Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure?

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Hematopoietic stem cells (HSCs) are the earliest cells of the immune system, giving rise to B and T lymphocytes, monocytes, tissue macrophages, and dendritic cells. In animal models, adoptive transfer of HSCs, depending on circumstances, may cause, prevent, or cure autoimmune diseases. Clinical trials have reported early remission of otherwise refractory autoimmune disorders after either autologous or allogeneic hematopoietic stem cell transplantation (HSCT). By percentage of transplantsations performed, autoimmune diseases are the most rapidly expanding indication for stem cell transplantation. Although numerous editorials or commentaries have been previously published, no prior review has focused on the immunology of transplantation tolerance or development of phase 3 autoimmune HSCT trials. Results from current trials suggest that mobilization of HSCs, conditioning regimen, eligibility and exclusion criteria, toxicity, outcome, source of stem cells, and posttransplantation follow-up need to be disease specific. HSCT-induced remission of an autoimmune disease allows for a prospective analysis of events involved in immune tolerance not available in cross-sectional studies. (Blood. 2002;99:768-784)
localized within secondary lymphoid tissues (spleen and draining lymph nodes). Transfer of antigen by immune cells to secondary lymphoid regions may be important to induce T-cell activation. For example, allogeneic tissue grafts are not rejected in mice that lack secondary lymphoid tissue.

Besides the requirement for costimulation, a variety of mechanisms maintain peripheral tolerance. Some of these mechanisms are similar to intrathymic tolerance but occur in the periphery, including peripheral T-cell deletion and/or anergy induced by T-cell interaction with parenchymal cells. Other checks to maintain peripheral tolerance include activation-induced cell death, suppressor or regulatory cells, and peripheral antigen avidity (i.e., antigen persistence, concentration, and affinity). Theories on peripheral tolerance explain how a T-cell repertoire selected intrathymically for reactivity to self maintains peripheral tolerance. A further extension of tolerance to what has been termed the “danger signal” explains the context in which costimulation arises.

The danger metaphor proposed by Matzinger involves the use of the innate immune system (neutrophils, natural killer cells, and macrophages) to break peripheral tolerance. T-cell–mediated immunity, known as adaptive immunity, is an evolutionary development of vertebrates. Adaptive immunity involves the rearrangement of a limited number of germ line genes to produce a highly diversified repertoire of approximately $10^4$ to $10^8$ somatically mutated T-cell (immunoglobulinlike) receptors and B-cell immunoglobulin receptors. These T cells undergo deletion and anergy within the thymus. However, the innate immune system does not have pathogen-receptor repertoire diversity. Response to infection is intrinsic to a limited number of germ-line receptor genes that recognize pathogen-specific molecular patterns. These patterns include receptors for conserved pathogen structures like lipopolysaccharides, mannans, bacterial DNA, and lipoteichoic acids. Receptor-mediated phagocytosis of pathogens by macrophages leads to release of proinflammatory cytokines and expression of costimulation molecules, along with MHC presentation of pathogen-derived peptides, leading to T-cell activation. Thus, pathogen stimulation of innate immunity can lead to activation of the adaptive immune system.

In animal models, active immunization with self-epitopes requires an adjuvant (immune stimulant) to break tolerance. Adjuvant is often nothing more than homogenized pathogens such as mycobacterium, which provides the danger signal for activation of innate APCs such as macrophages. Presentation of coinjected self-proteins by adjuvant-activated APCs initiates antigen–specific autoreactive T cells. Once activated to self by innate immunity, how is the adaptive immune system prevented from causing autoimmune disease? This question may be approached by viewing the immune system as dynamic and constantly fluctuating.

In all prior theories of tolerance, lymphocytes are viewed as responding or not responding, like a light switch that is on or off. The perturbation theory postulated by Grossman and Singer and Grossman and Paul proposes that lymphocytes are dynamically tuned much like a rheostat dims or brightens a room. Lymphocytes selected intrathymically may maintain a steady tone by repeated interaction with peripheral tissue. It is the sudden change in dynamic homeostasis that is perceived as a perturbation. By analogy, blood is always dynamically fluctuating between clotting and lysis. In steady state, blood may be erroneously perceived as static. The immune system may also be dynamically fluctuating between autoimmunity and tolerance in a dynamic steady state not readily appreciated. A steady state that may be controlled by clonal selection, activation, feedback inhibition, and intracellular receptor and signal transduction tuning. It is conceivable, but unproven, that immune ablation followed by infusion of hematopoietic stem cells (HSCs) may “reset the immune rheostat.”

### Breaking tolerance by environmental exposure

All processes involving tolerance, even deletion, are ongoing recurring events and may be broken. Both central and peripheral T-cell tolerance may be broken by environmental exposure. Classic agents capable of breaking tolerance include drugs and infections.

### Drug-induced autoimmunity

Numerous drugs may cause autoimmunity by affecting thymic TCR antigen interaction or TCR signal events. A common drug associated with lupuslike manifestations is procainamide. When the metabolite procainamide-hydroxylamine is injected into the thymus of an animal or added to primary thymic organ cultures, chromatin-reactive T cells emerge. Procainamide-hydroxylamine may alter the avidity of TCRs for self-antigen, preventing deletion of some autoreactive T-cell repertoires. Cyclosporine is an immunosuppressive medication that inhibits TCR-mediated signaling. By inhibiting peripheral T-cell activation, cyclosporine suppresses autoimmunity but by interference with thymic TCR signaling may also inhibit thymic deletion of autoreactive T cells, causing a T-cell autoimmune sclerodermalike disease termed syngeneic graft versus host disease (GVHD).

Drug-induced disruption of central tolerance implies existence of a functional thymus throughout adulthood. By using the membrane protein CD45 to differentiate naive (CD45 RA) from memory (CD45RO) T cells, thymic-dependent T-cell production appears to diminish markedly after puberty, presumably because of thymic atrophy. If the thymus involutes, new adult T cells would then be derived exclusively from peripheral expansion of existing memory cells. However, with the advent of newer DNA assays, the accuracy of differentiation between naive and memory T cells by CD45 has been questioned.

During TCR thymic development, rearrangement of TCR genes leads to excision of circular DNA termed T-cell receptor rearrangement excision circles (TRECs). TRECs are episomal, unique to T cells, and do not duplicate during mitosis. Because TCR rearrangement occurs during thymic development, TRECs may be used as a marker for recent thymic emigrants. In the early post–hematopoietic stem cell transplantation (HSCT) period, there is a substantial increase in peripheral blood TREC-positive T cells. Although an inverse correlation exists between age and TREC production after HSCT, TREC numbers increased in all age groups. Therefore, thymic-dependent generation of T cells occurs in all ages. A drug or environmental-related disruption of thymic tolerance, which alters TCR antigen avidity or TCR cytoplasmic or nuclear signaling events, may allow escape of autoreactive lymphocytes. Once in the periphery, long-lived autoreactive cells could cause a persistent autoimmune disease.

### Infection-induced autoimmunity

An infectious agent has been associated with virtually every autoimmune disease, including diabetes mellitus, ankylosing spondylitis, multiple sclerosis (MS), myocarditis, rheumatoid arthritis (RA), and SLE. These associations are suggested by epidemiologic studies and serology that connect disease onset or...
flare to various infectious agents, cross-section of virus or pathogen epitopes and self-proteins, and occasional isolation of an infectious agent in affected tissue.

An infection could precipitate an autoimmune disease by breaking self-tolerance through molecular mimicry,98,99 determinant or epitope spreading,100,101 or bystander activation.102 Molecular mimicry is the capacity of a lymphocyte activated to an infectious pathogen to cross-react with a similar host determinant. Because memory lymphocytes are long lived, the infectious agent that initiated molecular mimicry to self does not need to persist for autoimmunity to occur. This situation may be one reason for difficulty in proving an infectious etiology for autoimmune disorders. Bystander activation arises when activation of T cells specific for antigen X occurs during an immune response against a nonhomologous antigen Y. In contrast, molecular mimicry is targeted toward self-peptides homologous to the initiating determinant on a viral or other infectious agent. Immunization with adjuvant and peptide is an example of bystander activation to the co-injected nonhomologous peptide.103

Infection-related inflammation is associated with tissue destruction and presentation of self-epitopes, as well as up-regulation of APC costimulatory molecules that may also lead to bystander activation of T cells to self-determinants. Theiler murine encephalomyelitis virus (TMEV)–induced demyelination, an autoimmune demyelinating disease that mimics MS, is an example of viral-induced bystander activation.104 TMEV is a picornavirus (small RNA virus) that infects gray matter neurons but, through bystander activation of the immune system, leads to an autoimmune-demyelinating white matter disease.105

Superantigens may also cause bystander activation. Superantigens are bacterial, mycoplasma, or viral proteins that activate polyclonal groups of T cells.106-112 Polyclonal activation arises by cross-linking the side of a MHC molecule to the Vβ portion of a TCR. Superantigen binding occurs outside the MHC peptide-binding groove and outside the TCR CDR3 antigen-specific recognition site. Activation by superantigen results in overexpression and/or deletion of entire Vβ families, resulting in skewing of the T-cell repertoire. Superantigen activation of T cells has been suggested to initiate or cause a flare of various autoimmune diseases, including myocarditis, diabetes, MS, and psoriasis.107

Once molecular mimicry, bystander activation, or superantigens initiate an autoimmune disease, the immune response spreads over time to epitopes that are distinct and non–cross-reactive to the inducing epitope, a phenomenon termed determinant or epitope spreading.113 Epitope spreading has been documented for both T- and B-cell immune responses. A hierarchical order of epitope spreading occurs according to immune dominance of the epitope. Determinant spreading may occur to different regions on the same protein (intramolecular epitope spread) or to a protein distinct from the protein containing the disease-initiating epitope (intermolecular epitope spreading). Temporal spreading of immune responses to other epitopes has been demonstrated in numerous animal autoimmune disorders, including experimental autoimmune encephalomyelitis (EAE),114 diabetes in nonobese diabetic (NOD) mice,115 and experimental autoimmune myasthenia gravis.116 Determinant spreading is suspected to be associated with several human autoimmune diseases, including MS,117 SLE,118 bullous skin diseases,119 myasthenia gravis,120 diabetes,121,122 and chronic rejection of organ allografts.123-125

The mechanism of epitope spreading may be related to costimulation, because in some models blocking CD28/B7 costimulation may prevent epitope spreading.100 Whatever the mechanism, epitope spreading makes it difficult to retrospectively determine the inducing epitope or antigen. Effectiveness of targeted immune interventions directed against one TCR or epitope may be limited by the phenomenon of epitope spreading.

Genetic susceptibility to breaking tolerance

MHC autoimmune-associated genes

MHC antigens were initially referred to as tissue transplantation antigens. They were discovered, as the name implies (major histocompatibility complex), to have a major role in rejection of transplanted organs. As later discovered by Zinkernagel and Doherty,126 the MHCs are peptide-presenting molecules resulting in MHC/peptide restriction for T-cell recognition.127 It is not, therefore, surprising that many autoimmune diseases are associated with particular MHC genotypes.

Numerous suspected autoimmune disorders (such as MS, RA, spondyloarthropathies, diabetes, myasthenia gravis, Crohn disease, primary biliary cirrhosis, autoimmune hepatitis, SLE, vasculitis, pemphigus vulgaris, and Sjögren syndrome) are associated with MHC alleles.128 Because combined MHC/peptide presentation is essential for T-cell activation, a MHC association may be indirect evidence for an immune pathogenesis. RA-prone MHC alleles, their frequencies vary for different ethnic groups, share a similar amino acid epitope sequence (LLEQKRAA or LLEQRRAA) encoded by codons 67 to 74.129-131 The HLA sequence 67 to 74 is a HLA contact site for both peptide and TCR binding. This finding suggests HLA presentation of a common infectious or self-antigen to T cells is involved in the pathogenesis of RA. Spondyloarthropathies are linked with only some molecular subtypes of HLA-B27.132 Similar to RA, peptide-binding differences may explain differences in disease susceptibility. HLA-B27 may even present its own B27-derived peptides. In which case, the putative arthritogenic peptide may be a component of the HLA-B27 molecule.

The autoimmune etiology for scleroderma is questionable because of poor response to immune suppressive medications. Similarly, scleroderma also has a relatively weak MHC association that may indicate only partial immune pathogenesis or weak linkage of scleroderma genes to MHC alleles or the absence of an autoimmune etiology.133 Although MHC genes correlate with autoimmune disease susceptibility, most patients with disease-associated MHC genes remain disease free throughout their lifespan. Environment and/or non-MHC genes must, therefore, contribute toward development of disease.

Non-MHC autoimmune genes

Multiple non-MHC genes that regulate cell proliferation (oncogenes), cell signaling (tyrosinases), immune response (costimulatory molecules, interleukins, and cytokines), and apoptosis (fas) may play a role in development of autoimmunity.134 Analysis of the diabetic-prone NOD mouse has revealed at least 18 insulin-dependent diabetes prone genes.135 SLE occurs in various strains of mice, including Murthy Roth lymphoproliferative (MRL/lpr) mice and New Zealand Black X New Zealand White F1 hybrid (NZB/NZW) mice.136 Various mating crosses of lupus-prone mice, as well as backcrosses to normal mice, have linked murine lupus to 38 different genomic loci.137 Some loci are associated with glomerulonephritis, others with vasculitis, some with anti-ds DNA, some with antichromatin antibody, some with lymphoproliferation, and others with splenomegaly. No single gene is sufficient to cause
disease. Various combinations of SLE-prone genes among different patients may explain why patients with SLE can have highly variable organ involvement and clinical symptoms. Collagen-induced arthritis in rats is a model for RA and can be induced by injection of collagen and adjuvant. At least 14 genomic intervals or collagen-induced arthritis (CIA) loci are associated with collagen-induced arthritis.

Although autoimmunity involves MHC and numerous non-MHC genes, environmental interactions with these genes are essential to manifest disease. Approximately two thirds of syngeneic twins with MS, RA, SLE, or type I diabetes are discordant for clinical disease. Although a concordance rate of 33% is much higher than the general population, it remains significantly below a predetermined dominant Mendelian penetrance of 100% and suggests that environmental factors continue to have a significant role in polygenic autoimmune diseases.

**Induction of tolerance by immune ablation and autologous stem cell transplantation**

**Animal models and anecdotal case reports**

Animal autoimmune diseases that are induced by immunization with adjuvant or self-peptide and adjuvant may be ameliorated by syngeneic or pseudo-autologous HSCT.

Immunization with adjuvant and either myelin basic protein or proteolipid protein peptides induces a T-cell–mediated demyelinating disease. EAE, that, depending on the animal model, may be monophasic, relapsing-remitting with secondary progression, or progressive from onset. EAE in Swiss Jackson Laboratory/Jackson (SJL/J) mice is a relapsing, remitting, and secondarily progressive disease. Several investigators have demonstrated cure, decreased relapse rates, or decreased disease severity in EAE animals undergoing syngeneic HSCT. Because of the expense of long-term animal housing, most experiments in EAE are performed before disease onset to abort disease initiation or shortly after disease onset to ameliorate its course. It is unlikely that such experiments are applicable to patients with a long duration of MS with accumulated disease burden and tissue damage. Syngeneic HSCT performed in mice with chronic EAE, unlike the results in acute EAE, failed to demonstrate neurologic improvement. Histologic analysis revealed chronic scarring with glial proliferation that is unaffected by HSCT. To be effective as therapy for EAE, HSCT needs to be performed early in the disease course.

**Mobilization of HSCs**

Collection of stem cells from patients with autoimmune diseases is based on methods already established for patients with nonautoimmune disorders. The complications and risks of the procedure appear greater in patients with autoimmune disease and are specific for the autoimmune disease and involved organ system. The most common peripheral blood stem cell (PBSC) mobilization regimens are single-agent granulocyte colony-stimulating factor (G-CSF) or cyclophosphamide and G-CSF.

Flares of MS and RA have occurred while patients were taking G-CSF for mobilization. MS flares have resulted in serious and irreversible neurologic deterioration. G-CSF–related flares of RA are relatively mild, being manifest as a transient increase in the number of swollen or tender joints that resolves with or without an increase in corticosteroid dose. The only complications of G-CSF PBSC mobilization in patients with scleroderma are transient telangiectasia that spontaneously resolves. In other diseases, such as SLE, there exists virtually no data on PBSC with G-CSF as a single agent. The simultaneous administration of G-CSF and steroids has been used in a limited number of patients without disease exacerbation.

To prevent G-CSF–related disease flare, combined cyclophosphamide and G-CSF (Cy/G-CSF) may be used for mobilization. However, combined Cy/G-CSF PBSC mobilization has been complicated by neutropenic-related infection and disease-specific fatal visceral organ toxicity. Infections with opportunistic organisms may be more common in patients who have been on high-dose corticosteroids for prolonged intervals, such as patients with refractory SLE. Scleroderma patients with cardiac and/or pulmonary involvement undergoing PBSC with 4.0 g/m² cyclophosphamide have succumbed to cardiac arrest and/or pulmonary alveolar hemorrhage. No significant regimen-related organ damage has been reported at doses of 2.0 g/m² or for doses of 4.0 g/m² in nonscleroderma patients. This finding emphasizes the importance of avoiding high-dose PBSC mobilization and preferentially using Cy/G-CSF.
of adjusting the mobilization regimen based on disease and organ involvement for the minimum mobilization-related morbidity.

Although cyclophosphamide-based mobilization is generally associated with more toxicity from infection or organ damage, autoimmune diseases are generally ameliorated by the immune suppressive effects of cyclophosphamide.167 The duration of improvement from cyclophosphamide-based PBSC mobilization is unknown because most patients proceed within a relatively short time interval from mobilization to HSCT. As an exception, in at least one autoimmune disease (Evans syndrome), cyclophosphamide-based PBSC resulted in rapid and fatal acceleration of disease activity.170 This acceleration was attributed to a rapid cyclophosphamide-induced suppression of otherwise compensatory and accelerated hematopoiesis in the presence of persistent peripheral destruction from residual immunoglobulins against red blood cells and platelets.

There is no single optimal mobilization regimen for PBSC in patients with autoimmune disease. The PBSC method should be individualized for the disease and organ system involved. Newer mobilizing agents such as stem cell factor, thrombopoietin, chemokines, and/or high-dose corticosteroids and G-CSF need to be evaluated to collect progenitor stem cells with minimum mobilization-related morbidity.

After collection of progenitor cells, most but not all centers perform ex vivo lymphocyte depletion.167 Because the existence or identity of suppressor cells remains vague, graft depletion techniques are nonspecific without attempts at conserving regulatory cells. Positive enrichment for CD34+ cells has been performed by using either CEPRATE (CellPro, Bothel, WA), Isolox (Nexel, Irvine, CA), or CixIIMACS (Miltenyi, Bergish Gladbach, Germany) cell separation systems. Negative selection was performed with T-cell antibodies by e-rosette or Nexel Isolex CD4/CD8 selection.

Insufficient clinical data are currently available to compare an unmanipulated versus a T-cell–depleted graft in terms of disease response or relapse. Aggressive lymphocyte depletion may adversely affect immune reconstitution against pathogens, increasing the risk of serious posttransplantation opportunistic infections such as cytomegalovirus, fungemia, Pneumocystis carinii pneumonia, or Epstein-Barr virus posttransplantation lymphoproliferative disease (PTLD).

**Conditioning regimens and the role of immunosuppressive versus myeloablative conditioning for reinduction of self-tolerance**

The first convincing evidence that intense immunosuppression may cure life-threatening autoimmune diseases was obtained in a patient with mixed cryoglobulinemia in end-stage renal failure with a cryocrit level of 60%.171 In the early 1970s, a patient with monoclonal immunoglobulin (Ig)M and polyclonal IgG was treated with a combination of cyclophosphamide and azathioprine. Treatment was complicated by lymphocytopenia and sepsis because of neutropenia, but the patient recovered with no stem cell support. After recovery, renal function normalized in parallel with elimination of the cryoglobulinemia, and the patient is alive and disease free for more than 25 years.171 This case represents the longest observation of a patient with induced self-tolerance after elimination of self-reactive lymphocytes and reestablishment of immunity from uncommitted stem cells.

Brodsky et al172 extended this early observation by treating a variety of autoimmune diseases with high-dose cyclophosphamide (200 mg/kg) without HSC infusion.172 For some autoimmune diseases such as SLE, early results from high-dose cyclophosphamide without stem cell support are encouraging. Although the response rate is high, depending on disease, relapse is common. With the exception of some diseases such as SLE, a more intense and myeloablative regimen with stem cell support may be required for durable responses. Infusion of mobilized HSCs shortens the duration of neutropenia by 5 to 7 days, theoretically decreasing the risk of serious infections. Ex vivo expansion of HSCs before infusion may completely eliminate neutropenic-related infections. For these reasons, a trial that randomized between cyclophosphamide with or without stem cell support is not currently being planned, and the rest of this review will be devoted to immune suppression with HSC support.

Ideally, the conditioning regimen should be able to eliminate immune cells without neutropenia. Such a regimen does not exist. The more immune ablative a regimen becomes, the more likely it is to be myeloablative and require stem cell support for reconstituting hematopoiesis. The conditioning regimens being used in autoimmune transplantations were empirically developed for use in malignancies. Autoimmune conditioning regimens include cyclophosphamide (Cy)172-177; cyclophosphamide and polyclonal antilymphocyte antibodies such as antithymocyte globulin (ATG) or humanized monoclonal rat antihuman CD52 (Campath-1H) antibodies (Cy/ATG or Cy/CamPath-1H, respectively)178-182; carbamustine, etoposide, cytarabine, and melphalan (BEAM)183-185; cyclophosphamide and total body irradiation (Cy/TBI)185; cyclophosphamide, TBI, and antithymocyte globulin (Cy/TBI/ATG)184,185; busulfan and cyclophosphamide (Bu/Cy)186,187; busulfan, cyclophosphamide, and ATG (Bu/Cy/ATG)188; cyclophosphamide and thiopeta (Cy/T)189,200; and fludarabine-based regimens.

Cy or Cy/ATG is the most common conditioning regimen used for HSCT of SLE.181-183,188 Pulse cyclophosphamide (500-1000 mg/m²) is a standard treatment for SLE. It is, therefore, reasonable to escalate cyclophosphamide to transplantation doses as the conditioning regimen for SLE. To avoid cardiac injury, transplantation doses of cyclophosphamide are limited to 200 mg/kg usually divided into 50 mg/kg per day. Cyclophosphamide is often used to mobilize stem cells before HSCT at doses of 2.0 to 4.0 g/m². If cyclophosphamide is used in both the mobilizing and conditioning regimen, either the conditioning regimen dose may be decreased or the interval between mobilization and HSCT may be delayed by several weeks to minimize the risk of cardiac toxicity from total cyclophosphamide dose. When the conditioning dose of cyclophosphamide is decreased, some centers add another agent such as thiopeta.188-200 Most patients with SLE eligible for HSCT are corticosteroid dependent and markedly cushingoid. There is a marked discrepancy between ideal and actual weight in terms of calculating cyclophosphamide dose. For safety reasons, in cushingoid patients, the dose is generally based on ideal or an adjusted ideal rather than actual weight.

Cy and Cy/ATG are conditioning regimens for scleroderma176,178,194 and RA.173-175,180 High-dose cyclophosphamide may be associated with high cardiopulmonary mortality in patients with scleroderma.167 Volume shifts and infections that stress cardiovascular reserve are the likely culprit of HCT-related cardiopulmonary collapse in scleroderma-associated pulmonary artery hypertension. In RA, organ function is generally normal, and cyclophosphamide-related toxicity is less problematic. The toxicity of a conditioning regimen, therefore, depends on the disease and disease-related organ dysfunction.

Bu/Cy regimens have been used in a limited number of HSCTs for MS197 and RA.196 Busulfan is fat soluble and readily crosses the
blood-brain barrier to the site of MS plaques. Busulfan is administered orally with variability in absorption and first-pass hepatic metabolism. Busulfex is an intravenous formulation that gives more uniform and less toxic serum levels. For RA, it may be equally important for efficacy that the conditioning regimen target not only lymphocytes but also synovial macrophages. Theoretically, HSCT results may be improved in RA by adding a more effective antimacrophage agent such as busulfan to a cyclophosphamide-based regimen.201 There are special concerns about the use of Bu/Cy in RA and MS. Patients with RA may have disease-related interstitial pneumonitis with little reserve for busulfan-related lung injury. The effects of alkylation agents on demethylated neurons are unknown. In MS, the neurotoxicity of high-dose alkylating-based conditioning regimens remains unknown.

BEAM and Cy/TBI are common lymphoma regimens being used to treat MS.189-191,193 TBI was selected because, unlike most agents, radiation reads across the blood-brain barrier. To avoid TBI-related pulmonary injury, radiation is generally given in the anteroposterior and posterioranterior position with 50% lung blocks with full dose to the mediastinal lymph nodes and spinal cord. A comparison of BEAM versus Cy/TBI regimen-related toxicity has not been performed. In general, TBI regimens are not used in RA because trials of nonmyeloablative total nodal irradiation in RA were associated with unexpected late complications such as myelodysplasia.202

Cy/TBI/ATG has been used as a conditioning regimen in the United States for scleroderma195 and MS,169 and in Europe for juvenile chronic arthritis (JCA).194 For patients with pulmonary scleroderma, TBI without lung shielding has been associated with lethal pulmonary deterioration.195 If attenuated with partial lung shields, TBI-related scleroderma lung injury appears less likely. Cy/TBI/ATG has been associated with lethal PTLD.208 The investigators attributed PTLD to use of high-dose rabbit ATG. Lower and less immune-suppressive doses of rabbit ATG or the use of horse ATG has not been reported to cause PTLD in autoimmune diseases.

Independent of the conditioning regimen (Cy or Cy/TBI/ATG), when combined with aggressive T-cell depletion, HSCT in JCA has been complicated by lethal macrophage activation syndrome (MAS), manifesting as fever, lymphadenopathy, hepatosplenomegaly, and disseminated intravascular coagulation.186 MAS is a reactive hematophagocytic lymphohistiocytosis and has been associated with JCA independent of HSCT.203 The diagnosis is confirmed on bone marrow aspirate by macrophages (or histio-
cytes) actively phagocytosing hematopoietic cells and may arise from immune dysregulation perhaps in response to viral infections. To date, posttransplantation MAS appears to be a complication unique to JCA.

No reports exist of late regimen-related organ toxicity from HSCT in autoimmune diseases. All patients need to be warned of late toxicities such as cataracts attributed PTLD to use of high-dose rabbit ATG. Lower and less immune-suppressive doses of rabbit ATG or the use of horse ATG has not been reported to cause PTLD in autoimmune diseases.

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Mortality

Transplantation-related mortality (TRM) for all autoimmune diseases has been reported to be 8.6%.205 TRM is disease specific, in order of highest to lowest TRM: scleroderma, SLE, MS, and RA. This mortality is higher than expected because of phase 1 trials that selected patients with advanced end-organ dysfunction and/or active and refractory disease. Judicious selection of patients earlier in disease or in remission, but with a high risk of relapse or further progression, will diminish TRM. Variability in TRM based on the center performing the transplant, also known as the center effect,206 may be occurring for autoimmune diseases. Many factors affect TRM, including patient selection, supportive care, conditioning regimen, degree of lymphocyte depletion of the graft, use of disease-specific versus generic protocols, and so forth. A lower mortality in centers dedicated to autoimmune HSCTs may be obscured within the variability of multicenter registry data.

Posttransplantation immunization

After HSCT, a patient’s titer from prior immunizations (eg, diphtheria, measles, tetanus, hepatitis B, etc) is often low or undetectable. As discussed in the “Breaking tolerance by environmental exposure” section, immunization could, theoretically, reduce autoimmune disease. The risk of relapse may vary according to the type of immunization. For example, there was concern that onset and flare of MS may be associated with hepatitis B vaccination, although recent studies have shown no association.207 Although the risk of infection-related mortality or infection-induced autoimmunity in a nonimmunized individual probably outweighs any theoretical risk of immunization-induced disease relapse, guidelines on posttransplantation vaccination in autoimmunity have yet to be written.

Specific diseases

MS, SLE, RA, and scleroderma will be discussed further because phase 3 autologous HSCT trials are being prepared in these diseases. In Europe, the European Bone Marrow Transplant/European League Against Rheumatism (EBMT/EULAR) autoimmune committee is designing these trials. In the United States, the trials are funded by the National Institutes of Health and are being designed by disease-specific working groups composed of transplant physicians, rheumatologists, and neurologists.

Autologous HSCT for MS

MS is a relatively common North American and European disease with a prevalence of approximately 1 in 1000 people.208 It is at onset an immune-mediated disease confined to the central nervous system. The disease is characterized by a variable course.209-211 Patterns are (1) relapsing-remitting MS defined as relapsing disease without progression between relapses with or without residual neurologic deficits from each relapse, (2) secondary progressive MS defined as continuous (often insidious and steady) neurologic deterioration with or without superimposed relapses after an initial relapsing-remitting course, and (3) primary progressive MS defined as steady continuous deterioration from onset. At onset, approximately 15% of the cases are primary progressive and 85% are relapsing-remitting.209-211 Within 10 years, 50% of relapsing-remitting cases become secondary progressive, and by 25 years, 90% have progressive disease. Relapse frequency in the first year of diagnosis influences time interval to disability.209-211 The median time to difficulty ambulating without unilateral assistance (an extended disability status score [EDSS] of 6.0) is 7 years for 5 or more relapses; 13 years for 2 to 4 relapses; and 18 years for 1 to 2 relapses.

Accepted immune-modulating agents for MS are interferon beta (Avonex, Betaseron)212-216 or Copaxone (copolymers 1 or glatiramar
acetic acid) known as ABC therapy. Avonex and Betaseron are different formulations of interferon-β and Copaxone is an oral mixture of random peptide sequences containing L-glutamate, L-lysine, L-alanine, and L-tyrosine, thought to mimic myelin peptides. The ABCs of MS therapy are approved for relapsing-remitting disease and, although not approved by the U.S. Food and Drug Administration (FDA), are often used for progressive forms of MS. The immune suppressive chemotherapy drug mitoxantrone received FDA approval for secondary progressive and progressive-relapsing MS. The need for new interventions in MS is evident from the deserialization of patients who in some studies have a higher suicide rate compared with the general population.

Natural history magnetic resonance imaging (MRI) studies have demonstrated that neurologic progression can continue despite lack of new demyelinating events on MRI. Although early relapse frequency within the first year of diagnosis appears to correlate with onset of late disability, Conforte et al. reported that relapse frequency was independent of disease duration and EDSS scores. This finding indicates that treatment designed to prevent relapses (ie, immune-modulating therapy) used late in disease is probably not adequate to prevent progressive disability. Demyelination alone does not adequately explain the progressive disability that occurs in patients with progressive MS. Yet, the most important therapeutic goal is to prevent disability and maintain neurologic function. An evolving amount of literature on MS supports the concept that, although initially an inflammatory demyelinating pathogenesis, MS transitions into or is also an axonal degenerative disease.

HSCT for MS was suggested in 1995. In general, initial HSCTs were phase I studies and captured patients with progressive disease and high disability (EDSS scores) (Table 1). The Thessaloniki group has reported a 3-year progression-free survival for primary progressive MS (39%), which appears significant for secondary progressive (92%). In an Italian study, Mancardi et al. reported 10 subjects undergoing HSCT followed with a frequent MRI protocol who demonstrated lack of enhancing lesions and accumulation of T2 burden of disease over an observation period of 4 to 30 months. A second study with a 5-year follow-up has noted a discordant response between MRI and clinical results. Some patients had clinical progression of disability, defined as an increase in the EDSS by one or more points but no new attacks or change on MRI in terms of T2 disease burden. The patients whose EDSS increased despite lack of MRI changes had significant pretransplantation disabilities (EDSS of 7.0 to 8.5). Although longer follow-up is necessary, it appears that HSCT slows or halts acute attacks and further immune-mediated demyelination but not progressive disability, especially in disease of increasing duration or higher disability scores.

Two possible phase 3 MS trial designs are being proposed to run simultaneously. For secondary progressive MS, the trial would be aimed at suppressing relapses in patients with progressive disability. Patients with accumulated baseline deficits, but still inflammatory disease, could be considered candidates. This group could include ambulatory patients with an EDSS of 3.5 to 6.0 and continued relapses (or MRI evidence of active disease) randomized between a TBI and Cy regimen with or without low-dose ATG and CD34-selected HSCs versus mitoxantrone every 3 months for 2 years. However, suppression of relapses may be insufficient to halt progressive neurologic impairment, particularly as the duration of disease and the level of disability increase.

For relapsing-remitting MS, the protocol would be aimed at suppressing relapses in patients at risk for progressive disability. Patients with relapsing-remitting disease who have failed interferon may be randomized between cyclophosphamide (200 mg/kg, with or without low-dose ATG) with CD34-selected HSC support versus standard therapy (ie, continued interferon or interferon and adjuvant immunotherapy) (azathioprine, methotrexate, mitoxantrone, or cyclophosphamide). Because patients in this study would be earlier in the disease course, a safer conditioning regimen that does not include TBI would be indicated. Efficacy of earlier intervention in MS is supported by the Controlled High-risk Subjects Avenox Multiple Sclerosis Prevention Study (CHAMPS), in which a 3-year interval treatment with interferon after the first clinical event significantly lowered the probability of developing clinically definite MS. If early intervention before onset of progressive disease is important in preventing late disability, a safe but intense immune suppressive regimen might be indicated in patients with relapsing-remitting MS who have failed interferon.

Although the primary outcome of these trials would be progressive disability defined by the EDSS, other outcome measures would include clinical status by the Neurologic Rating Scale and Multiple Sclerosis Functional Composite, measurement of accumulated atrophy on MRI of the brain and cervical spinal cord, and potentially measures of whole brain N-acetyl-aspartate on magnetic resonance spectroscopy that reflects neuronal and axonal integrity.

### Table 1. Results of autologous/syngeneic hematopoietic stem cell transplantation in patients with multiple sclerosis

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients*</th>
<th>EDSS baseline</th>
<th>Regimen</th>
<th>Progressed</th>
<th>Follow-up, mo (median range)</th>
<th>Treatment-related deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fassas et al190, 191</td>
<td>24</td>
<td>6.0 (4.5-8.0)</td>
<td>BEAM + ATG</td>
<td>5/23</td>
<td>40 (21-51)</td>
<td>1</td>
</tr>
<tr>
<td>Burt et al179, 133, 220</td>
<td>27</td>
<td>7.0 (3.0-8.5)</td>
<td>Cy/TBI/ATG</td>
<td>4/25</td>
<td>14 (2-58)</td>
<td>0</td>
</tr>
<tr>
<td>Nash et al193</td>
<td>20</td>
<td>7.0 (5.0-8.0)</td>
<td>Cy/TBI/ATG</td>
<td>2/13</td>
<td>5 (3-24)</td>
<td>1</td>
</tr>
<tr>
<td>Carreras et al225</td>
<td>10</td>
<td>6.2 (5.0-6.5)</td>
<td>BEAM + ATG</td>
<td>2/10</td>
<td>18 (16-32)</td>
<td>0</td>
</tr>
<tr>
<td>Kozak et al189</td>
<td>8</td>
<td>6.5 (6.5-7.5)</td>
<td>BEAM + ATG</td>
<td>1/8</td>
<td>8.5 (1-16)</td>
<td>0</td>
</tr>
<tr>
<td>Openshaw et al197</td>
<td>5</td>
<td>6.5 (5.5-7.5)</td>
<td>BU/Cy + ATG</td>
<td>1/4</td>
<td>18 (17-30)</td>
<td>2</td>
</tr>
<tr>
<td>Mandello et al223</td>
<td>1 (identical twin)</td>
<td>6.5</td>
<td>Cy/TBI</td>
<td>0/1</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

EDSS indicates extended disability status score; BEAM, carmustine, etoposide, cytarabine, melphalan; ATG, antithymocyte globulin; Cy/TBI, cyclophosphamide and total body irradiation; and BU/Cy, busulfan and cyclophosphamide.

*Actual patient number is based on updated communication with the author and may be higher than the number reported in the reference.
the first 5 years, the main cause of death is active disease (neurologic, renal, systemic) or infection. Thereafter, causes of death tend to be infectious or cardiovascular events (strokes and/or myocardial infarction) related to hypertension and hyperglycemia/hypercholesteremia because of chronic corticotherapy.

Three consecutive but separable levels of etiologic, ethiopathogenesis, and pathogenesis have been considered for SLE. It has been thought imperative to identify the specific molecular defects as the only way to design and use any novel and rational treatments. In practice, however, SLE is treated with a variety of drugs, mainly immunosuppressive, that have been discussed recently. Along with corticosteroids, intravenous pulse cyclophosphamide has been used in a National Institutes of Health–developed protocol specifically directed toward lupus nephropathy.

At the pinnacle of the lupus iceberg, however, there are cases of refractory-relapsing (“intractable”) disease. For such patients, following the considerable experimental evidence discussed formerly and also on the basis of serendipitous case reports of coincidental diseases, HSCT was proposed in 1993. Several cases of concomitant SLE and malignancy have been treated with HSCT and published. They include chronic myeloid leukemia/SLE, non-Hodgkin lymphoma (NHL)/SLE, and Hodgkin disease/SLE. The first patient eventually died of his leukemia without any evidence of active SLE. In another case, the NHL did not relapse, but ITP surmounted in conjunction with an anticoagulant antibody.

The first patient with SLE received a transplantation of her own T-cell–depleted marrow in 1996. The first report on HSCT for SLE in the United States was published 1 year later in 1997. There are now several fully published case reports of nonconcomitant SLE patients having undergone HSCT (Table 2). All received transplantations of cyclophosphamide and G-CSF–mobilized CD34+ cells, and conditioning regimens varied from Cy/TT to Cy/ATG (200 mg) to BEAM. All patients reached complete remission, but in several there was a serologic antinuclear antibody (ANA) relapse after 2 to 3 years from transplantation. In the patient with the longest posttransplantation follow-up, after 3 years of corticoid-free remission, there was a reappearance of ANA/DNA antibodies, and, after another year, there was also a mild proteinuria, which is currently being treated with a combination of corticosteroids and mycophenolate mofetil.

In the most extensive single-center clinical study published to date, 9 patients underwent stem cell mobilization with cyclophosphamide 2.0 g/m² and G-CSF 10 μg/kg. Two patients were excluded from transplantation because of infection (one death from disseminated mucormycosis), and 7 received autotransplantations after conditioning with cyclophosphamide (200 mg/kg), 3.0 g methylprednisolone, and 90 mg/kg equine antithymocyte globulin. All patients were seriously ill, with SLE disease activity indices of 17 to 37, including 1 case with alveolar hemorrhage and 4 with World Health Organization class III-IV glomerulonephritis and nephrotic syndrome. Lupus remained in clinical remission, and ANA became negative in all patients with 1 to 3 years of posttransplantation follow-up.

Phase 3 trials are being designed in the United States to compare autologous HSCT with a control arm. The standard of care for the control arm has generated a great deal of discussion and controversy within the working group. Potential controls could be intravenous pulse cyclophosphamide, oral cyclophosphamide, mycophenolate mofetil, or an open control of best available care. American experience with oral cyclophosphamide or mycophenolate mofetil in SLE is limited. Pulse cyclophosphamide (500-1000 mg/m²) has a long track record and is generally considered the standard of care. If HSCT candidates are selected for failure to pulse cyclophosphamide, it is difficult to continue failed therapy as on the control arm. One solution is to offer HSCT earlier in disease. Eligible patients with nonrenal visceral involvement need only fail corticosteroids and 3 months of pulse cyclophosphamide. For patients in whom the indication is nephritis, active disease must be present despite at least 6 cycles of monthly pulse cyclophosphamide. Enrolling patients earlier in disease who are less ill would also decrease the morbidity and mortality of HSCT. A second solution is to allow patients enrolled on the pulse cyclophosphamide arm who continue to fail to crossover to HSCT.

Numerous SLE disease activity indices exist to measure disease activity including the British Isles Lupus Assessment Group scale (BILAG), Systemic Lupus Erythematosus Disease Activity Index, Systemic Lupus Activity Measure, and the Lupus Activity Index. The index used depends on institutional and investigator familiarity. In the American phase 3 trial of HSCT for SLE, the disease activity instrument will be the BILAG. BILAG is one of the more useful instruments for characterizing disease stage because BILAG score correlates with necessity to treat and has been validated as an instrument to measure disease activity. The evaluation is based on a 5-category classification, characterizing the degree of symptoms attributed to active lupus for 86 questions based on the patient’s history, examination, and laboratory results. The 5 categories of response are the following: not present, improving, same, worse, and new. The 86 questions are grouped into the following 8 systems: general, mucocutaneous, neurologic, musculoskeletal, cardiovascular and respiratory, vasculitis, renal, and hematologic. For each of the 8 systems, a severity grade (A to E) is

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients receiving transplants</th>
<th>Regimen</th>
<th>Results</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmont et al</td>
<td>1</td>
<td>TT/Cy</td>
<td>Clinical remission for more than 3 y, serologic relapse</td>
<td>0</td>
</tr>
<tr>
<td>Burt et al</td>
<td>9</td>
<td>Cy/ATG</td>
<td>Clinical remission for up to 4 y, 2 relapsed at 3 y and 3.5 y, respectively</td>
<td>1/12 mobilized</td>
</tr>
<tr>
<td>Traynor et al</td>
<td>1</td>
<td>BEAM</td>
<td>Clinical remission for 1 y; ANA negative at 6 mo but positive at 9 mo</td>
<td>0</td>
</tr>
<tr>
<td>Fouillard et al</td>
<td>3</td>
<td>Cy/ATG</td>
<td>Complete remission of active disease</td>
<td>0</td>
</tr>
<tr>
<td>Rosen et al</td>
<td>1</td>
<td>Cy/ATG</td>
<td>Posttransplantation low ANA titer and low Coombs positive at 8 mo but anti-ds DNA negative and anticoagulant antibody negative</td>
<td>0</td>
</tr>
</tbody>
</table>

TT/Cy indicates thiotepa and cyclophosphamide; Cy/ATG, cyclophosphamide and antithymocyte globulin; BEAM, carmustine, etoposide, cytarabine, melphalan; ANA, antinuclear antibody; and anti-ds, anti–double strand DNA antibody.
calculated according to the scores. The following list indicates interpretation of each of the grades for each system: A, disease is active enough to need treatment; B, disease has the potential to need treatment soon; C, disease currently does not meet grade A or B criteria; D, disease has satisfactorily resolved; and E, disease has never been involved. Because a crossover arm is tentatively planned in the American phase 3 trial, the primary endpoint will be need to treat as defined by a BILAG grade A.

**Autologous HSCT for RA**

RA affects 1% of the North American population. It is an immune-mediated disease that involves joint synovium with formation of an inflammatory pannus that erodes cartilage and bone. The characteristic joint lesion in RA includes an increase in the numbers of both fibroblastlike and macrophagelike synoviocytes in the synovial intimal lining, infiltrating lymphocytes, plasma cells, monocytes, and macrophages. T cells comprise about 30% to 50% of synovial tissue cells. Synovial T cells have been demonstrated to have a restricted repertoire and self-antigens such as type II collagen epitopes. Synovial macrophages produce IL-1 and tumor necrosis factor α (TNF-α). RA fibroblastlike synoviocytes can proliferate in an anchorage-independent manner, escape contact inhibition, aggressively invade into cartilage when coimplanted into severe combined immune deficient mice, and have somatic mutations of the p53 tumor suppressor gene. These complexities underscore the shortcomings of previous approaches designed to eliminate only one set of immune cells.

The most common rheumatoid symptoms are joint pain, swelling or deformity, morning stiffness, elevated sedimentation rate, and a positive rheumatoid factor. Extra-articular symptoms may occur, including rheumatoid nodules, vasculitis, and pulmonary interstitial fibrosis. Patients with more than 20 to 30 involved joints have a 5-year mortality of 40% to 60%. Despite newer therapeutic agents like anti-TNF drugs, about 5% to 10% of patients with RA continue to have a desperate need for more intensive yet highly myeloablative regimens such as busulfan and cyclophosphamide, or the use of a more intense myeloablative regime such as busulfan and cyclophosphamide.

A European approach being proposed for phase 3 trials uses the current cyclophosphamide mobilization (2.0 to 4.0 g/m²) and cyclophosphamide conditioning (200 mg/kg) with posttransplantation immune modulation. The nontransplant arm will be cyclophosphamide mobilization only followed by maintenance methotrexate (John Snowden, verbal communication, May 2001). This approach assumes that RA is not curable but is more easily controlled with conventional therapies after HSCT. Continued posttransplantation immune suppression may increase the risk of posttransplantation opportunistic infections. The Australians, rather than comparing HSCT with another therapy, are randomizing patients with RA to HSCT with or without T-cell depletion of the autograft. The American and Israeli approach is to pilot phase 1/2 autologous HSCT studies by using more intense myeloablative regimens (fludarabine plus oral busulfan or intravenous Busulfex and cyclophosphamide) in the hope of inducing more durable remissions, while simultaneously developing mini-allogeneic HSCT protocols for patients with HLA-matched siblings.

**Autologous HSCT for scleroderma**

Scleroderma is a rare disorder with a prevalence of anywhere from 2 to 100 per one million people. Two subsets of scleroderma are generally recognized, limited and extensive cutaneous scleroderma. Limited cutaneous scleroderma is characterized by cutaneous involvement of acral areas (hands, face, feet, forearms) but not the trunk. Limited scleroderma generally has a good prognosis. Diffuse cutaneous scleroderma is characterized by truncal and acral symptoms.

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**Table 3. Results of autologous hematopoietic stem cell transplantation in patients with rheumatoid arthritis**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>Conditioning</th>
<th>Comment</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joske et al175</td>
<td>1</td>
<td>Cy</td>
<td>Marked improvement at 6 mo follow-up</td>
<td>0</td>
</tr>
<tr>
<td>Snowden et al174</td>
<td>8</td>
<td>Cy</td>
<td>Cohort I, cyclophosphamide 100 mg/kg-response for 1-2 mo</td>
<td>0</td>
</tr>
<tr>
<td>Burt et al179,180</td>
<td>4</td>
<td>Cy/ATG</td>
<td>Marked improvement up to 18 mo but 2 relapsed</td>
<td>0</td>
</tr>
<tr>
<td>Pavletic et al178</td>
<td>2</td>
<td>Cy/ATG</td>
<td>Relapsed at 5 and 7 mo</td>
<td>0</td>
</tr>
<tr>
<td>Durez et al198</td>
<td>1</td>
<td>BU/Cy</td>
<td>Remission &gt; 10 mo</td>
<td>0</td>
</tr>
<tr>
<td>McColl et al171</td>
<td>1</td>
<td>Cy/ATG (identical twin)</td>
<td>Remission &gt; 24 mo</td>
<td>0</td>
</tr>
<tr>
<td>Munro et al178</td>
<td>1</td>
<td>N/A</td>
<td>Marked improvement for 1 y</td>
<td>0</td>
</tr>
<tr>
<td>Verburg et al177</td>
<td>12</td>
<td>Cy</td>
<td>Marked improvement in 6/12 patients with follow-up, ranging from 7-21 mo</td>
<td>0</td>
</tr>
</tbody>
</table>

Cy indicates cyclophosphamide; Cy/ATG, cyclophosphamide and antithymocyte globulin; BU/Cy, busulfan and cyclophosphamide, and N/A, not applicable.
skin involvement and early visceral (lung, renal, cardiac, gastrointestinal) involvement. For all patients with diffuse scleroderma, 5-year mortality is 25% to 30%. High skin scores, pulmonary, renal, or cardiac involvement is associated with a higher mortality of 40% to 50% within 5 years.

Scleroderma is characterized by fibrosis (ie, excessive deposition of collagen in skin and visceral organs). The etiology of scleroderma is unclear, and an autoimmune pathogenesis remains controversial. Unlike MS, RA, and SLE, the MHC association is weak. Randomized trials of D-penicillamine, interferon-α, or methotrexate either are no better than placebo or improve skin score with little beneficial effect on visceral organ function. An exception is pulse intravenous cyclophosphamide, which appears to ameliorate scleroderma-related pulmonary abnormalities. Scleroderma may be a vasculopathy, connective tissue disorder, and/or immune-mediated disease. Raynaud phenomena, nail fold capillary abnormalities, and elevated plasma von Willebrand antigen are indications of a vasculopathy with endothelial injury that may secondarily lead to ischemia and fibrosis. Scleroderma may be a connective tissue disease. The tight skin mouse, which is an animal model for scleroderma, is a genetic connective tissue disease because of a defect in the fibrillin 1 gene. Support for an immune-mediated etiology include a variety of autoantibodies, including antitopoisomerase (ScI-70) antibodies and anticentromere antibodies. Chronic GVHD is an immune-mediated disorder that is clinically and histologically similar to scleroderma. Similar to scleroderma, chronic GVHD is associated with tissue fibrosis and is slow to respond to immune suppression. GVHD is caused by allogeneic lymphocytes, and patients with scleroderma have been reported to have an increased incidence of allogeneic hematopoietic cellular microchimerism. Transplantation of fetal lymphocytes to the mother may lead to mixed chimerism in postpartum females. Transplantation of maternal lymphocytes to the fetus may cause mixed chimerism in males and nonparous females. Similar to scleroderma, GVHD is also associated with endothelial damage and elevated von Willebrand antigen. The perceived failure of immune therapies in both chronic GVHD and scleroderma may be due to neglect in recognizing or effectively treating an early inflammatory phase. Late fibrotic processes may progress and regress more slowly.

Regardless of etiology, because of its poor prognosis and lack of effective therapies, patients with scleroderma are being enrolled in HSCT protocols. Early results indicate improved skin scores and activities of daily living but unchanged renal, cardiac, and pulmonary function. In a study of mostly European patients by using a variety of conditioning regimens, skin score generally improved with stabilization of lung function. Overall mortality was 27% because of 10% disease progression and 17% transplantation-related mortality. These results suggest that more careful selection of patients earlier in disease is necessary in the design of phase 3 trials. Phase 3 randomized trials of HSCT versus monthly pulse cyclophosphamide are accruing in Europe and are being designed in the United States. The primary endpoint of these trials is overall survival.

**Induction of tolerance by allogeneic HSCT**

**Animal models**

Animal autoimmune-like diseases that occur spontaneously (without known precipitating infection or immunization) are not cured by a syngeneic HSCT. In fact, disease may be transferred to a normal strain of mice by HSCT from the autoimmunopne donor. Syngeneic HSCT in spontaneous-onset lupuslike disease of MRL/lpr mice resulted in only transient disease amelioration. Curing a spontaneous-onset autoimmune-like disease requires allogeneic HSCT from a nonautoimmune-prone donor. Murine spontaneous-onset lupuslike disease is cured by allogeneic HSCT from a normal donor strain. Most patients maintain remission indefinitely after discontinuation of immune-suppressive prophylaxis for autoimmune diseases.
GVHD. An occasional patient has relapsed despite being chimeric (ie, 100% donor hematopoiesis). Chimeric analysis of peripheral blood for residual host hematopoiesis may, however, be falsely negative. Separation and analysis of lineage-specific subsets, such as only T cells, may reveal mixed chimerism (both donor and host cells) in only the T-cell lineage. The clinically asymptomatic donor may also have subclinical disease, such as rheumatoid factor positive, that could adoptively transfer the same disease for which the recipient received a transplant. Alternatively, because the patients are MHC matched, the donor and recipient may have similar non-MHC autoimmune genes that in the presence of host “factors,” such as a persistent latent infectious agent or recurrent environmental exposure, may initiate de novo disease.

HLA-matched sibling allogeneic transplantations have already been successfully performed for some hematologic autoimmune diseases, including a case of hemolytic anemia, pure red cell aplasia, and Evans syndrome. Phase 1 allogeneic HSCT trials using mini-conditioning regimens with and without lymphocyte-depleted grafts are being suggested and designed for autoimmune diseases. Just as in autologous HSCT, protocols will need to be tailored for each disease.

Summary

HSCT of autoimmune disorders has raised new expectations, opportunities, and questions. What is the best mobilization regimen? What is the optimal conditioning regimen? Does T-cell depletion of the graft result in self-tolerance and decreased relapse, or rather result in an increased risk of infections? Can we predict candidates likely to relapse after autologous HSCT? Is relapsed disease responsive to previously refractory therapy and easier to control? Is HSCT cost effective? What is the mechanism(s) of posttransplantation remission? Which, if any, diseases may be cured by an autologous graft and which will require an allograft?

Encouraging phase 1 trials have propelled this field to phase 3 trials in MS, SLE, RA, and scleroderma. Completion of these trials should determine if autologous HSCT is better than current standards of care. Nonmyeloablative or reduced-intensity allogeneic transplantation protocols are being written, and advances in ex vivo stem cell expansion will soon be applied to autoimmune diseases to eliminate regimen-related neutropenia.

Historically, most autoimmune diseases are incurable, and it was impractical to define complete remission. HSCT, whether allogeneic or even autologous, may change this axiom. Initial results suggest that clinical tolerance, that is no evidence of disease off all immune-suppressive medications with normal third-party immune responsiveness, is being achieved in at least some patients. However, further improvement of the efficacy and safety of both allogeneic and autologous stem cell transplantation procedures need to be developed, and larger cohorts of patients need to be studied to assess the full benefits of stem cell transplantation as a most promising new armamentarium for the treatment of autoimmune diseases.

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Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure?

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