytes that survived the conditioning regimen or from the stem cell compartment. Therefore, transfer of a marking gene to hematopoietic stem cells (HSC) would be preferable. Such stem cells are self-renewing, so that gene-modified HSC persist long term, perhaps indefinitely, and give rise to cells that contribute to all hematopoietic lineages, including T cells, monocytes, macrophages, and antigen-presenting cells.

Choice of Vectors

Moloney-based retroviral vectors have been widely used in clinical trials to transduce genes into stem cells (19). Expression of such vectors has been demonstrated in both stem cells and their mature progeny, including T cells, B cells, and monocytes. Although clinical or therapeutically useful levels of gene transfer have been difficult to obtain, levels adequate for marking can be obtained. In addition, recent advances in transduction technology may improve marking levels further. Despite their advantages, retroviral vectors have limitations in human stem cell transduction. For example, their transfer is cell-cycle dependent, and few HSC are in cycle at any given time.

The neo^r *Retroviral Vector*

For two autoimmune diseases, multiple sclerosis (MS) and systemic lupus erythematosus (SLE), we will be isolating posttransplant antigen-specific and disease-associated T cells, respectively. We need, therefore, to use a retroviral construct that is not immunogenic. To date, *no* human retroviral transduction protocol using a construct containing only the *neo^r* gene have demonstrated immunogenic rejection of transgenic cells. This is important since absence of the transgene is *un*informative. T and B cells may have arisen from the stem cell compartment but those containing the foreign transgene could be eliminated by immunologic mechanisms. Only a positive result, that is, detection of the transgene will be informative. Presence of transgenic antigen-specific or disease-associated cells will have significant relevance to determining if an autologous transplant can be expected to result in a "disease-cure." The presence of the *neo^r* gene will allow easy identification of transduced cells by PCR. Since *neo^r* is a positive selectable marker, the detection threshold of transgeneic cells may also be increased by incubation of cells with G418. Our construct is a retroviral vector derived from the Moloney murine leukemia virus (MoMLV). This vector contains the bacterial neomycin resistance (*neo^r*) gene transcribed

from an internal SV40 (simian virus 40) early promoter (LTR–SV40–*neo*^{r}–LTR) in an LXSN backbone. This vector has been modified for increased safety by alteration of the gag start codon to a stop codon and by elimination of viral sequences to minimize the potential for the development of replication-competent virus from producer cells which contain the vector.

EGFP Retroviral Vector

For the autoimmune disease rheumatoid arthritis (RA), we will biopsy synovium to analyze characteristics of infiltrating cells *in situ*. In order to determine if these cells regenerated from the stem cell compartment, we would prefer a marker that is readily detectable *in situ*. Green fluorescent protein (GFP) has previously been used as a marker gene in mammalian cells (20, 21). The green fluorescent protein gene is produced naturally by the Pacific Northwest jellyfish, *Aequorea victoria*. The cloned wild-type gene expresses a 27-kDa protein which is excited by exposure to blue light (450–490 nm) and emits a green light fluorescence in prokaryotes. However, wild-type GFP is expressed at undetectable levels in most eukaryotic systems. In order to overcome this limitation, the wild-type gene was codon optimized or "humanized" for maximal eukaryotic translation (22). Such mutants, termed enhanced GFP (EGFP), drastically improve the fluorescent activity of GFP expressed from mammalian vectors. EGFP transduced cells are readily detected with FITC microscopy by viewing cryopreserved thin cross sections. In addition, direct quantification of transduction is obtainable by FACS analysis of collagenase digested cells. EGFP transduced lymphocytes may also be separated by surface characteristics such as adhesion molecules, activation markers, and T cell receptor repertoire.

Sources of Stem Cells

An important clinical advance in stem cell transplantation was the realization that HSC could be mobilized from marrow into peripheral blood (23). Large numbers of $CD34^+$ HSC circulate if patients are treated for 4–6 days with hematopoietic growth factors such as G-CSF. These cells can be harvested by leukapheresis and used for autologous transplantation. They induce more rapid engraftment than do marrow-derived stem cells. Peripheral blood stem cells (PBSC) have been used for gene transfer studies, and genetically modified PBSC reinfused into the patient will produce successful long-term engraftment.

Safety Concerns About Transgene Expression in Stem Cells

While there are sound reasons for selecting stem cells as the targets for gene therapy, there remain concerns about the possible consequences of this manipulation. Retroviral transduction of stem cells in prior transplant trials has not impaired the kinetics of reengraftment or immune reconstitution. Since retroviral transduction will be attempted on only a portion (approximately one-third) of the stem cells, damage or immunologic rejection of transduced cells would not be anticipated to cause graft failure. Transgenic hematopoietic stem cells do present two theoretical safety concerns: insertional mutagenesis and production of replication competent retrovirus (RCR). Mutagenesis may arise by chance insertion of the retrovirus within a suppresser oncogene, resulting in disruption of a normal apoptotic or antiproliferative signal. Alternatively, the retrovirus may insert upstream of an oncogene resulting in retroviral promoter driven transcription of the oncogene. To date, no human malignancies or evidence for insertional mutagenesis have been reported in human gene therapy trials. Replication competent retroviruses is ruled out by both *Mus dunni* assays and RT-PCR for envelope gene prior to release of supernatant for *ex vivo* transduction.

Immune-Mediated Rejection

Immune-mediated rejection of cells carrying the transgene is possible. Riddell *et al*. marked $CD8^+$ HIV-specific cytotoxic T cells (CTL) with a retrovirus encoding gene (HyTK) that permits both positive (hygromycin phosphotransferase) and negative (thymidine kinase) selection (24). The HIV-specific autologous CTL disappeared rapidly when infused into HIV-positive patients. Disappearance was accompanied by anti-Hy and anti-TK CTL responses. Therefore, the immune system may eliminate genetically altered cells expressing foreign proteins. Unlike the results with lymphocytes, stem cells infused after transplant with a retroviral vector containing *neo*^{r} have never been documented to be immunologically rejected. It may be that the intense immunologic suppression/ablation of transplant conditioning regimens followed by presentation of a foreign protein in hematopoeitic cells does not lead to immune rejection or that *neo*^{r} is not immunogenic. We do not, therefore, anticipate problems with immunologic rejection of *neo*^{r} transgenic cells in the lupus and MS trials. In the rheumatoid arthritis proposal, the immunogenicity of EGFP transgenic cells arising after the intense immunologic sup-

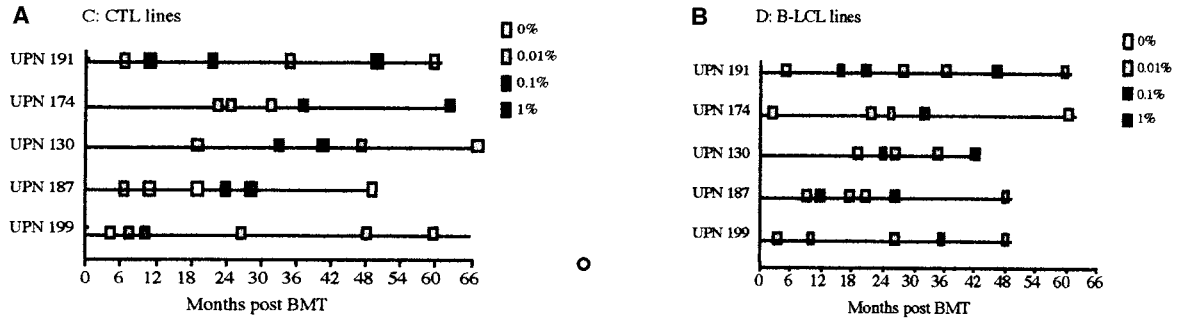

Fig. 1. (A) Persistence of transgene (*neo*^{r}) in peripheral blood T cells after transplant with retrovirally transduced stem cells. (B) Persistence of transgene (*neo*^{r}) in peripheral blood B cells after transplant with retrovirally transduced stem cells.

pression of a transplant regimen is unknown. If rejection should occur, we may switch constructs.

Evidence that Human Hematopoietic Progenitor Cells Can Be Transduced and Will Differentiate

The key to the success of this project is the ability to transduce human hematopoietic progenitor cells. We anticipate that even low levels of transfer will be adequate should disease relapse because of the exceptional capacity of T lymphocytes to proliferate *in vivo* in response to appropriate antigenic stimuli should disease-associated T cells arise from the stem cell compartment. We, nonetheless, have taken strides to improve the transduction efficiency of hematopoietic stem cells by coupling combinations of growth factors with physical methods to bring hematopoietic stem cell targets and retroviral vectors into close apposition. The best results were obtained by coculturing $CD34^+$ stem cells with IL-6, SCF, Flt3 ligand, and TPO for 3 days and then transducing the cells by pulling retroviruses through a polycarbonate membrane and layering the target cell population on top. This protocol of flow-through transduction has yielded levels of gene transfer three to five times higher than can be obtained with conventional procedures performed in solution. Flow-through transduction enhances the frequency of transgene-positive cells, which is now between 15 and 30%, relative to the 0.2 to 2% being obtained in prior gene-marking trials (25, 26).

Although gene transfer to primitive progenitor cells can be enhanced, this does not prove that the transduced cells will repopulate patients or that transgene expression will persist. Indeed, a number of studies with murine models suggest that transgene expression declines progressively after retrovirally mediated gene transfer, owing to an effect termed "silencing." Malcolm Brenner *et al*. have obtained data on patients treated 6 or more years ago with gene-modified progenitor cells that demonstrate the persistence of transgene expression in long-term repopulating cells. Six of these patients have now been followed for more than 6 years. In all of them, the marker gene (*neo*^{r}) can be detected by quantitative PCR amplification, in multiple lineages in peripheral blood. Figure 1 shows that the transgene can be readily detected in both T and B cell lines prepared from peripheral blood at different time points after transplantation.

MULTIPLE SCLEROSIS

We have performed T cell-depleted transplants in 10 patients with progressive MS over the last 3 years and have previously reported results on the first 6 of these patients (2, 6). Although posttransplant follow-up is short, no patient has had clinical progression despite discontinuation of corticosteroids and interferon. Based upon our prior transplant experience, candidates in the proposed gene-marking study will be eligible if they have more than three acute motor or cerebellar exacerbations over a 24-month interval despite interferon-γ therapy (Table II).

Outcome Measure

Our primary outcome measure is treatment failure (TF). The definition of TF will be disease relapse or progression of disability from pretransplant baseline by

Table II. Eligible Candidates for the MS Proposal Would Meet All of the Criteria Listed

(1) Clinically definite MS by Poser criteria
(1) EDSS score more than 4.0 but less than 7.5
(2) More than three motor or cerebellar relapses over a 24-month interval despite interferon.
(3) Less than 60 years old
(4) Certification of eligibility by the Outside Panel
(5) Normal function of other organ systems

1.0 Extended Disability Status Scale (EDSS) point. In order to allow for fluctuation in neurologic status due to the stress and intercurrent illnesses that may occur with a transplant (i.e., to allow for transient changes due to transplant-related morbidities), evaluation for treatment failure will not start until 3 months after transplant. Secondary outcome measurements will include the NRS score, an 8-meter timed walk, a nine-hole peg test, neuropsychological functions, MRI results, the number of relapses, the time to progression, and the number of steroid treatments. MRI studies of the brain with and without gadolinium will be performed prior to transplant to provide baselines. MRI studies will be repeated at 3 and 6 months after transplant and then every 12 months for 5 years.

Immune Parameters: Myelin-Specific T Lymphocytes

The role of the immune system in MS remains unclear. Restricted T cell clones may be associated with MS and vary by HLA and/or epitope spreading associated with disease progression. Some T cell repertoires may cause disease, while others regulate or suppress disease activity or are merely incidental bystanders. Alternatively, the same T cell repertoire can cause or prevent disease depending on other factors such as the cytokine profile. Finally, MS T cell repertoires and phenotypes may be unrelated to disease activity.

The T cell response specific to a number of previously characterized candidate antigens or peptides will be studied from pre- and posttransplant samples. The antigens to be tested are peptides of myelin basic protein (MBP), myelin oligodendrocyte protein (MOG), proteolipid protein (PLP), 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), and myelin oligodendroglia basic protein (MOBP). The precursor frequency for T cells responding to these antigens will be determined based on IL-7-stimulated primary proliferation and split well cloning. Further characterization of the clones will include (a) HLA restriction, (b) TCR usage, (c) the surface marker profile, (d) the cytokine profile, and, in posttransplant samples, (e) the presence of the *neo*^{r} transgene.

The clones derived from the pretransplant sample are candidates for being disease-associated T cells. However, the importance of these clones will be increased if similar or identical clones can be derived at the time of relapse. Samples from patients who show an exacerbation and disease progression will be a very important source of information about the nature of T cells responding to MBP and associated proteins. This clinical study provides an absolutely unique opportunity to longitudinally and prospectively follow the development of T cells recognizing CNS antigens that have heretofore been thought to be important in the pathogenesis of MS. It can be easily envisioned how different experimental outcomes can be informative. The variables that could change would be the precursor frequency or the clonal phenotype [e.g., surface molecules, sensitivity to stimulation via second or third signals, change in avidity of T cell receptor (TCR) complex, and cytokines produced]. Observation of identical changes in a number of clones and in a number of patients would help cement this as a possible mode of disease progression.

Identification of the *neo*^{r} gene within the clones would be conclusive data for regeneration of disease-associated cells from the stem cell compartment. This would lead one to question the long-term value of autologous hematopoietic stem cell transplantation as therapy for MS. On the other hand, some myelin-reactive clones arising from the stem cells may be of a particular phenotype (e.g., Th2 cytokine profile) associated with clinical remission.

Table III. Eligible Candidates for the Lupus HSCT

(1) Patients must have at least 4 of the 11 American College of Rheumatology (ACR) classification criteria for SLE.
(2) For lupus nephritis, patients must fail pulse methylprednisolone and pulse cyclophosphamide (500 to 1000 mg/m^2 × 6 months). Failure is defined as failure of serum creatinine to return to normal with biopsy confirmation of active disease, or nephrotic syndrome (24-hr urine protein >2.6 g), or relapse of nephritis within 3 years of treatment with pulse cyclophosphamide, or a continued requirement for pulse cyclophosphamide to control disease.
(3) For visceral organ involvement other than nephritis, patients must have evidence of clinically active disease (SLEDAI score >10 or BILAG score >15) or biopsy or radiographic evidence of active disease despite treatment. Patients must also fail corticosteroids (either oral prednisone >0.5 mg/kg/day for more than 6 months or pulse methylprednisolone for at least one cycle of 3 days) and at least one of the following; azathrioprine at 2–3 mg/kg/day for at least 6 months, mycophenolate mofetil at 2 g daily for more than 3 months, cyclophosphamide intravenously or orally for at least 3 months, or cyclosporin at at least 3 mg/kg/day for at least 3 months.

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Seven patients with SLE have undergone autologous hematopoietic stem cell transplantation on our phase I protocol (4, 6). Two of these patients have been reported previously. This study has provided the foundation for a phase II trial using genetically marked autologous hematopoietic stem cells. Potential candidates are listed in Table III.

Outcome Parameters

Primary outcome measurements will be the change in activity scores comparing pretransplant to 1, 3, 6, 9, 12, 18, and 24 months and then yearly posttransplant. Disease activity measures correlate with a patient's perception of quality of life, with physician decision to change treatment, and a few of the indices have been shown to be valid and reliable across different ethnic groups. The measuring instruments will be the SLE Disease Activity Index (SLEDAI) (Appendix VII) and the British Isles Lupus Assessment Group (BILAG) (Appendix VII). The BILAG has the advantage of including more body systems and also, because of its A to E scale, can be used as a measure of response to intervention.

Secondary parameters will include the Systemic Lupus International Collaborating Clinics (SLICC), a damage index that has been validated for damage to major organs, the number of relapses (the usual flare rate for SLE is 0.65 per year, where a flare would be a change by 3 or greater in the SLEDAI score), as well as a health assessment questionnaire (SF-36 measure of health status), and antihypertensive and immunosuppressive medication usage. Laboratory parameters will include antinuclear antibody, anti-ds DNA antibody titer, complement (C3, C4, CH50), and evaluation of involved organ systems.

Immune Parameters

T cells are central in the development of disease pathology in lupus patients either by providing cognate and noncognate help to B cells to produce autoantibodies or by directly infiltrating tissues (27–38). Numerous lupus-associated T cell abnormalities exist. Peripheral T cells from patients with lupus display activation markers (upregulation of CD69 on resting T cell) and provide help to B cells to produce immunoglobulin (31). T cell lines from patients with lupus nephritis provide help to autologous B cells to produce cationic anti-dsDNA antibody (32, 38). Fresh T cells and T cell lines from patients with lupus display enhanced TCR-mediated intracellular free calcium concentration $[Ca^{2+}]_i$ responses (34). Patient-derived autoantigen-specific clones display increased $[Ca^{2+}]_i$ and protein tyrosine phosphorylation following stimulation with anti-CD3 antibodies (37). Lupus T cells fail to produce IL-2 and proliferate in response to tetanus toxoid (28). Finally, T cells from patients with active lupus display a Th2 cytokine skewing.

Collectively, lupus T cells present with aberrations that may be directly related to the pathology of the disease. These studies, in conjunction with the accompanying clinical and laboratory (e.g., serology) studies, will shed much needed light on the role of cellular abnormalities in the expression of clinical disease. Analysis of pre- and posttransplant T cell abnormalities will establish in the most definitive manner the importance of T cell abnormalities in the development of lupus, a quest that has been impossible in cross-sectional studies.

Table IV. RA Patients Eligible for Autologous Hematopoietic Stem Cell Transplantation

Patients must fulfill *all* of the following criteria:
(1) Clinical diagnosis of RA by the American College of Rheumatology criteria
(2) Failed a trial of methotrexate and TNF inhibitor. Failure would be defined as at least 6 swollen joints and either 20 involved (tender, deformed, painful, or swollen) joints or the inability to answer at least 25% of HAQ-ADL questions without difficulty
(3) Be less than 65 years of age (since the risk of regimen-related mortality increases with age)
(4) Other organ function within normal limits

Lymphocytes arising from the reinfused stem cells will be determined by culture in G418 to select for neo^r-containing cells. Identification of the transgene in T cell lines that help or inhibit B cells to produce anti-ds-DNA antibody or have alterations in signaling or lymphokine abnormalities that are characteristic of lupus would indicate that the potential disease-causing or disease-suppressing autoregulatory T cells arose from the stem cell compartment. Scenarios can be imaged where T cells which survived the conditioning regimen and expand in the periphery display abnormalities consist with lupus, while neo^r gene-marked T cells that arise from the stem cell compartment do not display these abnormalities or are actually autoregulatory and inhibit B cell anti-ds DNA antibody production.

RHEUMATOID ARTHRITIS

We have previously reported on four patients with RA treated by autologous HSCT (6, 39). Based on what we *have* already learned, we are now beginning a phase II study (Table IV) of intense immunosuppression and autologous hematopoietic stem cell transplantation using gene marked stem cells.

Clinical Outcome

Time to treatment failure will be the primary outcome. Many studies in RA define failure as inability to maintain an ACR 20 (American College of Rheumatology response for a 20% improvement). Despite the lack of toxicity in the patients we have treated to date, transplan-

tation will be considered a "draconian" therapy and most rheumatologists would consider an ACR 20 to be a failure of this therapy. In contrast an ACR 50 is a truly meaningful response and has a much lower placebo effect compared to an ACR 20 (3 versus 20%). For these reasons, in our proposal, treatment failure will be failure to maintain at least an ACR 50.

Secondary Outcome. Secondary outcome will be the duration of an ACR 70, complete remission, progression or improvement in hand and knee radiographs, sedimentation rate, CRP, HAQ-ADL, morning stiffness duration, patient and physician assessment of disease, tender and swollen joint counts, and immunosuppressive medication usage and duration.

Immune Parameters: Analysis of Synovium

We have chosen to evaluate peripheral blood T cell clones in patients with MS and T cell lines in patients with lupus. For patients with RA, we opted to evaluate immune cells within the diseased organ system (synovium). This is because peripheral blood antigen-specific T cells or T cell disease-associated abnormalities are not well defined for RA. In contrast, it is relatively easy and uncomplicated to biopsy the involved organ in patients with RA. It is also possible that the characteristics of peripheral blood lymphocytes are different from those that "home" or traffic to diseased organs. It would be highly informative to analyze an involved organ system *in situ* by obtaining pictures at different posttransplant time points. This is especially important in open trials where one seeks hard evidence of a therapeutic effect reflected in synovial biology.

New blood vessel proliferation provides the framework upon which rheumatoid synovitis can build. Angiogenic factors (including fibroblast growth factor and IL-8) stimulate new capillary formation, and cytokines such as IL-1 and TNF-α induce formation of adhesive proteins on the endothelial cell surface that attract and bind circulating polymorphonuclear leukocytes and lymphocytes. Beneath the lining layer are infiltrating lymphocytes, plasma cells, monocytes, and macrophages. Histopathologic studies of RA reveal an abundance of T cells, which comprise about 30–50% of the synovial tissue cells. The predominant T-cell subset in the sublining of RA patients is the $CD4^+$ helper/inducer lymphocyte and the CD4/CD8 ratio ranges from 4:1 to 14:1. While the T cell is traditionally considered as a central player in rheumatoid arthritis, alternative models implicate macrophage-derived IL-1 and TNF-α and fibroblast-like synoviocytes (FLS). Considerable data have accumulated suggesting that FLS in RA have certain characteristics of transformed cells (40). For instance, RA FLS can proliferate in an anchorage-independent manner. Another property of RA synoviocytes suggesting partial transformation is that they can escape contact inhibition and form microfoci *in vitro*. The most convincing evidence is that they aggressively invade into the cartilage matrix when coimplanted into SCID mice with cartilage explants (41).

Stem cell transplant studies will open a window into the basic pathological processes that occur in inflammatory arthritis and possibly provide predictors for therapy. Perhaps the most important observations will involve examination of those unfortunate individuals that relapse, where we will have the opportunity to evaluate prospectively the evolving T cell repertoire, cytokine production, and FLS gene expression.

Transduction of a portion of $CD34^+$ stem cells with a marker gene (green fluorescence protein (EGFP) will enable us to track its distribution through tissue. For instance, it will be critically important to ascertain whether new T cells, B cells, monocytes, or macrophages that infiltrate the synovium in patients that relapse are derived from the stem cell compartment. EGFP transducted cells are readily detected with FITC microscopy by viewing cryopreserved thin cross sections. In addition, direct quantification of transduction is obtainable by FACS analysis of collagenase digested cells. EGFP transduced lymphocytes may also be separated by surface characteristics such as adhesion molecules, activation markers, intracellular cytokines, and T cell receptor repertoire to determine the lineage and location of the highly fluorescent GFP-bearing cells. More intriguing, it is possible that synoviocytes in the intimal lining will be derived from the infused stem cells. This would suggest that the intimal lining is constantly being replenished from newly formed bone marrow-derived cells.

SUMMARY

What if our relapse rate is unacceptably high for one or all three diseases after HSCT? This result, especially when coupled to the immune assays being performed, would be highly informative for designing future therapies. Gene marking of stem cells is designed to better understand the origin of posttransplant lymphocytes and to characterize the phenotype of immune cells arising from the infused stem cells. Demonstrating the feasibility of this approach may allow for future insertion of inducible regulatory genes within the stem cell compartment. The choice of which regulatory gene to use may also be clarified by analysis of the regenerating post-

transplant immune system and correlation of changes with disease activity.

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