

Induction of Tolerance in Autoimmune Diseases by Hematopoietic Stem Cell Transplantation: Getting Closer to a Cure?

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Abstract

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 transplantations 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.

1. Autoimmunity: Definition

Autoimmunity arises from the pathologic reaction of B-cell derived antibodies and/or T cells to self-epitopes. Proof of an autoimmune pathogenesis requires adoptive transfer of disease by either immune cells or antibody [1,2]. Transplacental or iatrogenic transfer of autoreactive antibodies may cause disease. This condition was first shown in Harrington's self experimentation using plasma from a patient with idiopathic thrombocytopenic purpura (ITP) [3]. Mothers with ITP, myasthenia gravis, and/or systemic lupus erythematosus (SLE) with SSA-Ro-SSB/La immunity may transfer antibodies to their fetus, resulting in neonatal disease [4-7]. Allogeneic stem cell transplantation from donors with autoimmune disease

may also transfer the disease to recipients [8-13].

2. Theories of Tolerance

Clinical tolerance is failure of an organism to reject an antigen or tissue without use of immune-suppressive medications but with intact normal rejection of third-party or foreign antigens. The oldest theory of tolerance, and now viewed as orthodoxy, is clonal selection of lymphocyte repertoires [14]. Self-reactive lymphocytes are deleted and not allowed to mature. Clonal selection as an explanation for tolerance was first proposed by Burnet [15] in 1957 in regards to antibody formation and self-recognition and non-selfrecognition. Subsequently, this concept was extended to selection of T cells by deletion of autoreactive clones within the thymus [16-21]. T-cell precursors emigrate from the marrow to the thymus. In the thymus, if self-antigen of sufficient concentration and affinity for their specific T-cell recep-

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tor (TCR) repertoires is present, the T cells undergo apoptosis (deletion) or anergy (functional silencing) [22-25]. Because lymphocyte progenitors are continually generated from HSCs, clonal selection would have to be an ongoing process occurring throughout life.

Thymic editing includes not only negative selection to delete self-reactive clones but also positive selection to allow maturation of self-reactive clones [17,26]. If a particular TCR fails to engage a major histocompatibility complex (MHC) peptide/complex, or binds it too tightly, it undergoes apoptosis. If it recognizes an MHC/peptide complex with moderate avidity, it is positively selected and undergoes further maturation. The avidity (concentration and binding affinity) of an MHC/peptide complex appears to play a role in positive versus negative selection of T lymphocytes [27,28]. Intrathymic selection and anergy as a mechanism of maintaining tolerance of autoreactive repertoires was, therefore, amended by theories concerning peripheral tolerance [29,30].

Mechanisms of peripheral tolerance revolve, in part, around the 2-signal hypothesis of self-discrimination and non-self-discrimination introduced by Bretscher and Cohn [31] in 1970. T cells, positively selected within the thymus, remain anergic unless antigen is presented with a second signal (ie, a costimulatory signal). Basically, antigen presentation to a T cell without costimulation maintains anergy, whereas TCR engagement of antigen combined with costimulation results in T-cell activation [32-35].

The traditional costimulatory molecule for T-cell activation is CD28, a ligand for B7-1 (CD80), and B7-2 (CD86) receptors on T cells [36]. CD28 binding increases transcription of interleukin 2 (IL-2) [35,37]. A variety of other molecules, including CD40L, inducible costimulator (ICOS), and various adhesion molecules, also provide secondary or tertiary signals to facilitate T-cell activation [38-43]. Requirement of costimulation for activation may place some constraints on peripheral sites for cellular activation. Antigenpresenting cells (APCs) that express costimulatory molecules are 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 [44]. For example, allogeneic tissue grafts are not rejected in mice that lack secondary lymphoid tissue [45].

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 [46,47]. Other checks to maintain peripheral tolerance include activation-induced cell death [48], suppressor or regulatory cells [49-51], and peripheral antigen avidity (ie, antigen persistence, concentration, and affinity) [52,53]. 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 [54].

The danger metaphor proposed by Matzinger [54] 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 [55]. Adaptive immunity involves the rearrangement of a limited number of germ line genes to produce a highly diversified repertoire of approximately 10^{14} to 10^{18} 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 pathogenreceptor repertoire diversity [56]. Response to infection is intrinsic to a limited number of germ-line receptor genes that recognize pathogenspecific 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 [57-59].

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 [60] and Grossman and Paul [61,62] 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".

3. 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 [63-65].

3.1. 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 [66-68]. 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 [66]. Procainamide-hydroxylamine may alter the avidity of TCRs for self-antigen, preventing deletion of some autoreactive T-cell repertoires [68]. 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 [69-72], causing a T-cell autoimmune sclerodermalike disease termed syngeneic graft versus host disease (GVHD) [72].

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 (CD45^{RO}) 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 [73-75].

During TCR thymic development, rearrangement of TCR genes leads to excision of circular DNA termed T-cell receptor rearrangement excision circles (TRECs) [73]. 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 [74]. 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.

3.2. Infection-induced Autoimmunity

An infectious agent has been associated with virtually every autoimmune disease, including diabetes mellitus [76-79], ankylosing spondylitis [80], multiple sclerosis (MS) [81-86], myocarditis [87-89], rheumatoid arthritis (RA) [90-96], and SLE [97]. These associations are suggested by epidemiologic studies and serology that connect disease onset or flare to various infectious agents, cross-reaction 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 coinjected 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 autoimmunedemyelinating 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 V β 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 overexpansion and/or deletion of entire V β 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.

4. Genetic Susceptibility to Breaking Tolerance

4.1. 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 HLAB27 [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.

4.2. 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 is induced by injection of collagen and adjuvant [138,139]. At least 14 genomic intervals or collagen-induced arthritis (CIA) loci are associated with collagen-induced arthritis [140,141].

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 [142]. 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.

5. Induction of Tolerance by Immune Ablation and Autologous Stem Cell Transplantation

5.1. Animal Models and Anecdotal Case Reports

Animal autoimmune diseases that are induced by im-

munization with adjuvant or self-peptide and adjuvant may be ameliorated by syngeneic or pseudo-autologous HSCT [143-155].

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 [146, 149-151]. 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 [146]. Histologic analysis revealed chronic scarring with glial proliferation that is unaffected by HSCT [146]. To be effective as therapy for EAE, HSCT needs to be performed early in the disease course during its inflammatory stage and before accumulation of disease burden. A principle that may also be important for MS.

Murine bone marrow transplantations are performed by killing and removing the femur from the donor and using a syringe to flush out the marrow cells. It is technically difficult and inhumane to perform a murine autologous transplantation because the surviving recipient's legs would have to be amputated. However, marrow could be harvested from a syngeneic donor in the same active stage of EAE as the recipient, referred to as a pseudoautologous transplant. HSCT of EAE using pseudoautologous donors suggests that infused lymphocytes contaminating the graft may contribute to relapse [147]. This suggestion indicates that lymphocyte depletion of grafts may be important in decreasing posttransplantation relapse after autologous HSCT.

Besides immunization with myelin peptides, demyelinating central nervous system disease may be induced with viruses such as TMEV [156]. Autologous HSCT of TMEV-induced demyelinating disease causes a high mortality from viral superinfection of the central nervous system during the postconditioning pancytopenic period [156]. Autoimmune disease mediated by an infectious agent can be rapidly fatal after autologous HSCT but only if the infectious agent is still present at the time of transplantation.

Several other environmentally induced animal autoimmune diseases are improved or cured by syngeneic HSCT. These diseases include experimental autoimmune myasthenia gravis [153], adjuvant arthritis [154,155] and collagen-induced arthritis [145]. Encouraging results of syngeneic and pseudoautologous HSCT in animal-induced autoimmunity supported the design of autologous and syngeneic HSCT trials in patients with severe

autoimmune disorders.

Anecdotal case reports of patients with a coincidental autoimmune disease and a malignancy provided further support and rationale for trial design [157-166]. Refractory autoimmune diseases entered remission sometimes for several years. Because the indication for transplantation was a malignancy, and the outcome was reported retrospectively, in most cases a detailed pretransplantation evaluation by a rheumatologist or neurologist is missing. The autografts were usually not purged of lymphocytes, and the transplantations were not tailored as therapy for an autoimmune disease. Duration of response appeared shorter for RA compared with SLE. Too few patients have been reported for other autoimmune diseases, and long-term results of response to treatment in those that relapse, as well as duration of remission in those who had not relapsed, remain unknown.

5.2. 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 [167]. 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 [167,168]. 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 [167]. The only complications of G-CSF PBSC mobilization in patients with scleroderma are transient telangiectasia that spontaneously resolves [167]. 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 [169].

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 [167]. 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 [167]. 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 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), Isolex (Nexel, Irvine, CA), or CliniMACS (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).

5.3. 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 reinduced self-tolerance after elimination of self-reactive lymphocytes and reestablishment of immunity from uncommitted stem cells.

Brodsky et al [172] 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) [173-177]; 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) [178-188]; carmustine, etoposide, cytarabine, and melphalan (BEAM) [189-192]; cyclophosphamide and total body irradiation (Cy/TBI) [193]; cyclophosphamide, TBI, and antithymocyte globulin (Cy/TBI/ATG) [194,195]; busulfan and cyclophosphamide (Bu/Cy) [196,197]; busulfan, cyclophosphamide, and ATG (Bu/Cy/ATG) [198]; cyclophosphamide and thiotepa (Cy/TT) [199,200]; and fludarabine-based regimens.

Cy or Cy/ATG is the most common conditioning regimen used for HSCT of SLE [181-184,188]. Pulse cyclophosphamide (500-1,000 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 time 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 thiotepa [199,200]. 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 scleroderma [176,187,188] 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 HSCT-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 MS [197] 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 alkylating agents on demyelinated 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 readily crosses the blood-brain barrier. To avoid TBI-related pulmonary injury, radiation is generally given in the anteroposterior and posteroanterior 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 non-myeloablative 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 scleroderma [195] 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 [358]. 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 histiocytes) actively phagocytosing hematopoietic cells and may arise from immune dysregulation perhaps in response to viral infections. To date, post-transplantation 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 infertility and of regimen-specific late toxicities such as cataracts from TBI. Late malignancies are also possible [204]. Similar to mobilization regimens, conditioning regimens must be uniquely designed for the disease, organ impairment, disease-specific infection susceptibility, and extent of prior immune suppressive medication related infectious risk to ensure minimum regimen-related mortality.

5.4. 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 I 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.

5.5. 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, reinduce 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.

6. 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.

6.1. Autologous HSCT for MS

MS is a relatively common North American and European disease with a prevalence of approximately 1 in 1,000 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 (copolymer 1 or glatiramar acetate) [217,218] 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 [219,220]. The need for new interventions in MS is evident from the desperation of patients who in some studies have a higher suicide rate compared with the general population [221].

Natural history magnetic resonance imaging (MRI) studies have demonstrated that neurologic progression can continue despite lack of new demyelinating events on MRI [222,223]. Although early relapse frequency within the first year of diagnosis appears to correlate with onset of late disability, Confavreux et al [224] reported that relapse frequency in disease of longer duration and EDSS scores more than 4.0 do not correlate with disability. 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 disease, MS transitions into or is also an axonal degenerative disease [225-228].

HSCT for MS was suggested in 1995 [229]. In general, initial HSCTs were phase 1 studies and captured patients with progressive disease and high disability (EDSS) scores (Table 1)[189-191,230-233]. The Thessaloniki group has reported a 3-year progression-free survival for primary progressive MS (39%), which appears significantly lower than for secondary progressive (92%) [190]. In an Italian study, Mancardi et al [231] 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 [230]. 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,

Table 1.

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

Group	No. of patients*	EDSS baseline	Regimen	Progressed	Follow-up, mo, median (range)	Treatment-related deaths
Fassas et al [190,191]	24	6.0 (4.5-8.0)	BEAM+ATG	5/23	40 (21-51)	1
Burt et al [179,193,230]	27	7.0 (3.0-8.5)	Cy/TBI	4/25	14 (2-58)	0
Nash et al [169]	20	7.0 (5.0-8.0)	Cy/TBI/ATG	2/13	5 (3-24)	1
Carreras et al [232]	10	6.2 (5.0-6.5)	BEAM+ATG	2/10	18 (16-32)	0
Kozak et al [189]	8	6.5 (6.5-7.5)	BEAM+ATG	1/8	8.5 (1-16)	0
Openshaw et al [197]	5	6.5 (5.5-7.5)	BU/Cy+ATG	1/4	18 (17-30)	2
Mandalfino et al [233]	1 (identical twin)	6.5	Cy/TBI	0/1	26	0

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.

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 best 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 over a 3-year interval treatment with interferon after the first clinical event significantly lowered the probability of developing clinically definite MS [234]. 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.

6.2. Autologous HSCT for SLE

Although studies have suggested that SLE encompasses several genetic diseases with some clinical commonalities [235,236], the disease will be considered here as a single entity with protean clinical expressivity [237,238]. SLE has an overall prevalence that has varied from 12 to 50.8 cases per 100,000 persons [239]. Survival has improved dramatically, reaching a 90% 10-year survival and a 70% 20-year survival in the 1990s. Within 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/hypercholesterolemia because of chronic corticotherapy.

Three consecutive but separable levels of etiology, ethiopathogenesis, and pathogenesis have been considered for SLE [240]. It has been thought imperative to identify the specific molecular defects as the only way to design and use any novel and rational treatments [241]. In practice, however, SLE is treated with a variety of drugs, mainly immunosuppressive, that have been discussed recently [242]. Along with corticosteroids, intravenous pulse cyclophosphamide has been used in a National Institutes of Health-developed protocol specifically directed toward lupus nephropathy [243].

At the pinnacle of the lupus iceberg, however, there are cases of refractory-relapsing ("intractable") [244] 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 [245]. Several cases of concomitant SLE and malignancy have been treated with HSCT and published. They include chronic myeloid leukemia/SLE [166], non-Hodgkin lymphoma (NHL)/SLE [157], and Hodgkin disease/SLE [160]. The first patient eventually died of his leukemia without any evidence of active SLE. In another case, the NHL did not relapse, but ITP supervened in conjunction with an

Table 2.

Results of autologous hematopoietic stem cell transplantation in patients with systemic lupus erythematosus.

Reference	No. of patients receiving transplants	Regimen	Results	Mortality
Marmont et al [199]	1	TT/Cy	Clinical remission for more than 3 y, serologic relapse	0
Burt et al [179,183]	9	Cy/ATG	Clinical remission for up to 4 y, 2 relapsed at 3 y and 3.5 y, respectively	1/12 mobilized
Traynor et al [181]				
Fouillard et al [192]	1	BEAM	Clinical remission for 1 y; ANA negative at 6 mo but positive at 9 mo	0
Rosen et al [188]	3	Cy/ATG	Complete remission of active disease	0
Musso et al [185]	1	Cy/ATG	Posttransplantation low ANA titer and low Coombs positive at 8 mo but anti-ds DNA negative and anticardiolipin antibody negative	0

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.

anticentromere antibody [163].

The first patient with SLE received a transplantation of her own T-cell depleted marrow in 1996 [199]. The first report on HSCT for SLE in the United States was published 1 year later in 1997 [183]. There are now several fully published case reports of nonconcomitant SLE patients having undergone HSCT (Table 2)[181-184,192,199,246]. 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 [247].

In the most extensive single-center clinical study published to date [181], 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, myco-

phenolate mofetil, or an open control of best available care. American experience with oral cyclophosphamide or mycophenolate mofetil in SLE is limited. Pulse cyclophosphamide (500-1,000 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)[248], Systemic Lupus Erythematosus Disease Activity Index [249], Systemic Lupus Activity Measure [250], and the Lupus Activity Index [251]. 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 [252,253]. 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 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.

6.3. Autologous HSCT for RA

RA affects 1% of the North American population [254]. It is an immune-mediated disease that involves joint synovium with formation of an inflammatory pannus that erodes cartilage and bone [255]. 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 [256,257] and to react to a variety of microbial antigens [258] and self-antigens such as type II collagen epitopes [259]. Synovial macrophages produce IL-1 and tumor necrosis factor α (TNF- α) [260]. RA fibroblastlike synoviocytes can proliferate in an anchorage-independent manner, escape contact inhibition [261], aggressively invade into cartilage when coimplanted into severe combined immune deficient mice [262], and have somatic mutations of the p53 tumor suppressor gene [263]. 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 [264,265]. Patients with more than 20 to 30 in-

involved joints have a 5-year mortality of 40% to 60% [266-275]. Despite newer therapeutic agents like anti-TNF drugs, about 5% to 10% of patients with RA continue to have a desperate need for better and more definitive therapies [276]. Because RA may be associated with significant morbidity, oncogene mutation, loss of synoviocyte growth inhibition, and, in some patients, high mortality, it is perhaps surprising that it was not until 1997 that the first HSCT for RA was reported from Australia [173] and the first American HSCT for RA reported in 1998 [180].

In general, the procedure has been well tolerated without mortality (Table 3). HSCT offers an almost immediate relief of symptoms. Patients become pain free, sometimes for the first time in years. Activities required for daily living, such as buttoning a shirt or combing hair, rapidly return to normal. Morning stiffness resolves, rheumatoid nodules disappear, sedimentation rate normalizes, and rheumatoid factor may disappear. Although these studies demonstrated that high-dose cyclophosphamide was well tolerated with marked improvements (American College of Rheumatology [ACR] 50 or ACR 70), a complete remission was unusual and relapse within 1 to 2 years is common [173,175,180,277,278]. There are suggestions of a dose-response effect. A dose escalation study of cyclophosphamide at 100 mg/kg revealed transient 1- to 2-month responses but at 200 mg/kg response duration increased to 18 to 20 months [174]. Too few myeloablative transplantations, for example a busulfan and cyclophosphamide regimen, have been performed to determine if durable remissions are feasible.

For an intense and expensive treatment such as HSCT to be considered for RA, sustained complete remissions or 70% improvement as defined by the ACR (ACR 70) must be achieved [279]. Several modifications are being considered, including the use of the current easily tolerated nonmyeloablative yet highly immunosuppressive regimen with posttransplantation immune modulation, eg, a TNF inhibitor, cyclosporine A, and/or methotrexate; or the use of a more intense myeloablative regimen such

Table 3.

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

Reference	No. of patients	Conditioning	Comment	Mortality
Joske et al [173]	1	Cy	Marked improvement at 6 mo follow-up	0
Snowden et al [174]	8	Cy	Cohort I, cyclophosphamide 100 mg/kg-response for 1-2 mo Cohort II, cyclophosphamide 200 mg/kg, improved for 17-19 mo	0
Burt et al [179,180]	4	Cy/ATG	Marked improvement up to 18 mo but 2 relapsed	0
Pavletic et al [178]	2	Cy/ATG	Relapsed at 5 and 7 mo	0
Durez et al [196]	1	BU/Cy	Remission 10 mo	0
McCull et al [175]	1	Cy/ATG (identical twin)	Remission 24 mo	0
Munro et al [278]	1	N/A	Marked improvement for 1 y	0
Verburg et al [277]	12	Cy	Marked improvement in 8/12 patients with follow-up, ranging from 7-21 mo	0

Cy indicates cyclophosphamide; Cy/ATG, cyclophosphamide and antithymocyte globulin; BU/Cy, busulfan and cyclophosphamide, and N/A, not applicable.

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.

6.4. Autologous HSCT for Scleroderma

Scleroderma is a rare disorder with a prevalence of anywhere from 2 to 100 per one million people [280]. 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 skin involvement and early visceral (lung, renal, cardiac, gastrointestinal) involvement. For all patients with diffuse scleroderma, 5-year mortality is 25% to 30% [281]. High skin scores [282], pulmonary, renal, or cardiac involvement is associated with a higher mortality of 40% to 50% within 5 years [282-286].

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 [287,288]. 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 [289-291]. An exception is pulse intravenous cyclophosphamide, which appears to ameliorate scleroderma-related pulmonary alveolitis [292]. 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 [293-295]. 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 [296-298].

Support for an immune-mediated etiology include a variety of autoantibodies, including antitopoisomerase (Scl-70) antibodies [299,300], and anticentromere antibodies [301]. Chronic GVHD is an immune-mediated disorder that is clinically and histologically similar to scleroderma [302-304]. 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 [305]. Transplacental transfer of fetal lymphocytes to the mother may lead to mixed chimerism in postpartum females [306]. Transplacental transfer 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 [307]. 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 [176,195]. 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 [308]. 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.

7. Induction of Tolerance by Allogeneic HSCT

7.1. Animal Models

Animal autoimmunelike 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 autoimmune-prone donor [309]. Syngeneic HSCT in spontaneous-onset lupuslike disease of MRL/lpr mice resulted in only transient disease amelioration [310]. Curing a spontaneous-onset autoimmunelike disease requires allogeneic HSCT from a non-autoimmune-prone donor [311-321]. Murine spontaneous-onset lupuslike disease is cured by allogeneic HSCT from a normal donor strain [311,314,315,317]. Spontaneous-onset diabetes in NOD mice is prevented by allogeneic HSCT from a nondiabetic prone strain [316, 319,321] and cured by combined pancreas and allogeneic HSCT from the same donor [318]. In fact, the

“tolerizing” effect [322,323] of HSCs is best demonstrated by donor-specific organ tolerance when combining solid organ and marrow transplant from the same donor.

Donor-specific organ tolerance was initially performed by lethally irradiating animals to ablate their marrow followed by allogeneic donor bone marrow transplantation [324-326]. Although donor-specific tolerance is associated with hematopoietic chimerism, the cellular mechanism by which donor-specific tolerance arises is not fully understood [327]. Fas ligand is a surface protein that can signal other cells expressing Fas to undergo apoptosis. Fas ligand expression appears to be necessary for donor marrow to induce donor organ tolerance, because hematopoietic-induced donor-specific tolerance does not occur in Fas knockout mice [328]. Therefore, the mechanism of allogeneic HSCT-induced tolerance to solid organ grafts may be in part explained by donor-induced apoptotic deletion of graft reactive cells. It has been postulated that allogeneic HSCT may induce tolerance to autoimmune epitopes by a similar deletion of autoreactive repertoires, a phenomena termed graft versus autoimmunity (GVA) [329,330]. A graft-versus-disease effect has already been established as the mechanism of remission for several hematologic malignancies, first discovered in 1981 and termed graft versus leukemia [331,332].

A putative GVA effect is supported by experiments showing that allogeneic chimerism by using a sublethal conditioning regimen followed by allogeneic transplantation can prevent the onset of diabetes and even reverse preexisting insulinitis in NOD mice, whereas the same radiation protocol without allogeneic HSC is insufficient [333]. With nonmyeloablative-conditioning regimens, spontaneous animal models of autoimmunity have been cured in the setting of mixed chimerism [333-336]. These experimental findings support low-conditioning preparative regimens for allogeneic transplantations in human autoimmune diseases.

Although in theory a GVA effect may be beneficial, the most significant toxicity of allogeneic HSCT is an immunologic reaction of donor cells against normal host tissues, a complication known as GVHD. Mini-conditioning may be associated with less GVHD compared with the more hazardous high-dose transplantation regimens. A lower GVHD risk may be due to reduced regimen-related tissue damage, decreased inflammatory cytokine release, decreased exposure of hidden tissue epitopes, and veto of alloreactive donor lymphocytes by hematopoietic cells of host origin, particularly CD8⁺ cells [337, 338]. Mini-transplantations are less likely to provide the danger signal hypothesized by Matzinger [54] that is necessary to break peripheral tolerance.

7.2. Allogeneic HSCT in Patients with Autoimmune Diseases

Anecdotal case reports of patients undergoing allo-

geneic HSCT for malignancy or aplastic anemia and a coincidental autoimmune disease have in most cases resulted in long-term remission of the autoimmune disease [339-353]. Most patients maintain remission indefinitely after discontinuation of immune-suppressive prophylaxis for 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 [354], pure red cell aplasia [355], and Evans syndrome [356,357]. 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.

8. 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 autologous and allogeneic 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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