25

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The Experimental Basis for Hematopoietic Cell Transplantation for Autoimmune Diseases

Introduction

Autoimmune diseases (ADs) are a heterogeneous group of disorders that affect an estimated 3–5% of the population [1,2]. These diseases occur when there is breakdown in the signals that mediate immune tolerance to normal tissues. The result of such breakdown is activation of cellular effector mechanisms and subsequent tissue destruction. Theoretically, all tissue types can be targets of an immune response; however, it is not known why certain organs are involved more commonly than others (Table 25.1). Six of the most common ADs are rheumatoid arthritis, systemic lupus erythematosus (SLE), type 1 diabetes mellitus (T1DM), Graves' disease, multiple sclerosis and pernicious anemia. Collectively these diseases represent ~50% of the ADs [3]. Autoimmune responses are generally sustained, persistent with manifestations of chronic tissue damage, presumably because self-antigens are continually produced on the targeted tissue and, in severe cases, diminution of the response does not occur until the cells expressing the autoantigens are destroyed. More than 30 years ago it was demonstrated that transfer of hematopoietic cells can alter the course of ADs in rodents. Bone marrow transplantations (BMTs) were shown to both transfer disease from autoimmune prone rodents to unaffected ones [4–6] and, conversely, to prevent disease if the hematopoietic cells were transplanted from unaffected rodents to susceptible ones [6–9]. The goals of this chapter are to provide a basis for understanding how and why hematopoietic cell transplantation (HCT) may effectively treat severe ADs and to describe the preclinical studies that have contributed to this understanding. The chapter begins with an over-view of how normal immune responses are regulated followed by a discussion of why autoimmunity develops. Thereafter the studies on preclinical models using HCT for the treatment of autoimmune syndromes are presented.

The immune response

Induction perpetuation of responses

Induction of antigen specific immune responses occurs as a complex cascade of events. The first critical step in turning on a response requires the processing of antigen for presentation to $CD4^+$ T lymphocytes [10]. $CD4^+$ T cells are central to immune response activation because they form the critical link between recognition of foreign antigen and the induction of effector mechanisms that destroy the antigenic source. Most effector cells rely upon ancillary signals provided by activated $CD4^+$ T cells in order to proliferate and differentiate. Because the consequences of nonspecific or inappropriate stimulation of naive $CD4^+$ T cells are potentially disastrous, a number of criteria must be met to activate these cells [11–13]. Only certain specialized cells, designated professional antigen-presenting cells (APCs) fulfill these criteria. The professional APCs include B lymphocytes, macrophages and dendritic cells. As part of their function APCs take up foreign protein antigens by endocytosis. The antigens are then processed via an intracellular pathway into smaller peptide fragments, and the fragments are then bound to class II major histocompatibility complex (MHC) molecules that make their way to the surface of the APC.

The seminal event in the activation of a quiescent and circulating $CD4^+$ T cell is encounter with its cognate antigen, which consists of the appropriate peptide bound to a self-MHC class II molecule. In addition to the binding of a T-cell receptor (TCR) to its cognate antigen, activation cannot be achieved unless this interaction is accompanied by simultaneous delivery of a costimulatory signal(s). In the absence of a costimulatory signal, engagement of the antigen receptor can lead to T-cell anergy —a state of nonresponsiveness to the antigen. Only professional APCs express both high levels of class II MHC molecules on their surface and are capable of delivering the appropriate costimulatory signals.

Table 25.1 Associations of human leukocyte antigen (HLA) serotype with susceptibility to autoimmune disease (AD).

Disease	Affected organ	HLA association	Relative risk
Rheumatoid arthritis	Joints	DR4	4.2
SLE	Systemic	DR3	5.8
T1DM	Pancreatic islets	DR3/DR4	~25.0
Graves' disease	Thyroid	DR3	3.7
Multiple sclerosis	CNS	DR2	4.8
Ankylosing spondylitis	Joints	B27	87.4

CNS, central nervous system; T1DM, type 1 diabetes mellitus; SLE, systemic lupus erythematosus.

Costimulatory molecules expressed by APC include B7-1 (CD80) and B7-2 (CD86)—the ligands for CD28, CD40, inducible costimulator ligand (ICOS-L), and various adhesion molecules [11,12,14]. Following TCR binding plus costimulatory signaling naive T cells respond by rapidly proliferating and differentiating. As part of this process they begin to express new receptor molecules and synthesize and secrete a number of chemokines and cytokines. One of the most important cytokines is interleukin 2 (IL-2) [15–18]. The production of IL-2 determines whether or not a $CD4^+$ T cell will proliferate and continue along its differentiation pathway. IL-2 functions as both a growth hormone and influences the activation state of other T cells, and functions as an autocrine hormone that induces the synthesis and expression of high affinity IL-2 receptor on the $CD4^+$ T cell itself. Signaling through the high affinity IL-2 receptor triggers the cells to progress through the remainder of the cell cycle.

Following CD4 T-cell stimulation and production of IL-2, these cells differentiate into two distinct populations that are distinguishable by the cytokines they produce. One population produces interferon-gamma (IFN-γ), tumor necrosis factor-beta (TNF-β) and IL-2, and the other secretes IL-4 and its congeners. These cell populations have been designated T-helper type 1 subset (TH1) and T-helper type 2 subset (TH2) cells. TH1 cells produce cytokines that drive cell-mediated immunity, while TH2 which elaborate cytokines critical to B-lymphocyte differentiation, provide help for antibody production (humoral immunity) [19,20]. It is clear that these $CD4^+$ T-cell subsets can regulate the growth and effector functions of the opposite T-cell subsets.

Cellular and humoral-based mechanisms mediate the effector phase of an immune response by destroying the pathogenic organisms that bear the target antigens [10]. Effector populations include mature B cells, activated cytotoxic T cells and other inflammatory cells such as natural killer (NK) and phagocytic cells. The humoral components of the effector phase include antibodies and complement protein. Products of activated mononuclear cells include proteolytic enzymes, nitric oxide and oxygen radicals and cytokines such as TNF-α.

Control of immune reactivity

To insure that autoreactivity does not occur during the course of defending host tissues activation of T lymphocytes is highly regulated. In addition, there are at least four ways that self-reactivity is controlled. These mechanisms are termed *clonal deletion*, *immunological ignorance*, *anergy* and *regulation* [21,22] (see also Chapter 24).

Clonal deletion

During development all lymphocytes undergo a rigorous selection process to delete potentially self-reactive cells [23–25]. Hematopoietic stem cells (HSCs) give rise to all lymphoid progenitor cells. For T cells, the progenitors migrate to the thymus, which provides a specialized microenvironment for T-cell maturation and selection. Developing cells that are potentially self-reactive—i.e. those cells with TCRs that bind too strongly to self-peptides plus self-MHC molecules are eliminated, a process termed negative selection or clonal deletion. Only those T cells with receptors that have the potential to recognize self-MHC molecules plus foreign peptides (positive selection) can leave the thymus and enter the bloodstream. Immature B cells that express immunoglobulin receptors that bind too strongly to components of self either die within the bone marrow (BM) or become impaired in their ability to respond to antigen (anergic).

Immunological ignorance

Most self-proteins are expressed at levels that are too low to serve as targets for T-cell recognition, and thus cannot serve as autoantigens. It is likely that only a very few self-proteins contain peptides that are presented by a given MHC molecule at a level that is sufficient for effector T-cell recognition but too low to induce tolerance. T cells able to recognize these rare antigens will be present in the individual but will not normally be activated; they are said to be in a state of immunological ignorance [26,27]. Most autoimmunity likely reflects the activation of such immunologically ignorant cells.

Anergy

A third level of control against nonspecific or self-reactive immune responses occurs if the requirements for lymphocyte activation fail. Quiescent lymphocytes traffic through the blood, lymphatics, and lymphoid organs in search of the cognate antigen that will bind their antigen specific receptors. Engagement of these antigen receptors in the absence of appropriate costimulatory signals (see section above) leads to a state of lymphocyte unresponsiveness called anergy [11,14,21,22]. A lymphocyte that is rendered anergic has an elevated threshold for activation, and thus is more resistant to responsiveness if its cognate antigen is encountered at a later time. Anergy has been observed in both T and B cells.

Regulation or suppression

A fourth way unwanted immune responses can be controlled is through populations that function to actively suppress lymphocyte activity. Experiments from the early 1970s [28] supported the existence of $CD8^+$ cells that down regulated the reactivity of other T cells in an antigen-specific fashion. Although the phenomenon of immune suppression clearly exists the identity of suppressor cells and their mechanism of action was the subject of controversy for many years. More recently lymphocyte subclasses have been identified that demonstrate suppressive activity but have been given the more modern designation of "regulatory" cells [29–32]. Among the most widely studied regulatory cells are $CD4^+CD25^+$ [31,32] and NKT cells [29]. These populations have been identified in both rodents and humans. T cells that coexpress CD4 and CD25 (the IL-2 receptor α chain) are powerful inhibitors of T-cell activation both *in vivo* and *in vitro*. Convincing evidence that $CD4^+CD25^+$ cells suppress AD has been demonstrated in numerous mouse models. Cells that coexpress both NK receptors and TCRs qualify as belonging to a heterogeneous population termed NKT cells. Subclasses of NKT cells have been shown to suppress immune responses, including graft-vs.-host disease (GVHD) responses.

Another form of regulation that has been observed in many autoimmune models involves preferential activation of $CD4^+$ T-cell subsets (TH1 vs. TH2) [19]. There have been several reports showing that ADs are associated with activation of TH1 cells, which drive cellular responses mediated by activated macrophages and inflammatory processes. In certain animal models of AD it has been shown that the relative activation of the T-cell helper subsets can be manipulated to give either a TH1 response, which results in disease, or a TH2 response (humoral), which confers protection from disease. The preferential activation of TH1 and TH2 cells can be achieved by manipulation of the cytokine environment or by administration of antigen by particular routes (such as by feeding). Thus, a hypothesis arose (which predates the revival of regulatory T-cell subsets) designated the "TH1/TH2 paradigm". This hypothesis proposes that skewing of autoimmune responses towards TH2 predominant responses in preference over TH1 responses will be protective. The current consensus is that the TH1/TH2 paradigm is oversimplistic and begs reevaluation [33,34].

Autoimmune pathology

ADs arise when self-antigens become targets for immune destruction. The response may be directed against a single tissue type or a very limited number of tissues. Histologic studies have shown variability in the

Table 25.2 Animal models of autoimmune disease (AD).

Strain or designation	Disease model	Induction/manipulation
NOD mice	T1Dm	Spontaneous
BB rats	T1Dm	Spontaneous
(NZB/NZW)F1 mice	SLE	Spontaneous
MRL-*lpr*/*lpr* mice	SLE	Spontaneous
BXSB mice	SLE	Spontaneous
NZB/KN mice	Polyarthritis	Spontaneous
C57BL mice	Multiple sclerosis	Induced—peptide of MOG
SJL mice	Multiple sclerosis	Induced—MSCH
DBA1 mice	Rheumatoid arthritis	Induced—Type II collagen
Buffalo rats	Rheumatoid arthritis	Induced—Freund's adjuvant
BDC.2.5 TG mice	T1Dm	Transgenic—TCR
ε-IFN-γ-Tg mice	Myasthenia gravis	Transgenic—IFN-γ on nicotinic acetylcholine receptor
HLA-B27 TG rats	Ankylosing spondylitis	Transgenic—human HLA class II on MHC promoter

HLA, human leukocyte antigen; IFN-γ, interferon-gamma; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; MSCH, mouse spinal cord homogenate; NOD, nonobese diabetic; NZB, New Zealand black; NZW, New Zealand white; SLE, systemic lupus erythematosus; T1Dm, type 1 diabetes mellitus; TCR, T-cell receptor; TG, transgenic.

apparent causes of tissue destruction since predominance of antibodies, activated T or B lymphocytes, or nonspecific inflammatory cells can be seen in the inflammatory lesions. On the basis of such studies, it was concluded that different diseases are predominantly mediated by either humoral vs. cellular driven immune responses. A traditional categorization of immunologic diseases divides the syndromes into four types, designated as types I–IV hypersensitivity responses. Type I responses are caused by antibodies of the immunoglobulin E (IgE) isotype and are considered to be allergic responses, not classical autoimmune responses. Types II–IV involve tissue damage. Type II responses are mediated by antibodies directed against the targeted tissue, type III responses by antibody-antigen complex deposition and type IV by cellular processes. It should be emphasized, however, that these classifications do not illuminate the more fundamental and important issue of what triggers autoimmune responses since, by the time an autoimmune process becomes clinically evident and classifiable by this scheme, the initiating events are obscured by the downstream effector mechanisms causing the actual tissue damage. At least for the reactions of type II, III and IV, there appears to be a common pathway by which autoreactive lymphocyte clones develop and escape the controls that enforce self-tolerance. From our current understanding of how the immune system functions, and from the cumulative experience in the study of autoimmune syndromes in animals, it is thought that loss of T-cell tolerance is the central pathogenic event.

Genes and environment

Both genetic and environmental factors appear to be required in the development of ADs. Family studies, animal models and human epidemiologic studies all support the role of these factors in AD susceptibility. The importance of genetic predisposition was first identified by analyses of disease incidence in monozygotic twins. The concordance rates in twins ranges from ~15% for rheumatoid arthritis [3,35] to a robust ~57% for SLE [3,36]. Comparisons of these rates with disease incidence in the general population predict that genetic predisposition is a dominant factor. For example, the lifetime risk of developing T1Dm in the general population in the USA is 0.4%, whereas for monozygotic twins the concordance rate is in the range of 30–50% [37,38]. For siblings the rate is still significantly increased above the general population at ~6%, but is lower than for twins. This decrease in the sibling concordance rates as compared with monozygotic twins suggests that multiple genes contribute to genetic predisposition. Thus, while genetic susceptibility is a dominant factor, the pattern of inheritance of ADs is complex [1,3]. The diseases are polygenic, meaning that they arise from several independently segregating genes and, to date, the only clearly defined consistent genetic marker for susceptibility to any AD are certain alleles of the genes located within the MHC (Table 25.1).

Inbred rodent strains exist that reliably develop spontaneous ADs (Table 25.2). These animals are highly inbred, and thus genetically identical. Like human twins, many but not all animals in these inbred colonies develop disease. This lack of 100% concordance in genetically identical humans and rodents gives evidence for the essential role of environmental interactions on AD development. Observations made in rodents where environmental elements can be controlled reveal that some of the factors affecting disease incidence include exposure to infectious pathogens and diet [39–43]. For example, a germ-free environment has been shown to suppress or enhance autoreactivity in mice with spontaneously arising forms of multiple sclerosis [40] and diabetes [41], respectively. Furthermore, it is well known among investigators that raise nonobese diabetic (NOD) mice, a model for T1Dm, that certain common mouse pathogens, such as pinworms, leads to dramatically reduced incidence of diabetes. Oral ingestion of protein antigens has been shown to lead to marked suppression of systemic humoral and cell-mediated immune responses when animals are later immunized with the same antigen. This phenomenon is called oral tolerance [44]. A high-fat, high-protein diet has also been shown to increase the rate and severity of diabetes in NOD mice [45]. Another factor contributing to autoreactivity is sexual dimorphism. Sexual dimorphism refers to a pattern of skewing of disease incidence and/or severity towards one sex. It has been observed in many human ADs, such as SLE and autoimmune thyroid disease, that human females demonstrate a disproportionately higher incidence compared to males [46]. Rodents ADs, including NOD mice, show a similar pattern of sexual dimorphism. In NOD mice castration studies [47] and administration of exogenous male hormones [48,49] have shown that sex-related hormones contribute significantly to the dimorphism.

Data from human epidemiologic studies confirm the contributions of genetic and environmental factors on AD incidence. Such studies show clear associations with race, geography and susceptibility to disease.

Again using T1Dm as an example, the incidence of the disease is ~40 times higher in Finland than in Japan [38].

MHC genes and susceptibility to ADs

The only established genetic association for predisposition to ADs is the genotype of the MHC (reviewed in [50]). This association was noted in the mid-1970s. Initially, correlations were made with the class I MHC type and the spondyloarthropathies. Ankylosing spondylitis, an inflammatory and presumably AD of vertebral joints, was found to be strongly associated with the class I human leukocyte antigen (HLA)-B27 allele. Individuals who are HLA-B27 positive have a ~90–100 times greater chance of developing ankylosing spondylitis than do individuals that lack B27. Later, the emphasis shifted to associations with class II rather than class I MHC molecules since frequent associations were found in subsequent studies with class II gene products and other ADs, such as Graves' disease and T1Dm (Table 25.1).

In the last 20 years, the technology of HLA typing has advanced from serologic assays to the more sensitive molecular based assays that detect variations at the nucleotide level (see Chapter 4). As this technology for HLA genotyping has become more precise, allowing examination of specific regions of the MHC, the associations have become stronger. For example, it has been observed that up to 95% of Caucasians developing T1Dm express the *HLA* alleles *DR3* or *DR4* vs. about 40% of normal individuals, and that individuals heterozygous for both *DR3* and *DR4* have the highest risk of T1Dm development [51]. Subsequent to these observations, it was shown that, in fact, the *DQ*, rather than the *DR*, genotype is a more specific marker for T1Dm susceptibility, and that the previous correlation with *HLA-DR* is due to the fact that *DR* and *DQ* are the products of closely linked genes (linkage disequilibrium) [52]. Thus, for T1Dm the highest risk *DQ* alleles, DQα1*0501/DQβ1*0201 and DQα1*0301/DQβ1*0302, are invariably found in the *DR3* and *DR4* genotype, respectively. Individuals heterozygous for these two *DQ* alleles are at greatest risk of T1Dm development. Such individuals comprise 2% of the US population but 40% of the patients with T1Dm.

A similar evolution in the association of *HLA*-type and disease susceptibility has occurred for Graves' disease. Graves' disease was among the first autoimmune disorders noted to have an association with *HLA* haplotypes and the initial association was with the MHC class I genotype *HLA-B8*. Later, however, it became evident that the stronger association was with *HLA-DR3*, which is tightly linked to *HLA-B8* [53].

Function of MHC in AD pathogenesis

Although genetic associations are firmly established between ADs and defined MHC haplotypes, the way that MHC molecules contribute to autoimmune pathogenesis remains hypothetical. MHC molecules play central roles in both T-cell selection during T-cell ontogeny and in the presentation of antigen to T cells. Thus, it has been hypothesized that certain AD associated MHC haplotypes permit faulty selection of T cells during development and/or allow aberrant presentation of self-peptides to T cells that results in inappropriate T-cell activation [33,54,55]. MHC/peptide-restricted recognition by T cells results from the combined effects of the differences in peptide binding and of direct contact between allotypic portions of the MHC molecule. It is known that certain polymorphic amino acids that form the walls of the peptide-binding groove can result in profound differences in binding affinity of MHC molecules with self-peptides as well as affect the conformation of the MHC/peptide-complexes seen by the antigen specific TCR. Furthermore, other polymorphic residues of the MHC molecules can make direct contact with TCRs and thus affect antigen recognition. Therefore, it is possible that the disease associated MHC haplotypes make certain self-antigens appear foreign and/or the haplotypes generate a strong enough immune response to self-antigens to induce T-cell activation.

Support for this hypothesis of aberrant antigen binding and presentation by disease associated MHC molecules comes from sequence analysis of *DQ* genes from individuals with T1Dm. These analyses suggested that there is a critical single amino acid located in the peptide binding groove at position 57 of the DQβ chain that confers either susceptibility or resistance to T1Dm [56,57]. An aspartic acid, which is present in most persons at that position, appears to decrease the risk of T1Dm, whereas substitution of other amino acids at position 57 is associated with increased risk. Further evidence for the importance of this single amino acid was found in spontaneous diabetic NOD mice. These mice show a similar replacement of serine for aspartic acid at position 57 of the homologous mouse MHC class II molecule (I-Aβ chain) [58]. In humans, amino acid 57 is located at the distal end of the DQβ chain and forms part of the peptide-binding cleft of the DQβ molecule [57]. Aspartic acid, the protective amino acid at this position, forms a salt-bridge with a residue on the opposite side of the binding cleft, and replacement of an uncharged residue at this position disrupts the salt bridge formation.

The above hypothesis presumes that the association of ADs with MHC haplotype derives directly from the function of the MHC gene products. While this hypothesis has been amply supported by data from both humans and rodents, it is not conclusively proved. Disease association clearly maps to the MHC region; however, contained within this region are a number of other genes. Alternative hypotheses include that the MHC-haplotype serves only as a marker and that the true (and as yet undetermined) disease-associated genes are closely linked to the MHC alleles.

Non-MHC genes and susceptibility to ADs

The importance of genetic predisposition in AD susceptibility and the conclusion that several genes contribute to an AD phenotype has motivated the search for predominant non-MHC susceptibility genes [3,59]. It was hoped that genome-wide linkage analysis could aid in the identification of such genes. To achieve this goal international coalitions were formed aimed at collecting large cohorts of families afflicted with specific ADs and employing state-of-the-art technologies to scan their genomes for the location of susceptibility genes. These analyses have confirmed the complex nature of the genetic associations and supported the conclusion that defining the AD susceptibility genes is not easily amenable by such an analysis. The reason for the difficulty is that inheritance of AD susceptibility is multifactorial, arising not only from a combination of multiple contributing susceptibility genes, but that each gene has the possibility to interact with a poorly defined array of environmental and/or stochastic factors. Furthermore, identification of the AD susceptibility loci is complicated by two factors that commonly influence inheritance of multifactorial traits: genetic heterogeneity and epistasis.

Genetic heterogeneity

Genetic heterogeneity means that different combinations of individual genetic abnormalities are capable of causing a similar disease phenotype. Examples of genetic heterogeneity are seen by comparison of the genomic locations of susceptibility genes in separate mouse models of ADs such as T1Dm, SLE and a rodent form of multiple sclerosis (designated experimental autoimmune encephalomyelitis [EAE]) wherein the genomic locations of many susceptibility alleles vary between models. Although emphasis of genetic analyses has been on identification of co-localizing susceptibility genes, it has been determined that most of the genomic segments detected are not shared between the different animal models [3]. Even within the same AD syndrome such as the different rodent models of SLE, susceptibility is mediated by a heterogenous array of genes.

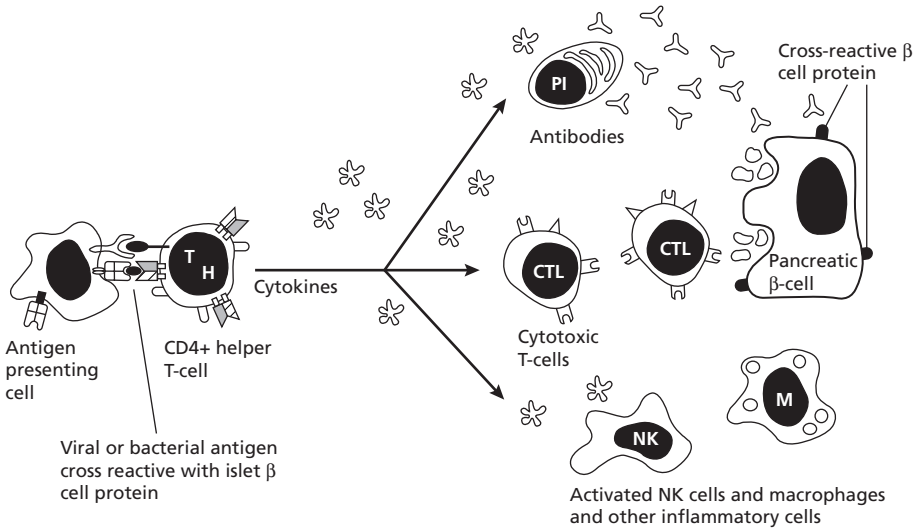

Fig. 25.1 Induction of autoimmunity. Schematic illustration of the events thought to cause an autoimmune response according to the hypothesis of molecular mimicry. A peptide derived from a pathogen is being presented to a $CD4^+$ T cell by a disease-associated class II major histocompatibility complex (MHC) molecule on the surface of a professional antigen-presenting cell (APC). The peptide sequence crossreacts with a component on pancreatic islet β cells. The $CD4^+$ T cell is activated upon receipt of this first signal from its cognate antigen plus MHC molecule, and a second costimulatory signal. Following activation $CD4^+$ T cells secrete a number of cytokines and/or make cell–cell contact with downstream effector cells inducing them to destroy the source of the antigenic stimulus. The pancreatic β cells are also destroyed since they express a protein that contains the same peptide sequence as the inciting antigen.

Epistatic interactions

Epistatic interactions classically refers to interactions in which the genotype at one locus affects the phenotypic expression of the genotype at another locus. A clear example of epistatic interactions in AD pathogenesis comes from a series of studies of congenic mice generated by Wakeland and colleagues [3]. Congenic strains of mice are defined as mice that are genetically identical at all loci except one. The loci may include one or several linked genes. Each strain is generated by repetitive backcrossing of mice carrying the desired trait onto a strain that provides the genetic background. C57BL/6 mice were used as the background strain for different primary susceptibility alleles derived from New Zealand white (NZW) strain mice that spontaneously develop a benign form of SLE. Some of these congenic mice, designated B6.*Sle*, develop non-pathogenic autoantibodies to nuclear antigens, but do not develop severe autoimmunity. However, when certain of the B6.*Sle* strains are bred, their bi-congenic F1 offspring develop severe systemic autoimmunity, which is ultimately manifested by fatal glomuerulonephritis [60]. This result is an example of epistasis between two susceptibility alleles leading to a greater increase in disease severity than would be predicted by simply adding together their individual phenotypes. Another type of epistatic interaction exists in which the autoimmune phenotype of the susceptibility alleles are suppressed by epistatic modifiers. Again the clearest example of this concept comes from the B6.*Sle* congenic mice. When three strains of the B6.*Sle* mice are crossed which results in triple congenic mice that contain the three susceptibility loci in their genome and are on the C57BL/6 background, nearly 100% of such mice develop fatal lupus nephritis. However, all three of the susceptibility loci were originally derived from NZW mice, a strain in which this genetic combination results in a relatively benign autoimmune syndrome [61].

What triggers autoreactivity?

Several hypotheses exist to explain what triggers and perpetuates AD pathology. Based upon the cumulative data that link autoimmune responses with MHC type, and the central function of T cells in the induction and perpetuation of antigen specific immune responses, these hypotheses have focused primarily on loss of T-cell tolerance, either through inappropriate presentation of antigens to T cells or through the failure to eliminate or silence self-reactive T-cell clones. Although T cells undergo a rigorous selection process during development in the thymus to eliminate self-reactive clones, it is thought that such clonal deletion is imperfect and that circulating self-reactive naive T cells exist which are controlled by the mechanisms of peripheral tolerance.

The predominant view is that one or a few self-peptides can trigger a cascade of cellular events that result in targeted tissue damage [33,62,63]. A typical immune response against a self- or foreign protein is usually focused on one or two peptide sequences (called epitopes) contained within that protein which are termed *dominant epitopes*. Once a response is triggered against the dominant epitope other peptide epitopes from the same protein become targets, thus expanding and perpetuating the immune response. This hierarchical extension of an immune response from dominant epitopes to subdominant ones is termed *epitope spreading* [63]. Most self-peptides cannot serve either as autoantigens simply because they are present at levels that are too low to be detectable by naive T cells. However, a few self-peptides that fail to induce tolerance may be present at high enough levels to be recognized by T cells. These peptides are the breakdown products of tissue specific proteins, and it is likely that only certain proteins can act as autoantigens since there are relatively few distinct autoimmune syndromes, and individuals with a particular AD seem to recognize the same antigenic targets. Thus, autoimmunity can occur if an APC picks up one of these proteins, and presents a dominant epitope of the protein in conjunction with costimulatory molecules resulting in activation of $CD4^+$ T cells. Once autoantigen specific $CD4^+$ T cells are triggered, then barring intervention by suppressive or regulatory subsets, the pathway is set towards elimination of the inciting antigenic stimulus.

Two hypothesis of how spontaneous ADs may be induced are by *molecular mimicry* or *tissue injury*. The hypothesis of *molecular mimicry* (shown in Fig. 25.1) suggests that immune responses directed against infectious agents can crossreact with self-antigens, causing autoimmune destruction. Thus, the inciting antigen could be a bacterium- or virus-derived protein that shares an amino acid sequence with a prevalent tissue-specific protein. Antibodies or cytotoxic T cells directed against the pathogen will also selectively destroy the normal tissue that expresses the crossreactive protein. Relevant examples are from studies in T1Dm where correlations exist between congenital rubella and coxsackievirus B4 [42]. For rubella, it has been shown that an immunogenic epitope for the virus capsid protein has structural similarities to an islet β cell protein [64]. In the case of coxsackievirus B4, there is a striking amino acid sequence homology with an enzyme found within β cells called glutamic acid decarboxylase (GAD) [65]. Autoantibodies against GAD may be found in the serum of prediabetic and diabetic patients. The *tissue injury* hypothesis attributes activation of localized inflammatory mechanisms in

response to organ injury as an inciting event. During inflammation the release of chemoattractants and cytokines recruits macrophages, lymphocytes and other effector cells. The result may be the release of tissue-specific antigens and uptake by APCs that can result in presentation of self-antigen at high enough levels to act as an immunogen.

Animal models of ADs

Rodent models of ADs have contributed significantly to the understanding of disease pathogenesis. Three major types of animal ADs serve as models for study: (i) ADs that arise spontaneously; (ii) ADs that are induced by immunization or by adoptive transfer of autoreactive mature immune cells; and (iii) ADs that are created by genetic engineering technology. Many of the concepts regarding the causes of autoimmunity have either originated from or have been confirmed by observations made in these animal models. Examples of these models and their homologous human diseases are shown in Table 25.2. The models rely on genetic homogeneity so that recipients and donors are from highly inbred strains.

Spontaneously arising ADs

Animals that spontaneously develop autoimmune syndromes have been instrumental in revealing the complex nature of genetic susceptibility to ADs and in understanding the cellular events that lead to tissue destruction. The observations that even in inbred animals there is reliable but not 100% development of the autoimmune syndromes underscores the importance of the interaction of genetic plus environmental factors in autoimmune pathogenesis [3,66,67]. The essential role of certain MHC alleles as the primary susceptibility genes, the interactions between other minor susceptibility genes, and the role of T lymphocytes in driving autoimmune pathogenesis have all been confirmed by studies in these animals. The most extensively studied models of spontaneously arising ADs are the mice that develop SLE-like syndromes and NOD mice that develop a disease resembling T1Dᴍ.

The lupus-prone mice include the F1 hybrid of New Zealand black (NZB) and NZW mice designated (NZBxNZW)F1, MRL-*lpr*/*lp*r mice and BXSB mice [67,68]. Like human SLE these animals develop autoantibodies to nuclear antigens and progressive severe glomerulonephritis. Extra-renal disease manifestations occur variably in the individual models and include lymphoproliferation with both splenomegaly and lymphadenopathy, hemolytic anaemia, autoimmune thrombocytopenia, vasculitis, thrombosis and arthritis. All of these lupus-prone strains exhibit premature thymic atrophy, the significance of which is unknown. In the (NZBxNZW)F1 model heterozygosity at the MHC (MHC designation $H2^{d/z}$) has shown to directly impact on disease severity. Data on the non-MHC genes linked with murine lupus comes primarily from the New Zealand hybrid model for which genetic crosses have demonstrated confirmed linkage with ~12 loci from the NZB or NZW strains [3,67]. In MRL-strain mice homozygosity for the *lpr* or *gld* mutations results in the acceleration of lupus autoimmunity [69]. *Lpr* is a spontaneous mutation of Fas (CD95) and *gld* is a mutation of Fas ligand. Binding of Fas ligand to Fas results in programmed cell death in the Fas expressing cells. Although the role of these molecules in apoptosis are the subject of intense investigation, the mechanism by which mutations in Fas lead to accelerated autoimmunity is not known. Regardless, the MRL background has been shown to significantly contribute to expression of the lupus-like disease, and neither the genes for Fas or Fas ligand appear to overlap with any of the New Zealand disease loci mapped thus far [69]. BXSB mice carry the *Yaa* (Y chromosome-linked autoimmune acceleration) gene [67,70], which results in more rapid and severe lupus-like disease in male vs. female BXSB mice. This skewing towards higher disease severity in males contrasts the more common pattern of severity seen in female (NZBxNZW)F1 mice, NOD mice and in many human ADs where disease severity is often skewed towards females.

NOD mice develop a syndrome resembling human T1Dᴍ and are the most exhaustively studied model of an animal AD [66,71]. The disease pathogenesis begins with the infiltration of mononuclear cells into the insulin producing islets of Langerhans at ~3–4 weeks of age. Infiltration of the islets (termed insulitis) progresses slowly over the course of several months until the islets are destroyed and the mice manifest symptoms of hyperglycemia at ~6–9 months of age. NOD mice express only one MHC class II gene product. As described in detail in the Function of MHC in AD pathogenesis section above, the sequence of this class II molecule, designated I-A^{g7}, is unique to the NOD strain and it binds peptides poorly—a characteristic which has been suggested to explain the association of this genotype with autoreactivity [54,72]. This hypothesis, however, is currently undergoing reevaluation. Progression to overt diabetes can be blocked in these mice by prolonged administration of antibodies directed against $CD4^+$ T cell in the prediabetic phase demonstrating that NOD disease is $CD4^+$ cell mediated [73,74]. Pathogenic T cells capable of transferring the disease have been cloned from these mice and TCRs from such clones have been expressed in non-NOD background transgenic mice thus causing diabetes [75–77]. Once hyperglycemia develops NOD mice can only be cured of their disease with islet or pancreas transplantation.

Immunization and cell transfer models of ADs

Conventional strain rodents may also be induced to develop autoimmune syndromes by immunization with proteins or peptides derived from defined tissues, or by transfer of pathogenic lymphocytes (Table 25.2). Genetic susceptibility plays a major role since certain strains are more prone to mount pathogenic responses than others. One of the best-studied examples of an antigen induced AD is the rodent disease, EAE [62,78,79]. EAE affected mice or rats develop symptoms analogous to the human neurologic disease, multiple sclerosis. The animals show symptoms of paralysis that, like multiple sclerosis, can be either progressive or fluctuating in symptoms. EAE is induced by subcutaneous immunization with components derived from the spinal cord. These components range from the emulsified spinal cord itself, proteins derived from the spinal cord, or defined peptides from spinal cord proteins. The proteins that are known to induce EAE in mice include myelin-basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG) [80]. Defined peptides derived from these proteins can also induce EAE, and the exact sequence of such peptides depends upon the MHC haplotype of the mouse strain immunized. For any given mouse strain only certain peptide sequences are pathogenic and are termed immunodominant. Figure 25.2 illustrates induction of EAE using a peptide of MOG [35–55]. It was the study of immunodominant peptides in EAE that led to the hypothesis of epitope spreading [62,63] (see What triggers autoreactivity? section above) as a mechanism by which immune responses can be perpetuated and expand to other antigenic specificities. In order to induce pathogenic autoimmune responses by immunization with peptides or proteins it is critical that these antigens be administered as part of a mixture with adjuvants, such as Freund's adjuvant and/or pertussis toxin. Complete Freund's adjuvant is an oil-in-water emulsion containing dead mycobacteria which is thought to enhance immunogenicity in two ways. First, emulsification of antigens in adjuvant serves to convert soluble protein antigens into particulate forms, which are more readily ingested by APCs; and, second, the bacterial products in adjuvant are thought to induce the expression of costimulatory molecules on the surface of APCs so that responding T cells become activated rather than anergized.

Another way to reproducibly induce ADs is by transfer of lymphoid cells from affected to unaffected immunoincompetent recipients.

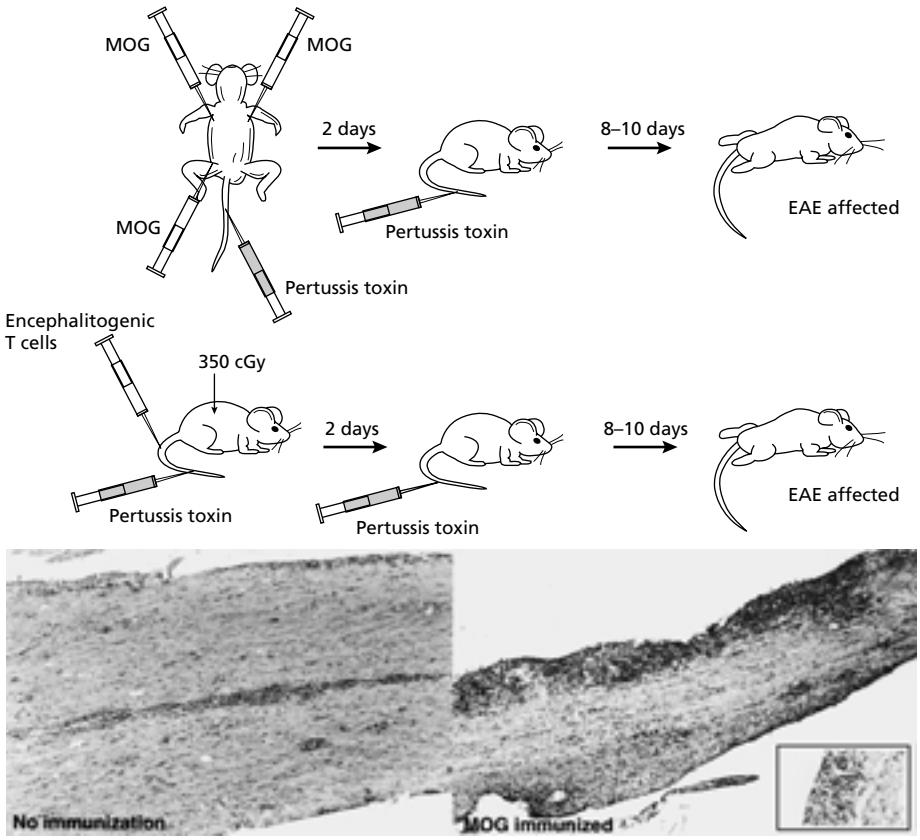

Fig. 25.2 Experimental approach to causing an autoimmune disease (AD). Experimental autoimmune encephalomyelitis (EAE), a rodent disease resembling multiple sclerosis, can be induced by immunization of normal mice with a peptide that crossreacts with components on the spinal cord.

The top panel shows a schematic for EAE induction using a peptide derived from myelin oligodendrocyte glycoprotein (MOG). The peptide is emulsified in Freund's adjuvant. On day 0 the mixture is injected subcutaneoulsy into an inguinal and bilateral axillary regions. Immunized mice receive an additional adjuvant injection (pertusis toxin) by the intravenous route on days 0 and +2. Alternatively, encephalitogenic T-cell lines or clones can be injected into mice prepared with 350 cGy plus pertussis toxin on days 0 and +2. Expected onset of disease is ~8–10 days following immunization or cell transfer. Clinical scoring of EAE is on a scale of 0–5 as follows: 0, no clinical signs; 1, loss of tail tonicity; 2, flaccid tail and hind limb weakness; 3, hind limb paralysis; 4, complete hind limb paralysis; 5, moribund or death.

The bottom panel shows hematoxylin and eosin stained spinal cords from (left) an nonimmunized mouse as compared with (right) a MOG immunized mouse. Note the intense mononuclear cell infiltrate is most prominent in the meningeal areas of the spinal cord of the immunized animal.

Populations capable of transferring disease include cells taken from peripheral lymphoid organs, such as the spleen or lymph nodes [81,82], or cells extracted from autoimmune target tissues, such as infiltrated pancreatic islets in NOD mice. It is also possible to clone pathogenic T cells of a single antigen specificity that can transfer disease [75,76,83]. Cloned T cells are particularly valuable for tracking immune responses *in vivo* since they express a monotonous TCR that can be identified by labeled monoclonal antibodies (MABs) that bind the variable region of the TCR β chain (*V*β). Receptors from such clones can also be used to generate transgenic animals. Interestingly, adult T cells from normal mice that are depleted of $CD4^+CD25^+$ T cells can transfer an autoimmune syndrome with a wide spectrum of organ specific manifestations including gastritis, oophoritis, orchitis and thyroiditis. Indeed, this latter observation was one of the ways the existence of regulatory $CD4^+CD25$ T cells was demonstrated [31,32].

Appropriate recipients for adoptive transfer studies are irradiated animals, or genetically immunodeficient strains that cannot produce T and/or B lymphocytes [84]. Two prominent examples of naturally occurring mutations that prevent normal lymphocyte development are a defect in a DNA repair gene resulting in mice with severe combined immunodeficiency syndrome (SCID), and a defect in the Wnt signaling pathway which results in mice that are both hairless and lack a thymus (*nude* mice). Mice with the *nude* defect cannot produce T cells. However, transfer of their BM progenitor cells to recipients with normal thymuses results in normal T development. BM from SCID mice cannot generate functional T or B cells even in a normal recipient. Genetically engineered knockouts of the recombination activating genes (*RAG-1* or *RAG-2*) required for T- and B-cell receptor rearrangement results in defects phenotypically similar to the SCID mutation in that the *RAG-1* or *RAG-2* knockout mice cannot generate functional T or B cells.

Genetic engineering of ADs

Transgenic mice expressing a variety of molecules are now regularly produced in order to model specific aspects of AD pathogenesis. There are four broad categories of the types of transgenic mice that have been studied: (i) MHC transgenic mice; (ii) lymphocyte receptor transgenic mice; (iii) double transgenic mice that express a particular antigen plus the receptor that binds the antigen; and (iv) transgenic mice expressing immunoregulatory molecules.

MHC transgenics

One of the earliest applications of transgenic technology was to ask if ectopic expression of MHC class II molecules on parenchymal tissues that do not normally express these molecules will elicit autoreactivity [85]. For example, chimeric transgenes that contained the insulin promoter fused with an MHC gene resulted in constitutive expression of MHC molecules on islet β cells. An earlier hypothesis had predicted that such ectopic express on nonhematolymphoid tissues would result in presentation of "hidden" tissue specific antigens to potentially autoreactive T-cell clones and induce an immune attack against the islets. The results of a series of independent experiments generating mice that expressed a variety of MHC molecules on the islet β cells were surprising. Although many of the transgenic mice became diabetic, there was no indication that immune reactivity was the cause of the diabetes phenotypes. Rather the data suggested that hyperexpression of the MHC molecules in the islets was detrimental to β cell function, and gave further evidence for the importance of costimulation in T-cell activation. More recently human AD associated MHC alleles have been expressed as xenogeneic proteins in mice [86]. Such mice provide both a surrogate *in vivo* model for studying the development of a TCR repertoire based on human MHC

molecules as well as tools to examine aberrant responses as has been shown in *HLA-B27* transgenic rats that develop a syndrome resembling ankylosing spondylitis [87].

Antigen receptor transgenics

Experimental analysis of autoreactivity and immune tolerance has been confounded by difficulty in following the fate of antigen specific cells during development and in the blood and lymphoid tissues where they encounter their cognate antigen. Transgenic technology has permitted a method for generating T and B lymphocytes with defined antigen specificity that dominate an animal's lymphocyte repertoire. MABs specific to the transgenic receptor allow the cells to be tracked throughout the life of an animal and assessed under different conditions, such as challenge with the known antigen. T- or B-cell receptor transgenic mice are generated by using as the transgene the rearranged receptors from antigen specific lymphocyte clones. Because these antigen receptor genes inhibit recombination of the other endogenous antigen receptor gene loci (a phenomenon termed *allelic exclusion*), a large fraction of the T or B cells in these mice express the introduced transgene encoded antigen receptor. Examples in the study of autoimmunity are mice that express a TCR specific for a protein in pancreatic islet β cells (as occurs in T1Dᴍ), a TCR specific for myelin basic protein (a target in EAE), and immunoglobulin specific for self-DNA (involved in the pathogenesis of SLE) [88–90]. While BM or lymphoid cells are routinely transferred from these transgenic animals to a variety of types of recipients, the converse experiment has rarely been done. The latter type of experiment wherein transgenic mice serve as recipients rather than donors should be pursued, since the results would allow study of the fate of residual recipient autoreactive cells following autologous or allogeneic HCT.

Double transgenics

A variation for tracking the fate of cells with transgenic antigen receptors is the generation of double transgenic animals that express both the lymphocyte and the cognate antigen (such as a virus) as transgenes [91,92]. The antigen may be expressed in different forms such as secreted, membrane bound or cytoplasmic. Additionally, antigen expression may be constitutive or driven by an inducible promoter. An example of such a system modeled molecular mimicry to show that infectious agents can trigger autoimmunity [93]. The double transgenic mice expressed both a viral nuclear protein driven by the insulin promoter (thus expressed primarily in the islets) and T cells that recognize the viral protein. Virus expression was low in the islet β cells; therefore, T cells that recognized the viral protein remained ignorant, meaning they were neither tolerant to the viral protein nor activated by it. However, when the mice were infected with the live virus, they responded by activating virus specific $CD8^+$ T cells and these $CD8^+$ virus specific T cells could then recognize the viral antigen on the β cells and destroy them causing diabetes.

Immunoregulatory transgenics

A variety of mice have been generated that express transgenes involved in lymphocyte activation or suppression, or that have had specific regulatory genes knocked out [90]. Targeted genes include cytokines, costimulatory molecules, Fas ligand and molecules involved in lymphocyte intracellular signaling pathways. Examples relevant to the study of autoimmunity are transgenic mice that express cytokines, such as IFN-γ in the islets of Langerhans, causing an immune-mediated diabetes [94], enhanced production or knockout in lymphocytes of transforming growth factor beta (TGF-β), which results in progressive glomerulonephritis in the transgenic mice and mononuclear infiltration of multiple organs in the knockout mice, and knockout of the Src tyrosine kinase, *Lyn*, which participates in B-cell receptor signaling results in antinuclear antibody production and glomerulonephritis [67].

HCT and the treatment of ADs

Historical perspective

Preclinical studies and case reports in the human transplantation literature support the use of HCT for the treatment of severe ADs [95–97]. The clinical literature on this topic (reviewed in Chapter 101) contains several case reports demonstrating that patients undergoing allogeneic HCT for conventional indications (i.e. hematologic malignancy) with a coincidental AD experienced long-term improved or even full remission of both disorders. Conversely, there have been case reports of transfer of ADs from AD affected allogeneic HCT donors into previously unaffected recipients. In evaluating the preclinical literature for its relevance to the treatment of human ADs it is important to bear in mind that heterogeneity exists among the experimental systems, and that often the studies were designed to answer basic questions about AD pathogenesis rather than to form a basis for direct translation to human therapy. A goal of the early investigators was to establish what cellular elements transfer or prevent disease. Thus, initial studies were directed towards the creation of allogeneic radiation BM chimeras to determine if transplantation of hematolymphoid elements could alter susceptibility to ADs in rodents.

It was known that certain mouse strains, such as the NZB, develop a syndrome resembling human SLE with manifestations that include production of antinuclear antibodies (ANA), immune complex glomerulonephritis, Coombs-positive hemolytic anemia, and a more general phenomenon of immunological hyperresponsiveness. In 1969, Denman *et al*. [5] demonstrated that transfer of BM or spleen cells from NZB mice to MHC-matched nonautoimmune prone BALB/c mice (both $H\text{-}2^d$) resulted in disease in the recipients. Later, in 1974 Morton and Siegel [4] demonstrated that BM transferred into irradiated recipients could, on the one hand, transfer disease from NZB donors into MHC-matched BALB/c or DBA/2 recipients and, on the other hand, BM from BALB/c or DBA/2 donors could result in transient normalization of ANA titres in NZB recipients. Donor chimerism was not measured in these studies, and one explanation proposed by the authors for the transient rather than persistent nature of ANA depression was that perhaps only short-term chimerism was achieved in the NZB recipients. Other investigators confirmed in different SLE mouse models that BM appeared to be the component capable of transferring disease [8,9]. Cumulatively, these studies were interpreted as demonstrating that the etiology of autoimmunity is determined by the innate properties of the HSC and its differentiated lymphocytic progeny, and independent of the host environment.

ADs as "stem-cell disorders"

These seminal experiments led to the concept that ADs are disorders of HSCs. Indeed, it was later observed by other groups and in different animal systems that the genotypic origins of the BM (i.e. from susceptible or nonsusceptible strains) determined whether or not the animal developed or was protected from disease. However, not all of the studies have been consistent with this concept. For example NOD disease has been transferred by NOD BM into F1 offspring of NOD mice crossed with different strains [82,98]. However, when recipients were genetically disparate such that they did not share one haplotype, radiation chimeras engrafted with NOD BM developed insulitis but most did not progress to overt diabetes [99]. These data show that while anti-islet reactivity can be transferred by BM, the host environment provides additional elements that permit the perpetuation of an immune response, which ultimately results in tissue destruction. That inconsistencies exist in this literature should not be surprising. As discussed in detail in the prior sections, autoreactivity arises from a combination of interacting genetic and stochastic factors that affect hematolymphoid cells as well as other tissues. Furthermore,

BM grafts are complex mixtures of cells with differing functions and the grafts give rise to heterogenous cell populations. Among the populations transferred by BM are those that control antigen specific immune responses—APCs and lymphocytes. APCs express the MHC restricting elements that are instrumental in shaping the T-lymphocyte repertoire. Thus, it is logical that the BM genotype contributes significantly to autoimmune susceptibility. However, the concept that ADs are solely disorders of HSCs is overly simplistic. The other complexities that influence immune reactivity should be considered when interpreting the studies reviewed in the next sections.

Rationale for HCT to treat ADs

Both autologous and allogeneic HCT have been studied in preclinical models of ADs. The rationale for efficacy differs between these two procedures.

The use of *autologous* HCT is based on the idea that near complete ablation of autoreactive cells, primarily T cells, can be achieved by high-dose therapy followed by rescue with a hematopoietic graft that contains few or no pathogenic cells. Such an approach is analogous to the treatment of cancer wherein the conditioning regimen results in cytoreduction of malignant cells and the patient is "rescued" with a hematopoietic graft that contains none or very few passenger cancer cells. It is thought that the aberrant events that induce autoimmunity (such as infection with virus that crossreacts with normal tissues) occur only rarely, and the manifestations of disease in AD patients reflect the perpetuation of effector responses which continue even though the inciting event has passed. Thus, lymphoablation and reconstitution with autologous grafts that lack mature lymphocytes is thought to "reset" the immune system, and the disease will not reoccur assuming that the likelihood a second pathogenic event will trigger autoreactivity is extremely low.

The rationale for *allogeneic* HCT is similarly based on the assumption that replacement of a defective immune system with a normal one will eliminate the autoreactive cells. In addition, donor hematolymphoid cells may express genes that modify immune responses favoring tolerogenic rather than immunogenic responses against autoantigens. The weight of evidence from preclinical animal models favor allogeneic HCT over autologous as the more efficacious approach. However, the current ongoing clinical trials in human disease have been exclusively directed to the use of autologous HCT (see also Chapter 101) [95,96]. The reluctance to perform allogeneic as compared to autologous HCT for ADs is based on concerns of unacceptable procedure-related morbidity and mortality in the former as compared with the latter [9,95].

Definition of terms and experimental approaches

Autologous, syngeneic, congenic

Genuine autologous HCT has only rarely been performed in rodents because of the pragmatic limitations of harvesting autologous hematopoietic cells from small animals. Instead syngeneic donors from the same inbred strain as the recipients, or congenic donors (see Non-MHC genes and susceptibility to ADs section above) that differ from the recipients by one or a limited number of nonhistocompatibility genes, are used. The advantage of using congenic donors is that the gene difference(s) allow determination of the origin of hematopoietic cells (residual host-vs.-graft derived) in the transplanted recipient. For example, mice that are genetically identical except for a congenic difference at the CD45 allele are common laboratory tools. CD45 (previously designated Ly-5) is expressed on all lineages of hematopoietic cells, and in mice there are two alleles, CD45.1 and CD45.2 [100,101]. MABs exist that can distinguish between the two alleles. Thus, staining assays employing labeled MABs, such as fluorescence activated cell sorter (FACS) analysis or immunohistochemistry permit detection of donor-vs.-host hematopoietic cells in transplant recipients. Male into female transplants or vice versa can also be used, in which case the chimerism analysis involves *in situ* hybridization looking for the presence or absence of the Y chromosome. Another type of donor has been termed pseudoautologous. Pseudoautologous means that in models wherein the disease in recipients is induced by antigen immunization [9], the congenic or syngeneic donors undergo similar immunization. Grafts from such donors may contain contaminating autoreactive cells with the potential to cause disease and therefore more accurately reproduce clinical autologous HCT. Grafts from congenic or syngeneic donor strains that develop spontaneous disease are not called pseudoautologous, although there is similar potential to transfer autoreactive cells in unmanipulated hematopoietic grafts. Here, the HCT studies are described as they were originally performed using congenic, syngeneic or pseudoautologous donors with the understanding that all of these graft types serve as models for autologous HCT.

Genetic differences and measurement of chimerism in allogeneic HCT

By definition allogeneic HCT uses donors that are genetically disparate from the recipients. Genetic disparity means that the donor and recipients are mismatched at multiple gene regions and are distinct from congenic pairs wherein the genetic differences are limited. Many of the studies were performed between donors and recipients that were derived from distinct ancestral strains, and thus differ at both MHC and multiple other minor histocompatibility antigen genes. Determination of donor chimerism has been possible using antisera or MABs in cytotoxicity or staining assays. For transplants involving MHC differences (MHC-mismatched and haplo-identical) reagents that recognize MHC determinants have been used. Detection of chimerism between MHC identical strains has been more difficult primarily because the reagents are more limited. In fact, studies that predate the late 1970s when allele specific MABs were developed did not evaluate chimerism. In order to use antibody based assays to measure chimerism allelic differences for defined gene products expressed at the cell surface must exist between donor/recipient strains. Furthermore, antibody reagents that distinguish the allele markers must be available and allelic markers must be expressed on all or on subsets of hematopoietic cells. Examples of antibody reagents used for this purpose are those against Lgp100 [102], a glycoprotein expressed on lymphocytes of certain strains, or CD45 [100,101], an allelic marker expressed on hematopoietic cells. More recently, polymorphisms identified within the mouse genome [103] can be used in polymerase chain reaction assays to differentiate hematopoietic cells between MHC-matched strains.

Preparative regimens

In the vast majority of rodent studies, recipients were prepared for transplantation with lethal radiation. Myeloablative radiation doses are strain specific and require titration studies to determine the dose(s) at which death occurs because of hematopoietic failure, and not from other organ toxicities [9,104]. At such doses mice that would otherwise expire, are rescued by infusion of syngeneic BM cells. For any given strain there is a range of doses that cause myeloablation without other toxicities [104,105], and thus some nonuniformity of radiation dose exists in the literature. Such dose variation can affect both the level of lymphoablation and the degree of resistance to engraftment of allogeneic hematopoietic cells. Therefore, comparisons of outcome between different experiments must take into consideration the potentially relevant effects of radiation dose variability.

Chemotherapy that includes reagents used in human patients such as cyclophosphamide (CY) and busulfan (BU) have been employed in studies wherein the goals were to explicitly model clinical transplantation [106,107]. CY is the best studied of the chemotherapeutic drugs in

rodents and reports from the early literature show that this agent alone without hematopoietic cell rescue is highly effective at ameliorating manifestations of Ads, such as EAE in rats [108], and antibody production and immune abnormalities [109,110] in SLE prone mice. Dimethyl myleran is an alkylating agent related to BU with profound marrow suppressive activity and little immunosuppression that has also been used for rodent HCT preparation [111,112]. Fludarabine, an agent that is now widely used in human nonmyeloablative regimens, has also been tested in animals. Unfortunately, mice are highly resistant to the lymphoablative effects of fludarabine and its congeners making it difficult to model homologous nonmyeloablative regimen in mice utilizing this drug. One major advantage of working in rodent systems is the availability of numerous antibody reagents that target specific immune cell subsets. Although there is no study demonstrating that antibodies alone can effectively allow engraftment of allogeneic hematopoietic cells, there are reports demonstrating that antibody treatment can permit engraftment at nonmyeloablative radiation doses in NOD mice [113,114] and other nonautoimmune strains (see also Chapter 24).

Hematopoietic graft types

Unfractionated or T-cell depleted (TCD) BM from wildtype donors or BM from mice with the *nude* defect have been the graft source in most studies. The reason for the use of *nude* mice as donors is that one manifestation of the *nu*/*nu* gene defect is the absence of a thymus [84]. Thus, BM grafts from *nude* mice do not contain conventional T cells, but when engrafted into recipients with functional thymuses their hematopoietic cells give rise to mature T lymphocytes. Transplantation of purified hematopoietic cell populations have also been studied [115]. Separation techniques to negatively select mature T, B and macrophages in combination with positive selection methods have been used to enrich for progenitor cells. One group of investigators used positive selection by binding to the plant lectin wheat germ agglutinin (WGA) [116]. Their rationale for this approach was the WGA-positive BM cells were enriched for stem and progenitor cells as well as an immunoregulatory "natural suppressor" population [117].

We have used the positively selecting markers Thy1.1, c-Kit and Sca1 in combination with negative selection for mature lineage markers (CD4, CD8, CD3, B220, Mac1, Gr-1 and Terr119) to enrich for an HSC population with a composite phenotype of $cKit^{+}Thy1.1^{lo}Lin^{-/lo}Sca\text{-}1^{+}cKit^{+}$ (KTLS) (see also Chapter 8) [115,118,119]. Quantitative assessment of the KTLS population revealed that these cells comprise one in 2000 cells in mouse BM and are, in fact, 2000-fold enriched for HSC activity as measured in *in vivo* radioprotection assays. Further, ~200 KTLS HSC rescues 100% of lethally irradiated mice across CD45 congenic barriers. The T-cell content of KTLS HSC grafts is reduced by >5 logs as compared with BM. Of note, the use of KTLS HSC or other manipulated BM populations in allogeneic transplantation results in profound differences in resistance to engraftment and chimerism outcome as compared to unmanipulated BM [115,120,121]. Thus, graft content can directly affect the outcome of autologous and allogeneic HCT in the treatment of ADs.

Timing of the HCT procedure

Since the progression of ADs in most rodent models is well characterized, it is possible to choose the time of HCT relative to the expected disease course. For example, in antigen induced models, immunized animals have been transplanted either before or following the onset of overt manifestations. In spontaneously arising ADs, the transplantations can be performed during the phase when mice have documented abnormalities in immune function but little clinical signs of impairment or at later disease stages. The studies show that animals are more consistently cured of their ADs if they undergo the HCT procedure at very early stages of disease, even when there is measurable pathology such as insulitis in NOD mice or evidence of glomerulonephritis in SLE-prone animals, but not at the point when they have suffered endstage organ damage. If a single organ has been destroyed which is itself replaceable by transplantation, then it is possible to perform simultaneous organ plus HCT transplantation (see Chapter 24). The best example of this approach in AD affected animals has been in older NOD mice that have undergone simultaneous allogeneic HCT plus donor matched pancreatic islets in order to cure them of overt diabetes [114,122].

Autologous HCT

There are conflicting reports in the preclinical literature regarding the efficacy of syngeneic or congenic transplantation in curing autoimmune syndromes [9]. Until the late 1980s it was generally believed that syngeneic HCT would have no effect on AD pathogenesis. In fact, mice transplanted from syngeneic donors served as negative controls in allogeneic HCT studies to differentiate the effects of the preparative regimen alone from effect of the allograft—a logical conclusion since syngeneic grafts were thought to merely perpetuate ongoing tissue destruction unless complete elimination of pathogenic cells was achieved. Indeed, in many studies [4,113,123–125] the animals in the syngeneic control groups showed no amelioration of disease whereas allogeneic HCTs were curative. However, a series of reports from a single group of investigators led by van Bekkum emerged beginning in 1989, demonstrating that significant remissions could be achieved with syngeneic transplantation in rats affected with antigen induced ADs [9]. In the original studies of arthritis caused by Freund's adjuvant, the syngeneic BMT "control" group was surprisingly noted to show equal resolution of disease as the allograft recipients [126]. Interestingly, rats that had undergone syngeneic BMT prior to adjuvant exposure developed disease equivalent in frequency and severity as naive rats, whereas reimmunization of rats that were disease affected at the time of syngeneic BMT did not reinduce disease. These data suggest that one element in the effectiveness of HCT may be due to disruption of an ongoing immune response and that antigen exposure at the time of procedure (and not before) is required for shifting the response from an immunogenic to a tolerogenic one. The positive findings were repeated in a genuine autologous transplant study wherein BM was harvested from arthritic rats by surgical removal of a femur, followed by preparation with myeloablative radiation and intravenous return of their own BM cells [127]. In order to reduce the suffering of the affected animals subsequent studies were then performed using pseudoautologous donors with the same stage of disease severity as the recipients at the time of transplantation.

These same investigators confirmed the effect of syngeneic and pseudoautologous BM transplantation in a different AD rat model—EAE. For the EAE studies, rat spinal cord homogenate (RSCH) mixed with Freund's adjuvant was used [107,128,129]. In the initial studies [129] the conditioning regimen of myeloablative radiation (850–1000 cGy) was begun shortly after the appearance of clinical symptoms and a short period of exacerbated disease occurred with the radiation. It was shown that myeloablative radiation followed by transplantation of syngeneic BM from either nonimmunized or disease affected immunized donors lead to complete remission in most rats. However, a certain percentage of these animals spontaneously relapsed. Less intensive conditioning with a nonmyeloablative regimen was also performed using 750 cGy of total body irradiation (TBI) plus CY. Although complete remissions were achieved in many rats, the rate of spontaneous relapse was much higher than what was observed with the myeloablative treatment. Thus, EAE appeared to be more resistant to the curative effects of syngeneic BMT compared with adjuvant induced arthritis.

Success using syngeneic BMT in treating mice with antigen induced EAE or spontaneously arising SLE (MRL-*lpr*/*lpr*) was also demonstrated

by Karussis and colleagues [130–132]. In their studies of EAE [130,131], transplantations were performed at different time intervals following immunization with mouse spinal cord homogenate (MSCH) and recipients received different preparative regimens. Conditioning was begun before to the onset of clinical symptoms on days +6 or +9 following the first immunization, or alternatively on approximately day +17 (2–3 days following the onset of paralysis). Preparative regimens consisted of high-dose radiation (900–1100 cGy) or single dose CY (300 mg/kg). The results were complicated since mice treated at the early (day +6) or later time points (day +17) demonstrated excellent protection from disease, and were resistant to relapse when later rechallenged with the encephalitogenic agent. However, mice treated on day +9 had delayed onset of severe paralysis by 1 week. The reason for this discrepancy is not clear.

One important result of these studies was the use of high-dose CY as a preparative regimen. Given several prior reports demonstrating the efficacy of CY alone in the treatment of rodent ADs [108–110,133] Karussis *et al*. [130] compared high CY with or without syngeneic BM rescue. Although both treatments were equally effective at ameliorating disease symptoms, survival was superior in the BMT groups. Comparisons of the outcome at the different radiation doses—900 vs. 1100 cGy—showed a superior outcome for the latter group. These investigators extended their studies on syngeneic BMT to the SLE-prone MRL-*lpr*/*lp*r mice [132]. Mice were prepared with either lethal TBI or high-dose CY followed by rescue with either TCD or unmanipulated syngeneic BM. Improved survival and amelioration of serological and pathological evidence of disease occurred in all treatment groups, unlike untreated controls. However, long-term follow-up at >20 weeks post-transplantation revealed significant incidences of relapse. Under both preparative regimens recipients of TCD BM grafts produced superior results than did unmanipulated BM.

Burt *et al*. [134] more recently carried out syngeneic BMT studies using a mouse model of EAE that was induced by adoptive transfer of lymphocytes reactive against a PLP peptide. Mice were treated at two time points—in the acute phase or during the chronic phase (day +14 and +74 post-lymphocyte transfer, respectively). Recipients were conditioned with regimens of myeloablative TBI (1100 cGy), TBI plus methylprednisolone, or fractionated TBI (1200 cGy delivered as 200 cGy over 3 days) plus CY (60 mg/kg) and were rescued with unfractionated BM. Histologic analyses of spinal cords were performed on selected mice. Treatment of mice in the acute phase resulted in clinical improvement and prevention of glial scarring in all syngeneic BMT groups as compared to untreated controls. In contrast, mice treated late in the chronic disease phase showed no clinical evidence of disease amelioration and had significant glial scarring. These investigators also measured *in vitro* proliferative responses to the disease associated PLP peptides, production of IFN-γ in splenocytes and an *in vivo* assay of delayed type hypersensitivity responses to peptide challenge. There was no correlation with the *in vitro* studies and clinical outcome, since responses were similar between clinically affected or nonaffected mice. However, the *in vivo* delayed type hypersensitivity assay was more predictive of clinical outcome. The data were interpreted as showing that success with syngeneic BMT in diseases such as multiple sclerosis will likely depend on the stage of disease at treatment—i.e. BMT may be highly effective when there is minimal chronic tissue damage, whereas late intervention initiated after significant tissue damage has occurred will likely not result in clinical improvement.

Autologous grafts

TCD grafts

It can be argued that removal of preformed autoreactive T cells from a hematopoietic graft will reduce the likelihood of relapse following autotransplantation. Indeed, the report of syngeneic BMT by Karussis *et al*. [132] in the spontaneously arising MRL-*lpr*/*lpr* model demonstrated improved for the TCD vs. non-TCD groups. The outcomes were different in an antigen-induced rat EAE model wherein comparisons were made between pseudoautologous, syngeneic and TCD BM. No differences were observed in inducing remissions and preventing spontaneous relapses among the various graft types [128]. There were, however, significantly higher incidences of spontaneous relapse in rats that received BM plus peripheral lymphoid cells from pseudoautologous donors. Rodent BM differs from human BM and human mobilized peripheral blood, since rodent BM is extracted directly from bone and has little to no contamination with peripheral T cells. Thus extrapolation from these data leads to the conclusion that T-cell depletion or positive selection of $CD34^+$ cells should be performed for humans undergoing HCT with autologous cells.

Purified HSCs

To test the importance of complete depletion of T cells in autologous HCT we have studied the use of purified syngeneic or congenic HSCs in transplantations into prediabetic NOD mice [113]. KTLS HSCs were isolated from mouse BM as described in the Hematopoietic graft types section above. In our studies doses of ≤1000 HSCs were infused. The T-cell content was, therefore, negligible in these grafts given that HSC purification resulted in a >5 log reduction of T cells from BM, and T cells comprised 2–3% of mouse BM. NOD mice with existing islet infiltrates (8 weeks old) were prepared with myeloablative radiation and transplanted with 200–1000 HSCs from Thy1.1 congenic NOD mice (wildtype NOD are Thy1.2). Donor chimerism was verified in the T-cell lineage by Thy1.1 staining. All HSC recipient mice were partial T-cell chimeras, although the absolute number of NOD Thy1.2 was reduced from ~2000/μL to ~500/μL. Despite this treatment, 80% of mice prepared with radiation and rescued with purified NOD.Thy1.1 HSCs developed hyperglycemia within 6 months post-transplant (Fig. 25.3). The age of diabetes onset was not significantly different from untreated NOD mice (p >0.42). Thus, these studies support the data showing that congenic HCT is ineffective in blocking AD pathogenesis, even if the recipients are rescued with highly purified HSCs.

We next asked if NOD T cells that persist following an irradiation-conditioning regimen are, in the absence of lymphocytes derived from a congenic graft, capable of causing islet destruction. To study this question NOD-SCID mice were used as donors. Because HSCs from NOD-SCID mice cannot give rise to T or B lymphocytes, such grafts are incapable of generating pathogenic T cells. Thus, diabetes could only occur if the residual host cells destroyed the islets. Figure 25.4 demonstrates that NOD-SCID HSC engrafted mice still developed diabetes within 6 months post-transplantation despite very low numbers of endogenous T cells. As compared with unmanipulated NOD mice, the age at which NOD-SCID HSC transplanted mice developed diabetes was delayed, but only by ~2 months. NOD-SCID engrafted mice had persistently reduced absolute counts of $CD3^+$ cells in their peripheral blood as compared to unmanipulated mice (Fig. 25.4). At 2 months post-transplantation, the $CD3^+$ cells remained significantly reduced (p <0.001). Thus, even very low numbers of residual NOD T cells are capable of mediating diabetes pathogenesis.

Translation of autologous HCT to clinical practice

Despite the conflicting results in the preclinical literature using autologous HCT to successfully treat ADs, there are currently a number of patient protocols underway testing if high-dose therapy with or without autologous HCT rescue can effectively treat a variety of human AD syndromes (see also Chapter 101) [95,96]. One hypothesis for the

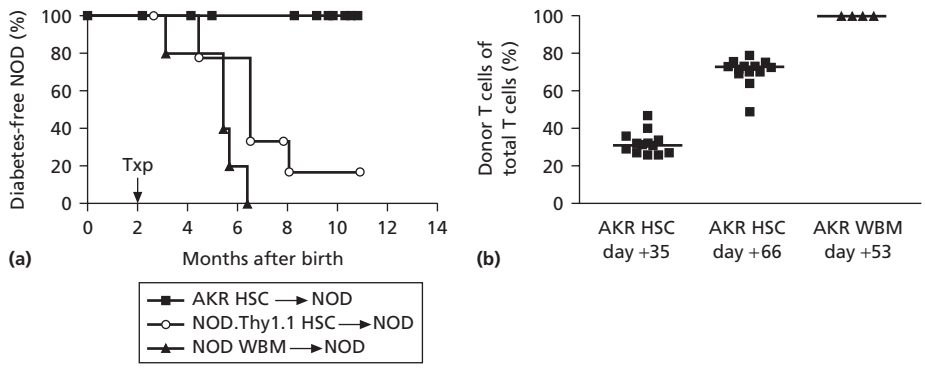

Fig. 25.3 Survival and chimerism in hematopoietic stem cell (HSC) and bone marrow (BM) transplanted mice. Shown in (a) is diabetes onset in nonobese diabetic (NOD) mice that were treated with 950 cGy and 200–1000 NOD.Thy-1.1 congenic HSCs (open circles, $n = 10$), NOD mice treated with 950 cGy plus α-CD4 and α-ASGM1 antibodies and 10^7 NOD syngeneic BM (triangles, $n = 10$), or NOD mice treated with 950 cGy plus α-CD4 and α-ASGM1 antibodies and 10^4 allogeneic AKR HSCs (squares, $n = 15$). Significant differences were noted for AKR HSC transplanted mice vs. mice in all other groups shown (all $p < 0.001$). (b) shows donor T-cell chimerism increased slowly in the blood of NOD mice transplanted with AKR HSC vs. AKR BM. Solid squares represent the percent donor T-cell chimerism as measured in the peripheral blood of individual HSC transplanted mice. The same set of mice were evaluated at 5 and 10 weeks post-HSC transplant, respectively ($n = 13$). These data are compared with AKR BM recipients (triangles; $n = 4$) that were complete T-cell chimeras early post-transplantation.

discrepancies encountered in the animal studies is that antigen induced diseases may be more amenable to treatment with autologous HCT than the spontaneously arising ones. Indeed, all of the reports showing successful treatment with syngeneic HCT have been in animals with antigen induced diseases. Van Bekkum has suggested [9] that antigen induced ADs are the more realistic models of human disease, since their etiology appears to more closely resemble events proposed to induce disease in human counterparts (i.e. exposure to antigens crossreactive to normal tissues). While this latter point is not proved, given the reported successes of autologous HCT in the treatment of rodent ADs, and the more recent results demonstrating positive outcomes in a proportion of patients undergoing the clinical protocols [95,96] (see also Chapter 101), the translation of the autologous HCT approach from the animal studies to clinical practice is not unwarranted.

Allogeneic HCT

In contrast to the conflicting preclinical literature supporting the use of autologous HCT for ADs, several studies have demonstrated that both spontaneous and induced forms of rodent ADs can be successfully treated by allogeneic HCT [7–9]. Indeed, the seminal studies by Morton and Siegel showing success with allogeneic but not syngeneic BMT motivated this area of research [4]. Variability exists among the experiments, including the degree of donor/host genetic disparity, measurements of donor chimerism outcome, the hematopoietic graft type, the preparative regimens and timing of the HCT procedure relative to the onset of disease manifestations. Given these heterogeneities it is, therefore, striking that rodents are consistently cured by an allogeneic HCT approach.

Treatment of advanced stage spontaneous ADs with allogeneic HCT

In 1985, Ikehara and Good began a large series of studies demonstrating that allogeneic BMT could successfully treat AD affected mice with overt clinical symptoms [7,8]. Similar to the prior reports these investigators transplanted mice with spontaneously arising SLE-like syndromes, including MRL-*lpr*/*lpr* (NZBxNZW)F_1 and BXSB strain mice [135,136]. Resolution of disease was achieved even though treatment was initiated at relatively advanced stages of disease. They further showed protection from progressive insulitis and development of diabetes in young NOD mice [137]. Mice in their series were prepared for transplantation with lethal (myeloablative) radiation and in the initial studies recipients were rescued with either TCD BM from wildtype or BM from mice with the *nu*/*nu* defect. Thus, mature T cells were not transferred, but the grafts gave rise to functional T cells. Such grafts were incapable of causing GVHD, but were also limited in their ability to eliminate host T cells (see Graft facilitating cells section below). Most of the studies were performed between MHC-mismatched donor/recipient pairs. Donor chimerism was evaluated by H-2 specific antisera plus complement assays, and uniformly revealed that >90% of spleen cells were of donor type. With the exceptions of the MRL-*lpr*/*lpr* SLE-affected mice [138] and NZB/KN mice that develop a spontaneous inflammatory polyarthritis [139], such allogeneic HCT was uniformly successful at not only blocking disease progression, but also in resolving already established inflammatory lesions [136].

In the case of MRL-*lpr*/*lpr* and NZB/KN mice it was noted that, while HCT resulted in initial reversal of the clinical manifestations and restoration of other immune aberrations) [7,8], the effects of the transplants were transient and mice regularly relapsed after transplantation [136]. H-2 typing of the relapsed MRL-*lpr*/*lpr* mice revealed correlation of relapse with the loss of donor chimerism. The major immunologic defect in MRL-*lpr*/*lpr* is greatly reduced expression of Fas leading to perturbations in lymphocyte apoptosis. These observations by Ikehara and colleagues [138] suggested a high level of engraftment resistance in these mice that the authors attributed to abnormal radioresistant HSCs. In order to enhance engraftment of MHC-mismatched BM, MRL-*lpr*/*lpr* mice underwent an intensified regimen of increased radiation plus the chemotherapeutic agent CY. In addition, MRL-*lpr*/*lpr* recipients received both donor BM infusion plus donor bone grafts [138]. The rationale for the added bone grafting was based upon their prior studies that showed colonization and proliferation of donor BM cells could occur in H-2 matched bone grafts [8]. They concluded that such colonization in H-2 compatible BM stroma may enhance hematopoietic cell engraftment. MRL-*lpr*/*lpr* mice that received this regimen survived long term and were disease free. The principle of simultaneous TCD BM plus bone transplantation from MHC-mismatched donors was also applied to the arthritic NZB/KN mice [139]. The combined transplantations resulted in prevention of joint disease and long-term remissions.

Allogeneic HCT in induced ADS

An important distinction between induced ADs and spontaneous ones is that the latter generally arise in the context of a genetic background with multiple immune system abnormalities, whereas the former are induced in wildtype mice and require purposeful immunizations to break T-cell tolerance. Nonetheless, susceptibility to develop an induced AD also appears to be strain specific, since it has been observed that for any given antigen immunization protocol certain rodent strains are more likely to develop autoimmunity while others are more resistant. Similar to the earlier studies in spontaneously arising ADs, BM transfer experiments were performed from susceptible to resistant rodent strains, and vice versa, in order to identify the cellular elements controlling responsiveness to autoantigens. Therefore, the studies were not designed to examine the curative potential of BMT (i.e. perform BMT after disease induction), rather they focused on disease induction in already established radiation BM chimeras. The majority of investigators reported results similar to those observed for the spontaneously arising ADs, i.e. the BM genotype seemed to determine susceptibility or resistance [9]. Furthermore, there were at least two independent reports from 1981 [140,141] wherein the authors surmised that the mechanism by which the BM genotype exerts its autoreactive or protective effects is at the level of antigen presentation to T cells.

There were, however, notable exceptions to the results demonstrating that BM genotype is the sole determinant of induced AD development. Korngold *et al*. [125] performed experiments in a model of acute EAE wherein MHC-matched hematopoietic chimeras were generated between SJL strain mice that are highly responsive to MSCH and the low responder B10.S strain. Challenge of chimeras with MSCH derived for SJL led to a high incidence of disease in the B10.S into SJL chimeras, but not in the SJL into B10.S mice. The outcome differed if chimeric mice were immunized with MSCH derived from B10.S mice—both B10.S into SJL and SJL into B10.S chimeras developed severe disease. These data suggested that nonhematopoietic factors, such as elements in the central nervous system, control the development of EAE. This same group published a separate study in a relapsing EAE model [124]. These experiments showed that immunization with MSCH derived from third party MHC-disparate BALB/c or B10.S mice resulted in disease in B10.S into SJL chimeras, but not SJL into B10.S chimeras, indicating once again that restriction in the development of EAE involves elements outside the hematopoietic system.

Treatment of advanced stage induced ADs with allogeneic HCT

Success in curing advanced stage induced ADs with allogeneic HCT was reported beginning in the late 1980s. van Bekkum and coworkers established the adjuvant induced arthritis and EAE models in rats and tested the efficacy of MHC-mismatched transplantations in conjunction with the autologous HCT studies described above (see Autologous HCT section and [9]). It was in the arthritis model that equivalence in the effect of syngeneic as compared with allogeneic BMT was first described [126]. Susceptible strain Buffalo rats (MHC designation $RT1A^u$) were lethally irradiated and transplanted with BM from nonsusceptible MHC-mismatched WAG/Rij ($RT1A^i$) or syngeneic BM at either weeks or many months after immunization with the adjuvant (M. tuberculosis). The most effective results were obtained when treatment was initiated shortly after evidence of clinical manifestations 4–7 weeks post-immunization. Animals treated at the later stage had limited recovery with stabilization of disease, but not complete regression. Scarring and permanent joint destruction likely limited the therapeutic effect. Equivalent responses were seen in disease affected recipients of allogeneic or syngeneic BM.

Comparative studies of syngeneic vs. MHC-mismatched allogeneic BMT were then performed in the rat EAE model [107]. RSCH plus adjuvant were used to induce disease and recipients were prepared with lethal radiation or, for some groups of allografted rats, BU plus CY. Syngeneic transplantation was initially effective in inducing complete remissions in all recipients. However, as discussed in an earlier section of this chapter, these results differed from the adjuvant induced arthritis studies, since syngeneic and pseudoautologous recipients demonstrated significantly increased incidences of relapse that occurred spontaneously or following rechallenge with RSCH. In contrast, allografted animals that were given TCD BM or BM from T-cell deficient *nude* rats showed improved outcomes with complete remissions in all animals and markedly reduced spontaneous and induced relapses. The superior outcome of allogeneic HCT was attributed to a subclinical graft-vs.-host reaction, an effect mediated by competent immune cells in the graft against recipient T cells. Turnover of donor CNS cells (perivascular microglial cells) that can potentially function as APCs was measured, and this parameter did not appear to correlate with spontaneous relapse. These EAE studies were extended to the use of largely MHC-matched donor rats using the same preparative regimens [142]. Again allogeneic BMT (unfractionated BM) induced complete remissions and low relapse rates as compared with pseudoautologous recipients. Mixed chimeras were created by using grafts of TCD syngeneic plus TCD allogeneic BM. It was observed that the mixed chimeras relapsed more frequently than the complete chimeras leading the investigators to conclude that clinical protocols for the treatment of multiple sclerosis should be designed for achieving full chimerism. Other studies (see next section), however, do not support the need to convert recipients to full donor type in order to achieve cures of ADs.

Transplantation of purified hematopoietic stem cells

MHC-disparate HSCs

Studies from our laboratory at Stanford University [113] have been directed towards identifying the cells within an allogeneic hematopoietic graft that confer disease protection. To address this question we have examined if grafts composed solely of purified allogeneic KTLS HSCs can block autoimmune pathogenesis in prediabetic NOD mice. Mice were prepared for transplantation with lethal radiation plus antibodies directed against NK and $CD4^+$ cells followed by infusion of MHC-mismatched HSCs. These antibodies were required in the preparative regimen because recipient NOD mice demonstrated high levels of resistance to the allogeneic HSCs, and the antibody treatment reduced this resistance allowing durable engraftment. Figure 25.3 shows that engraftment of purified MHC-mismatched HSCs or BM (not shown) conferred similar protection from diabetes development. Insulitis was also resolved in HSC and BM allografted mice. Of note, although both HSC and BM groups were protected from disease, the pattern of blood chimerism differed (Fig. 25.3). Mice transplanted with purified HSCs remained T-cell chimeras for an extended period of time post-transplantation, whereas BM transplanted animals were complete donor T-cell chimeras only shortly after the time of transplantation. In both groups the other blood cell lineages (B cells, macrophages, granulocytes) were 100% donor derived. The significance of persistent host T cells in animals with spontaneously arising ADs is that these remaining host cells theoretically have the potential for autoreactivity. Evidence that regimen-resistant T cells can, in the absence of an allograft, mediate disease was demonstrated by our studies using NOD-SCID mice as donors (Fig. 25.4). In those studies NOD recipients that underwent myeloablative radiation and rescue with NOD-SCID BM—a congenic graft source that could not contribute lymphocytes to the recovering immune system—developed diabetes. It, therefore, appears that purified allogeneic HSCs alone confer the ability to block autoimmunity and that these grafts have demonstrable effects

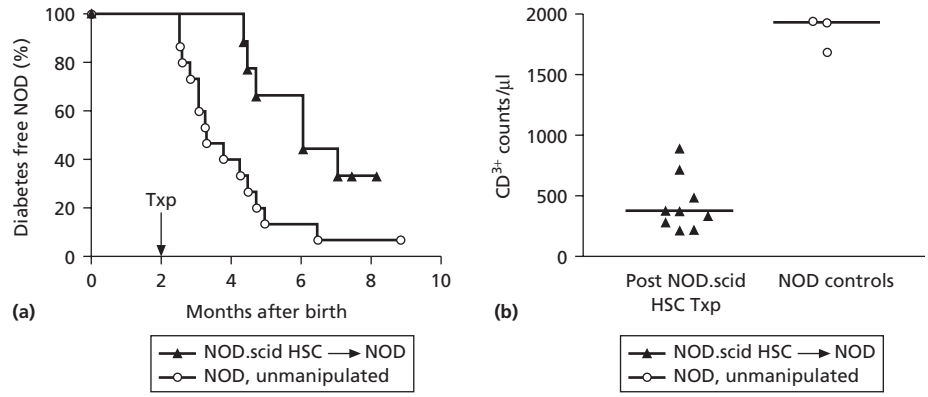

Fig. 25.4 Diabetes-free survival and absolute T-cell levels in nonobese diabetic (NOD) mice following transplantation of non-obese diabetic with severe combined immunodeficiency syndrome (NOD-SCID) hematopoietic stem cells (HSCs). (a) Diabetes onset in NOD mice that were conditioned with 950 cGy and transplanted with 1000 NOD-SCID HSCs (closed triangles, $n = 9$) was compared with untreated NOD control mice (open circles, $n = 15$). Shown in (b) are absolute counts of residual peripheral blood $CD3^+$ cells of NOD mice engrafted with NOD-SCID HSCs that were fourfold reduced even at 2 months post-transplantation (closed triangles, $n = 9$) as compared with unmanipulated NOD mice (open circles, $n = 3$) ($p < 0.001$).

on autoreactive T cells that escape the preparative regimen. This conclusion differs from the one reached by van Gelder *et al*. [142] since, in our hands, complete replacement of donor T cells does not appear to be required to obtain curative benefit from HSC transplantation.

MHC-matched HSCs

The use of purified allogeneic KTLS HSCs to treat prediabetic NOD mice has been extended in our laboratory to the use of donors that are matched at the MHC. Although the NOD H-2 congenic mice became available in 1992 [143], transplantations of MHC-matched BM into prediabetic NOD mice had not been previously done. The experiments were of particular interest because of the well-studied association of the class II MHC of NOD (IA^{g7}) with susceptibility to diabetes. Thus, it might be predicted that MHC-matched HCT would not confer protective effects comparable to the many prior studies using MHC-mismatched hematopoietic sources [99,113,114,123,144–146]. The donors in our studies were C57BL/6 mice congenic for the entire NOD MHC-region, generated by Wakeland and colleagues [147] and designated B6.H-2^{g7}. The donor were therefore matched at class I and class II loci of NOD mice but differed at multiple minor histocompatibility loci. Prediabetic NOD mice were prepared for transplantation with lethal radiation and infused with BM or KTLS HSCs. Unlike the MHC-mismatched HSC studies (see section above) further antibody treatment was not required in the preparative regimens because the genetic barrier was not as severe. Transplantation of either BM or HSCs from B6.H-2^{g7} donors resulted in 100% protection of prediabetic NOD mice from progression to hyperglycemia. Similar to the chimerism levels observed in the transplants of HSC in MHC-mismatched strains the recipients were partial T-cell chimeras with significant residual host T cells remaining. Thus, the MHC matched allograft also demonstrated the capability to modify the activity of residual host autoreactive cells. The extrapolation of these data to clinical transplantation suggests that inocula of purified HSC from matched related or unrelated donors have the possibility to effectively treat ADs. However, it is possible that not all MHC-matched donor/host combinations will be disease protective unless the critical background genes are homologous between mouse and human. The next important step that requires study in preclinical models is the identification of the background genes expressed in hematopoietic lineages that confer protection.

Graft facilitating cells

The studies demonstrating successful amelioration of NOD disease with purified HSCs show that these grafts are sufficient to protect from development of a spontaneous AD. Moreover, HSCs are the only cells that can permanently engraft in a recipient. Thus HSCs are likely the only population that can confer long-term disease protection. However, non-HSC elements in a hematopoietic graft are known to provide significant beneficial (as well as potentially deleterious) effects. From the studies in nonautoimmune strain mice using TCD BM and our studies with KTLS HSCs, it is evident that unmanipulated grafts contain cells capable of aiding or facilitating engraftment of HSCs. The important contribution of mature immune cells in engraftment is well known in clinical HCT, since it has been observed that TCD leads to higher incidences of graft failure [148,149]. Graft facilitating activity has, therefore, been loosely attributed to T cells. Studies aimed at more precisely identifying allograft-facilitating cells have shown that in MHC-mismatched mice the $CD8^+$ fraction of BM contains the majority of graft facilitating activity [120,150,151]. Studies by Weissman and colleagues [120] examined in detail the phenotypic characteristics of the $CD8^+$ BM cells that can facilitate engraftment of purified KTLS HSC. Those studies showed that the $CD8^+$ population was heterogenous in that there were two morphologically distinct populations that could enhance allogeneic HSC engraftment. One population expressed the α/β TCR, and appeared by microscopy to be conventional lymphocytes, whereas the second did not mark for the TCR and appeared morphologically distinct. This second population was larger than conventional T cells with a granular cytoplasm and low nuclear/cytoplasmic ratio. Cotransfer of the $CD8^+$ facilitating cells with HSCs in lethally irradiated mice enhanced survival and chimerism without GVHD. Chimerism studies comparing mice that received HSCs only vs. HSCs plus facilitating cells [120] or unfractionated BM [121] showed significant decrease in radiation resistant host T cells in the latter groups. Thus, the combined effects of grafts composed of HSC plus facilitating populations provided robust engraftment and significant depletion of residual host immune cells. Such engineered grafts should be considered specifically for treatment in clinical AD wherein engraftment of HSCs from an appropriate donor with depletion of host T cells is likely all that is required to achieve the desired outcome.

Nonmyeloablative allogeneic HCT

One limiting factor in treating human ADs with allogeneic HCT is concern about the morbidity and mortality of the high-dose chemotherapy and radiation used in the preparative regimens. In the last 5 years the field of clinical HCT has markedly changed since allogeneic hematopoietic cell engraftment can now be accomplished with nonmyeloablative conditioning regimens [152,153]. Proof that such an approach is feasible for the treatment of ADs has not been widely tested in different animal models. However, a few reports have been published on nonmyeloablative transplantation (with sublethal radiation) in NOD mice. Ildstad and colleagues [154] prepared 8-week-old NOD mice (H-2^{g7}) with titrated doses of radiation and infusion of MHC-mismatched B10.BR (H-2^k) or

B10 (H-2^b) unmanipulated BM. NOD mice are relatively radioresistant and, consistent with this observation, the investigators found that the nonmyeloablative radiation dose, which permitted engraftment of high quantities of BM cells ($6–24 \times 10^8$ cells/kg), was significantly higher in NOD mice as compared to the nonautoimmune strains that were tested (750 vs. 600 cGy). Control mice that receive these doses of radiation without hematopoietic cell rescue, recover blood-forming capacity without support. Engraftment correlated with high BM dose and despite the nonmyeloablative radiation nearly all engrafted mice exhibited high levels of donor chimerism (>95%). All chimeric animals were protected from disease as compared with the 39% that received radiation conditioning but no cell infusion. Insulitis was also attenuated in the chimeras.

We have also performed nonmyeloablative transplants in NOD mice using low-dose radiation (700 cGy) plus grafts of MHC-matched B6.H-2^{g7} unfractionated BM (1×10^7 BM cells). All NOD mice that received this nonmyeloablative treatment engrafted and all were protected from disease development. This regimen resulted in partial chimerism in the T-cell lineage that persisted for an extended period of time (>3 months post-transplantation). In contrast, the other white blood cell lineages converted to near complete donor type within 6 weeks post-transplantation. These studies show that even nonmyeloablative treatment and engraftment of MHC-matched hematopoietic cells can be curative of ADs and that this is a strategy that can be translated directly into clinical practice. Other studies have demonstrated that mixed chimerism, rather than full donor chimerism, is sufficient to protect NOD mice from progression to diabetes [145,155]. However, in those experiments mixed chimerism was achieved by lethal radiation and infusion of grafts that contained both NOD plus donor cells.

Cure of overtly diabetic NOD mice with a combined nonmyeloablative BMT and a donor matched islet graft has been reported [114]. A preparative regimen of sublethal irradiation plus anti-CD40 ligand mAb permitted engraftment with a resultant high level of donor chimerism (>99%) in most mice that received MHC-mismatched (BALB/c) BM. Diabetic chimeric mice were then transplanted with donor matched islets and achieved long-term normoglycemia. The high level of donor chimerism achieved in these studies may be necessary to permit long-term islet allograft acceptance in autoimmune diabetic recipients. We recently showed [113] that the disease outcome was significantly different in diabetic NOD mice that were near full donor chimeras vs. multilineage partial chimeras. NOD mice that received a myeloablative regimen and purified HSCs plus donor type islet allografts were permanently cured of their diabetes, whereas mice that received a nonmyeloablative regimen and developed stable partial chimerism in all white blood cell lineages rejected their donor matched islet grafts after several weeks.

Mechanisms by which allogeneic HCT abrogate autoreactivity

Although many investigators have shown success using allogeneic HCT to block AD pathogenesis, the understanding of the mechanisms that mediate these protective effects is still rudimentary. It is generally thought that allogeneic HCT interrupts AD pathogenesis by a combination of cytoreduction of host immune cells caused by the preparative regimen plus an effect that has been termed graft-vs.-autoimmunity [156]. The latter term refers to the analogous graft-vs.-leukemia effect of allogeneic HCT, wherein the graft mediates elimination of pathogenic cells. This explanation, plus the concept that the allogeneic hematopoietic source replaces a defective HSC leads to a formula that is pervasive in the literature and can be summarized as follows:

$$\text{Elimination of host immune cells} + \text{replacement of defective HSC} = \text{AD cure.}$$

While these two factors clearly play a prominent role in disease protection, the experiments reporting success in treating ADs with transplants of syngeneic, and more recently nonmyeloablative and purified HSC transplants, argue that the formula is overly simplistic. The superior outcomes in allogeneic as compared with autologous models shows that the donor cells, in fact, play a critical role in modifying recipient immune responses. However, the stochastic interactions that occur between a regenerating immune system and the nonhematopoietic factors that drive autoreactivity ultimately determine whether or not immune self-tolerance will be restored. In the broadest of terms it can be surmised that allogeneic HCT demonstrates a higher success rate in curing ADs over autologous HCT because certain hematopoietic specific susceptibility genes have been replaced by donor cells. Furthermore, given the dynamics of immune reactivity, there are likely to be a number of genes or genetic combinations that can favor a protective outcome.

Shifting from the more generalized view to the specific ways allogeneic HCT alters autoreactivity leads to a focus directed towards understanding the effects of the procedure on T cells. T lymphocytes are the primary mediators of pathogenic AD responses. It is therefore logical to conclude that the allogeneic HCT results in changes in the T-cell repertoire and/or T-cell reactivity. The mechanisms of T-cell tolerance described (see Control of immune reactivity section above and Chapter 24) that HCT grafts may affect include: (i) deletion of pathogenic T-cell clones; (ii) alteration in the threshold of reactivity in pathogenic T-cell clones as occurs in the induction of anergy; (iii) skewing of the T-cell response from a TH1 to TH2 type response; and (iv) the emergence of regulatory cells that suppress autoreactive cells.

Depletion of host T cells

Depletion of host T cells is the most extensively studied of the mechanisms by which allogeneic HCT can alter recipient immune function. The advances in antibody based technology has made the measurement of donor-vs.-host T cells accessible to perform. That said, it should be noted that many of the publications on the topic of HCT for the treatment of ADs predates the MAB era and, thus, T-cell chimerism was not directly assessed. There was an apparent assumption in the early reports that if animals survived lethal irradiation with BMT, conversion to donor type must have occurred. In the subsequent studies, particularly by Ikehara and colleagues [123,135–139] wherein chimerism of the blood or spleen was assessed, the data consistently showed chimerism levels of >90%. However, lineage subset analyses were not performed. From more recent studies, including our experiments using purified HSCs [113,121] (Fig. 25.4), clinical studies examining chimerism in patients of TCD grafts [157], and analyses of chimerism following nonmyeloablative transplantation [158], it is evident that the T-cell lineage is the most resistant lineage to conversion to donor type. Furthermore, HSCs and T-cell deficient BM lack graft facilitating populations that mediate elimination of residual host immune cell populations. Transplantation of allogeneic unfractionated BM into lethally irradiated mice results in near complete conversion to donor type shortly after transplantation, whereas HSC transplantation consistently results in partial T-cell chimerism which persists for many months post-transplantation [121,159]. Patients that have received TCD BM also demonstrate long-term mixed chimerism [157,160,161]. Thus, it is reasonable to assume that in the many experiments wherein T chimerism was not assessed, but in which TCD or BM from *nude* mice was used as the graft source, it is highly probably that those autoimmune prone recipients remained partial T-cell chimeras for extended periods of time post-transplantation. Complete deletion of recipient T cells therefore is not a requirement to achieve protection from ADs following allogeneic HCT.

Evidence that even in the absence of a strong graft-vs.-autoimmunity effect (i.e. the graft does not eliminate the host T cells) the donor

hematopoietic elements nonetheless modify residual autoreactive cells comes from comparing the results of the experiments in NOD mice using purified allogeneic vs. congenic NOD-SCID HSC grafts (Figs 25.3 & 25.4) [113]. In those experiments the recipients were prepared in an identical manner and following transplantation both groups had similar levels of surviving NOD T cells in the peripheral blood. HSC from NOD-SCID donors cannot contribute T cells to the regenerating immune system, yet recipients engrafted with NOD-SCID HSCs developed diabetes suggesting that the surviving recipient immune cells destroyed the islet tissue. In contrast, mice engrafted with allogeneic HSCs had comparable levels of surviving NOD T cells yet diabetes did not develop and insulitis was reversed.

Although complete depletion of host T cells is not required for disease protection, it is still possible that hematopoietic allografts selectively mediate deletion of autoreactive cells by negative selection. Negative selection is the process that eliminates developing T cells in the thymus whose TCR binds self-antigens too avidly. Although negative selection is thought to apply primarily to the immature T cells in the thymus, this process can also affect peripheral post-thymic cells [162]. BM derived APCs are the most efficient mediators of negative selection. It is virtually impossible to demonstrate directly negative selection of T cells for any particular self-antigen because, generally speaking, antigen specific T cells are too few in number to detect. However, this process can be measured for a class of nonconventional antigens called superantigens (reviewed in [163]). Superantigens are viral or bacterial proteins that bind tightly to both MHC class II molecules and a region of the TCR called the variable region of the β chain (*V*β). T cells can be divided into identifiable subsets (by MAB staining) based on this *V*β segment of their receptor. Some superantigens exist as stable endogenous genes in mice of certain strains. The superantigens induce exceptionally strong T-cell responses and T cells bearing the *V*β segment specific for the antigen die by apoptosis resulting in near complete elimination of all cells that are of the responding *V*β subclass. In mice that express endogenous superantigens, the T cells with receptors that have the *V*β segment capable of reacting to these "self-antigens" are deleted by negative selection. Thus, tracking T cells based upon *V*β staining in experimental systems in which exposure to superantigens can be manipulated (such as in BM chimera) serves as a surrogate assay for assessing negative selection.

We followed the fate of superantigen reactive cells in the NOD mice transplanted with purified allogeneic HSCs in order to determine if HSC grafts can mediate negative T-cell selection in these animals [113]. When NOD mice were engrafted with HSCs from a donor mouse strain (AKR/J) which expressed a particular superantigen different from NOD, all of the T cells expressing the *V*β segment ($V\beta_6$) capable of binding the superantigen were absent from the blood of the chimeras [113]. Of particular interest was the *V*β subset analysis of the NOD type cells. Since NOD mice do not normally delete $V\beta_6$ cells, the finding that $V\beta_6$ cells were absent in the blood of chimeras demonstrated that HSC grafts not only mediated the deletion of developing T cells, the grafts also caused the deletion of potentially self-reactive mature post-thymic T cells of NOD origin. Staining of all the different *V*β subsets in the blood of the chimeras revealed that only those *V*β subsets with the potential to bind the discrepant superantigen were deleted in the chimeras. These data, therefore, show that HSC allografts can selectively mediate deletion of potentially autoreactive cells by negative selection.

Replacement of susceptibility genes of the host

The assumption that one fundamental mechanism by which HCT allografts confer protection from autoimmunity is by replacement of susceptibility genes expressed in hematopoietic cells provides the impetus to characterize these genes. The only AD susceptibility genes identified with certainty in mouse and human are specific alleles of MHC molecules [164–166]. Experiments that directly addressed the significance of adding or replacing the MHC susceptibility gene were done by using transgenic technology or genetic approaches in NOD mice. Nishimoto *et al*. [164] and, later, others [167–169] showed that expression of non-NOD MHC class II transgenes (driven by MHC promoter regions) provided a high degree of protection from insulitis and diabetes. Later, Wicker and colleagues [66,143,170] generated by genetic outcrosses NOD mice that were congenic for non-NOD MHC products and formally showed that replacement of the NOD MHC resulted in failure to develop diabetes. The fact that many of the preclinical studies demonstrating successful treatment of advanced ADs with allogeneic HCT have been done with MHC-mismatched BM raises the very important issue of whether or not similar consistently positive outcomes will be seen when AD affected humans are treated by this approach using HLA-compatible HCT. We [113] and others [4,116,142], have shown that transplantation of MHC-matched HCT can confer similar levels of protection in preclinical models. However, more recent studies from our laboratory in Stanford have revealed that in a model of antigen induced EAE, affected mice prepared in an identical manner were consistently cured of their disease with MHC-mismatched, but not MHC-matched HCT.

There is a vast spectrum of non-MHC genes expressed in hematopoietic cells that can potentially alter the course of autoreactivity. Their identity is not yet known. These genes include those that affect overall immunoreactivity such as cytokines, cell proliferation, lymphocyte or APC cell activation and apoptosis. Another class of candidate genes includes those that affect antigen presentation and recognition. While identification of protective alleles is a formidable challenge, the continued advances in basic sciences such as gene expression profiling by microarrays, sequencing of the mouse and human genome, and other technologies, will aid the determination of these genes. One approach that we have taken is to obtain a series of NOD mice that are congenic at non-MHC gene regions. Although the genetic background in these animals is derived from the NOD (including the MHC) many of these strains do not develop diabetes. We have initiated a series of studies wherein these nondiabetes prone mice serve as HCT donors for prediabetic NOD recipients. We predict that some of the resultant chimeras will demonstrate protection from disease, which will direct us to the genes expressed by donor hematopoietic cells capable of mediating diabetes protective effects to the recipients.

Anergy, subset skewing and regulatory cells

Mechanisms of T-cell tolerance, which include the induction of anergy, skewing of T-cell reactivity to favor nonpathogenic subtypes and/or the emergence of predominant regulatory subsets, have not been studied in the context HCT in the treatment of ADs. Part of the reason for the paucity of data in this area relates to the difficulty in isolating and identifying pathogenic clones that can be followed before and after HCT. The identity of autoantigens has not been achieved with certainty in the spontaneously arising animal ADs, further confounding these types of mechanistic analyses. However, it has been possible to clone autoreactive T cells from animals with spontaneous or antigen induced diseases and adoptively transfer the clones to immune deficient recipients that develop the autoimmune phenotype. The extension of this technology has allowed the generation of transgenic animals that produce a predominant lymphocyte clone with a defined receptor type that develops spontaneous AD. Although these latter two types of animal models are somewhat artificial, they can be used to track the fate and function of autoreactive cells following HCT. Use of these models for this purpose has not been reported to date. Similarly, the emergence of regulatory subsets has not been reported in transplanted AD prone chimeras. In preliminary

studies from our laboratory we found no difference in relative numbers of peripheral $CD4^+CD25^+$ cells in unmanipulated NOD mice as compared with allogeneic hematopoietic chimeras. Clearly, these are areas of research that will be explored in the near future that are likely to provide further insights to how allogeneic HCT mediate AD protective effects.

Conclusions

The knowledge that autologous and allogeneic HCT results in profound alterations in immune reactivity has been present in the scientific and clinical literature for decades. The animal studies have provided the fundamental basis for understanding both the negative effects as well as the potential beneficial effects that HCT can provide. As summarized in this chapter, there have been numerous studies applying the use of HCT to treat ADs that form the platform for clinical protocols. We believe that these animal models will continue to provide guidelines for translation to clinical practice but, more importantly, they will continue to provide insight into how and why HCT exerts its immune altering effects.

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32

Stephanie J. Lee

Outcomes Research in Hematopoietic Cell Transplantation

Introduction

The field of hematopoietic cell transplantation (HCT) has grown dramatically since the first successful allogeneic transplant procedure was performed in 1968. However, as the subspecialty has matured, questions about costs and cost-effectiveness, quality of life (QOL), patient preferences, medical decision making and aggregation of different data sources to guide treatment decisions have become relevant. These questions are best addressed by "outcomes" research, a field of study focused on obtaining the best results, broadly defined, given the available medical knowledge and limited healthcare resources. A closely related discipline, health services research, is concerned with social and political determinants of outcome such as access to health care and quality of care. A chapter on outcomes research is new to the third edition of this book, and the research methods employed to study these issues may be new to many readers.

There are several features of HCT that make outcomes studies especially relevant: (i) HCT involves high treatment-related risks compared to other medical interventions; (ii) significant practice variation exists; (iii) costs are high; and (iv) the long-term results, considering both disease-free survival (DFS) and QOL, have much room for improvement. Issues of medical decision making, quality of care, resource allocation and QOL are material to HCT, and all fall under the rubric of outcomes and health services research.

On the other hand, several characteristics of HCT make outcomes studies challenging. For example, HCT patients are often not represented in large, administrative databases that collect standardized clinical, outcome and resource utilization data. The most active transplant centers still perform only several hundred procedures per year, and smaller centers may do fewer than 10. Thus, the overall impact of HCT on the health of the general population and health care finances is relatively small. Also, the field is changing rapidly, and adequate information on long-term results is available for few diseases and procedures.

This chapter will present a brief history of outcomes research in American medicine to help frame the research topics and methods. The remainder of the chapter is organized around several specific questions in HCT that outcomes research is well-suited to answer. Representative HCT studies are used whenever possible to illustrate the principles discussed. Readers are referred to Chapter 31 (Biostatistical Methods in Hematopoietic Cell Transplantation), Chapter 39 (Assessment of Quality of Life in Hematopoietic Cell Transplantation Recipients) and Chapter 49 (Hematopoietic Cell Donor Registries) for detailed information on related research methods and data sources.

Definition

It is difficult to state a precise definition of "outcomes research." At some level, all results can be considered outcomes; thus, most scientific investigation is concerned with measurement and interpretation of outcomes. However, generally excluded from the definition of "outcomes research" are phase I, II and III clinical studies addressing efficacy questions when the primary endpoints are toxicity, disease control and survival. Similarly excluded are clinical epidemiology studies that describe an institutional experience with a disease or treatment. However, when the research question begins to consider how well a treatment works outside of a clinical trial or institutional setting ("effectiveness"), subjective endpoints (e.g. QOL, patient preferences), nonbiologic influences on outcomes (e.g. access, quality of care, physician–patient communication, medical decision making), health care policy (e.g. economic evaluation), or aggregation of data from multiple sources (e.g. decision analysis, meta-analysis), then the title of "outcomes research" may be legitimately applied. A conceptual framework that distinguishes outcomes research from other types of clinical research is presented in Fig. 32.1 [1].

Major questions for outcomes research in HCT:

- What are the costs of HCT and how can they be reduced? (Resource utilization, cost minimization.)
- Are the clinical benefits of HCT worth the monetary cost? (Cost-benefit, cost-effectiveness, cost-utility analysis.)
- How can one combine knowledge available from several different data sources to reach broader conclusions about HCT than are possible from any one study? (Registry studies, decision analysis, quality time without symptoms of toxicity [Q-TWiST], meta-analysis, evidence-based medicine.)
- What is the patient's experience with HCT? (Qualitative research, QOL.)
- How are new tools to measure subjective or clinical endpoints developed? (Instrument development, scale development.)
- How can the practice of HCT be improved through health services research? (Access, quality of care.)

History

The ultimate goal of outcomes research is to improve the practice of medicine through the provision of data about the effectiveness, costs, risks and benefits of treatment options, incorporating both individual and societal level considerations [2,3]. Approaches to achieve this goal in the USA have varied over the decades. Initially, it seemed that funding large-scale research projects would help establish which clinical practices worked and which did not, so that effective practices could be promulgated.

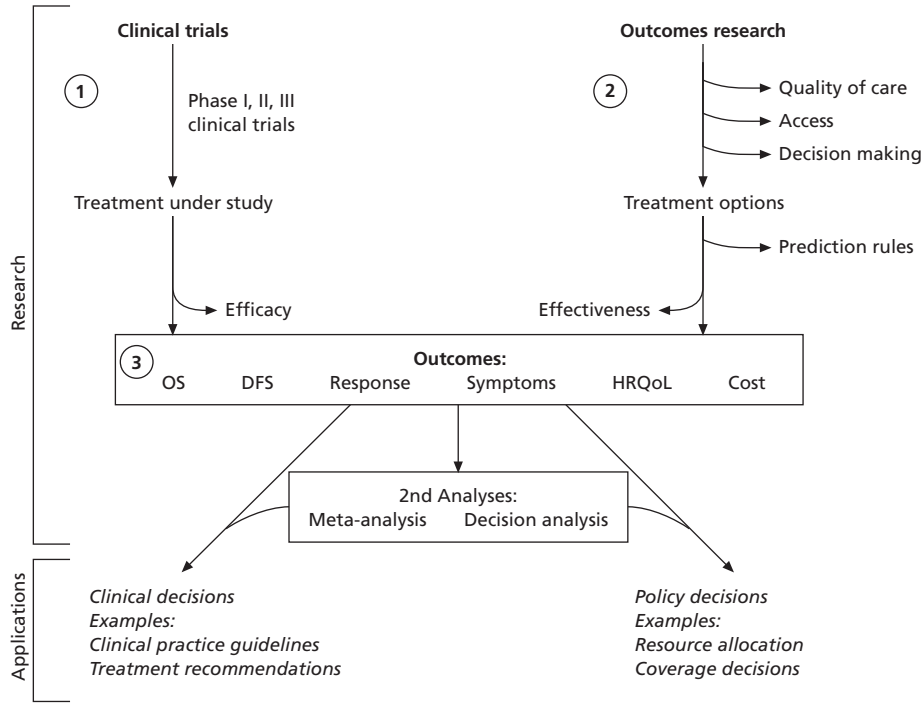


Fig. 32.1 Conceptual framework. Interaction is shown between research topics, end points, analytic techniques, and applications in defining outcomes research. In **1** are depicted the classic clinical trials and analytic techniques that are not outcomes research. **2** shows the study topics, end points and analytic techniques that are considered to be outcomes research. Outcomes depicted in **3** may or may not constitute outcomes research, depending on the context. For example, overall survival as measured in a phase III trial is not an outcomes study (efficacy), whereas it is if observed in a large community cohort (effectiveness). Symptoms have both efficacy and outcomes influences. Applications are indicated in *italic* and may emanate from either clinical trials or outcomes research. DFS, disease-free survival; HRQOL, health-related quality of life; OS, overall survival. Reproduced with permission from Lee *et al*. [1].

However, this attempt was soon followed by a realization that definitive conclusions about the most effective therapies were elusive because there were so many diverse factors (patient characteristics, patient preferences and societal priorities) to consider. More recently, outcomes researchers recognized that, while it is important to know what works at a population level and to establish treatment guidelines, scientific methods to incorporate patient values and individualize treatment are also important. This more encompassing view of the situation recognizes the complexity of medical decisions and the often contradictory influences affecting patient outcomes.

The "father" of the American outcomes movement was a surgeon named Ernst Codman who, as early as 1914, argued that the quality of hospitals could be judged only if procedure success rates were made available on a routine basis [4]. He advocated standardized measures of outcomes so that different institutions could be compared on a level playing field. In 1966, Avides Donabedian reintroduced the term "outcomes" when he developed his concept of quality assessment and its three components: *structure*, *process* and *outcome*. He broadened the endpoints of interest: "Although some outcomes are generally unmistakable and easy to measure (death, for example), other outcomes, not so clearly defined, can be difficult to measure. These include patient attitudes and satisfactions, social restoration, and physical disability, and rehabilitation." He echoed Codman in stating "Outcomes, by and large, remain the ultimate validators of the effectiveness and quality of medical care" [5].

In the 1970s and 1980s the need to understand medical outcomes reached political prominence because of its association with health care costs. Archie Cochrane, after whom the Cochrane evidence-based database is named, warned that the medical system would become bankrupt if expensive technologies were routinely applied without evidence of benefit [6]. In 1973, Wennberg and Gittelsohn documented surprising geographic variation in resource utilization, expenditures and rates of hospitalization and procedures [7]. For example, rates of tonsillectomy varied dramatically within the state of Vermont without seeming to influence health outcomes. This observation focused attention on practice variation and the possible savings that could be realized by eliminating unnecessary procedures.

Several national databases were established to study practice variation in the USA. These included the Patterns of Care Study (focusing on radiation therapy practices) [8,9], the National Cancer Data Base (focusing on surgical practice) [10] and the linkage of Medicare billing and Surveillance, Epidemiology and End Results (SEER) data (providing resource utilization and cancer-specific information on patients common to these databases) [11]. In addition, the Federal government entered the fray directly by establishing the Agency for Health Care Research and Quality (AHRQ). Although this organization has been renamed and refocused several times, it is probably most famous for developing the Patient Outcomes Assessment Research Teams (PORTs). The goal of this funding mechanism was to support large teams investigating the effectiveness of treatments for common diseases.

Discourse in the medical journals throughout this period reflected the outcomes movement. In 1988, Arnold Relman labeled "assessment and accountability" the "third revolution in medical care," following the earlier revolutions of health care expansion and the backlash of cost containment [12]. In 1990, Arnold Epstein further defined the "outcomes movement" as research efforts to address "the effectiveness of different interventions, the use of this information to make possible better decision making by physicians and patients, and the development of standards to guide physicians and aid third-party payers in optimizing the use of resources" [13].

The 1990s were a period of national economic growth in the USA, and concerns about health care financing for specific procedures faded into the background behind debate about the overall structure of health care coverage. Managed care and health maintenance organizations thrived, and physicians practiced in a more constrained setting with new concerns about financial risk. The incentive to save money may have replaced the original goal of outcomes research, which is to spend money wisely to improve overall health. Treatment guidelines proliferated in the late 1990s but these efforts grew out of a desire to standardize physician practice to improve patient outcomes rather than to contain costs.

It is difficult to tell what the future holds for outcomes research, especially in HCT. HCT is a highly specialized practice that for many will fall outside of health care policy and economic considerations. Nevertheless,

there are many aspects of HCT that may make outcomes research more relevant. Attention to long-term outcomes, patient decision making and guidance based on what is known about short-term outcomes is especially critical for the field.

Specific questions for the field of HCT

What are the costs of HCT, and how can they be reduced?

On a per patient basis, the costs of HCT are high relative to other available medical interventions, ranging from approximately $30,000 for an uncomplicated autologous procedure to $200,000 for an allogeneic, myeloablative procedure using an unrelated donor [14–16]. (For information regarding charges for the different HCT procedures, see Table 34.1 in Chapter 34.) The investment in infrastructure is immense, requiring support of the transplant centers and national resources such as the National Marrow Donor Program. In the USA, insurance companies have tried to limit access to some HCT procedures by designating them "experimental," but are often forced by state law or by threat of patient lawsuits to acquiesce and finance the procedures. When it comes to HCT, society has shown itself to be quite willing to follow "the rule of rescue," defined as the human imperative to help those facing an otherwise tragic death without regard for the resources consumed or the ultimate likelihood of success.

Well-established research methods are available for quantifying monetary costs [17–19]. "Direct medical" cost refers to the monetary value of goods and services provided. These costs are usually captured through administrative billing systems or other itemized methods of determining resource utilization. Units of goods and services are then converted to charges or costs. The distinction between "charges" and "costs" is important in the USA. Charges are the amount billed to the patient or insurance company, and are almost always higher than costs. In contrast, costs should reflect the actual resources needed to provide a service and are usually lower than charges. Because health care organizations offset one expense against another, and usually include some amount of profit, charges are not directly linked to the resources needed to provide a service.

Outcomes studies favor the use of costs since they reflect the actual resources expended. When costs are not available directly, conversion between charges and costs is determined by "ratios of costs to charges" (RCCs), a fraction recalculated on an annual basis. Institutions often aggregate logical groups such as clinical departments when setting their RCCs. Fixed costs (such as physical space, personnel) and variable costs (supplies) are totaled and divided by the amount billed by the department during the same period of time.

Direct *nonmedical* costs are expenditures related to health care, but not directly used for goods and services (e.g. transportation to the hospital, hotel charges for family members accompanying patients). These costs have proven much more difficult to quantify since they must be captured directly from patients through cost diaries or receipts. *Indirect* nonmedical costs are even harder to quantify and include time off work and the loss of future earnings. Which costs to include depends on the perspective of the analysis. The "societal" perspective includes all costs to the system regardless of who pays. Other perspectives can be imagined, such as the hospital, insurance company or patient, and would include costs borne by that payer.

One important feature of costs is that they vary by year because of inflation. Thus, it is important to consider the year in which the study was performed and the specific items that are included in the analysis. For example, health care inflation is calculated from a "basket" of goods similar to the methodology used for the Consumer Price Index, and at 3–9% has outpaced general inflation. These conversion factors, published by the Bureau of Labor Statistics by month and year, help to "inflation-adjust" costs and allow comparability of studies performed at different times (http://stats.bls.gov). "Discounting" is distinct from inflation-adjustment, and is normally set at 3% per annum. Discounting reflects the fact that costs and benefits in the future are valued less than those that are immediately available, and allows conversion of future dollars or future improvements in health to their current value.

Once the relevant costs are captured, they can be analyzed in a variety of ways. Some studies simply report the costs of an intervention. Others look for patterns of costs, predictors of costs or ways to decrease costs. Totals, breakdowns by specific categories, trends over time and association with clinical characteristics or treatments have all been reported in HCT. For example, several studies have evaluated the costs or lengths of stay associated with specific complications or patient characteristics [20–22].

Cost-minimization studies compare the costs of treatment approaches that result in similar clinical patient outcomes. In these cases, adoption of the least costly approach does not compromise patient outcomes. Table 32.1 shows some examples of cost-minimization studies in HCT [15,23–32].

Are the clinical benefits of HCT worth the monetary costs?

Deciding whether the clinical benefits of HCT are worth the monetary costs may seem to conflict with a physician's duty as a patient's advocate. However, in a society where health care dollars are constrained, spending money for one person's procedure ultimately means that another person may not receive some necessary treatment. As discussed below, the various forms of economic analysis (cost-benefit, cost-effectiveness and cost-utility) differ primarily in how they quantify clinical benefits.

Table 32.1 Examples of cost-minimization studies.

Ref.	Less costly approach	More costly approach
[15,23,24]	Peripheral blood progenitor cells for autologous HCT	Bone marrow
[25]	Delayed growth factor support in autologous HCT	Early growth factor support
[26]	Growth factor support in T-cell-depleted BMT	No growth factor support
[27]	Acute GVHD prophylaxis with T-cell depletion in unrelated donor BMT	Methotrexate, cyclosporine
[28–31]	Outpatient transplantation	Inpatient transplantation
[32]	Hyperhydration to prevent hemorrhagic cystitis after cyclophosphamide conditioning	Mesna

BMT, bone marrow transplantation; GVHD, graft-vs.-host disease; HCT, hematopoietic cell transplantation.

Table 32.2 Economic analyses.

Type	Equation	Illustration	Conclusion
Cost–benefit	Cost of providing treatment ($) minus benefits of treatment ($)	For treatment X: $100,000–0.50*$1,000,000 = –$400,000 For treatment Y: $50,000–0.30*$1,000,000 = –$250,000	Treatment X is the preferred approach, although society should support both treatments because they "save" money
Cost-effectiveness	(Cost of X minus cost of Y)/ (benefit of X minus benefit of Y)	($100,000–$50,000)/ (0.5 lives–0.3 lives)*20 years = $12,500/LY	Treatment X is cost-effective relative to other well-accepted medical procedures
Cost-utility	Same numerator as cost-effectiveness but denominator is (quality-adjusted benefit of X effectiveness quality-adjusted benefit of Y)	($100,000–$50,000)/(0.5 lives*0.85– 0.3 lives*1.0)*20 yrs = $20,000/QALY	Quality-adjustment raises the cost/effectiveness ratio of treatment X but it is still very favorable

For illustration purposes, consider treatment X and treatment Y. Treatment X costs on average $100,000 per patient but cures 50% of patients, while Treatment Y costs $50,000 and cures 30%. Survivors live for another 20 years. However, patients undergoing treatment X suffer from long-term complications, so that their utility is 0.85 compared to patients undergoing treatment Y who have a utility of 1.0. Separate studies suggest that for the purposes of cost–benefit analysis, a life saved through medical intervention is worth $500,000–$1,000,000 [33–35]. See text for details.
LY, life year; QALY, quality-adjusted life year.

However, all are designed to provide information that may be used by policy makers to allocate resources and maximize the health and welfare of the entire population. Table 32.2 contrasts the types of economic analyses [33–35].

Cost–benefit analysis requires that clinical benefits be converted into monetary values to determine the net financial impact of an intervention. This is sometimes straightforward (inexpensive prophylactic antibiotics may prevent costly infections later) but is often quite complicated and fraught with unpalatable value judgements. For example, what is the economic value of a life extended or saved? Attempts to use income as a surrogate lead to the uncomfortable conclusion that the lives of high-wage earners are more valuable than homemakers or retired people [17]. Placing monetary values on goods that are not normally for sale (such as medical procedures or health) can be performed by creating a hypothetical market for that good ("contingent valuation"), but the amounts derived from such market exercises have been questioned because they seem too high [36]. As a consequence, cost–benefit analysis is rarely performed in health care; it is much more common in business and environmental applications.

Cost-effectiveness analysis avoids such value judgements by calculating a "cost/effectiveness ratio" expressed as dollars per unit of clinical benefit [18]. To facilitate comparison across interventions, clinical benefit is usually measured in years of life gained (life-years, or LYs), but may be any clinically recognized unit of benefit (e.g. cases of acute graft-vs.-host disease [GVHD] prevented, days of hospitalization, lives saved). Cost/effectiveness ratios are by definition comparisons of one treatment approach vs. another (which may be "no treatment") since they are calculated as:

(cost of treatment X minus cost of treatment Y)/(benefit of X minus benefit of Y).

When several possible treatment options are available, one or more may be "dominated" (found to be both more costly and less effective than another option) and eliminated from further consideration. Because cost-effectiveness analysis are intended for policy makers, it is important to specify the perspective (e.g. government program, hospital, health plan, etc.) and time horizon (e.g. 1 year, 100 years, etc.) of the analysis in order to reflect which costs and benefits were included. The strategy most cost-effective for a health maintenance organization may not be the one that is best for a hospital, patient or society.

Many people use the term "cost-effectiveness analysis" and "cost-utility analysis" interchangeably. However, a *cost-utility analysis* specifically incorporates QOL considerations and usually has a denominator of quality-adjusted life years (QALYs). In these analyses, survival time is adjusted for the QOL associated with that survival. For example, for some people, a year of life in good health may be worth several years in poor health. These adjustment factors are called "patient utilities" and usually range from 0 (equivalent to being dead) to 1.0 (the year of life is fully valued). A utility less than 0 represents a health state worse than death. Utilities fulfill the mathematical condition of linearity so that one year of perfect health is considered equal in value to two years of life with a utility of 0.5. For example, some quoted patient utilities for health states are 0.98 for suffering the side-effects of beta-blockers [37], 0.8 when 1 year after autologous transplantation for non-Hodgkin's lymphoma (NHL) [38] and 0.5 following a stroke [39–41].

Patient utilities may be assessed using several techniques: standard gamble, time trade-off and multiattribute utility theory. *Standard gambles* ask people what risk of death they would accept to reach perfect health, with one minus risk of death equal to patient utility. For example, if a patient is willing to assume a 15% chance of death to reach perfect health, then the utility of their current, compromised health state is 0.85 (1.0 minus 15%). Assessment of patient utilities by standard gamble is limited by people's ability to consider life and death risks hypothetically and rationally. However, the decision to undergo HCT is very much like a standard gamble. Patients may either opt for best supportive care or standard chemotherapy, or they accept some chance of treatment-related mortality from the transplant procedure in order to cure their diseases. *Time trade-off* questions ask people how much life expectancy they would trade for perfect health in their remaining time, with utility equal to time in perfect health divided by time in current compromised state of health. For example, let's assume that a patient has a life expectancy of 10 years, but has a painful, debilitating disease. If that patient was willing to trade-off (i.e. give up) 1.5 years of life so that the remaining 8.5 years would be in perfect health, his utility would be 8.5/10 or 0.85. Similar to utility assessment, time trade-off questions require people to consider hypothetical scenarios in which perfect health is guaranteed, but at a cost of some decrease in life expectancy. *Multiattribute utility theory* calculates utilities from QOL or functional status data. The conversion equations are derived from studies in which subjects complete validated surveys and have their utilities assessed by standard gamble or time

Table 32.3 Examples of cost-effectiveness and cost-utility studies in hematopoietic cell transplantation (HCT) and medicine.

Ref.	Year of pub.	Treatment	Alternative	Cost/effectiveness ratio
[42]	1989	Allogeneic BMT for AML	Conventional chemotherapy	$10,000/LY
[43]	1992	Autologous BMT for HD in 2nd CR	Conventional chemotherapy	$26,000/LY
[44]	1997	Autologous BMT for relapsed NHL	Conventional chemotherapy	$9,200/LY
[16]	1998	Unrelated donor BMT for stable phase CML	IFN-α	$51,800/QALY
[45]	1999	Second allogeneic transplantation after relapse of acute leukemia	Conventional chemotherapy	$52,000/LY
[46]	2001	Autologous PBSCT for MM	Conventional chemotherapy	$23,300/LY*
[47]	1989	Smoking cessation program	No intervention	$1,300/LY
[48]	1987	Hemodialysis	No dialysis	$50,000/LY
[49]	1990	Captopril for hypertension	No therapy	$72,000/LY

*£0.64 = $1.00

AML, acute myeloid leukemia; BMT, bone marrow transplantation; CML, chronic myeloid leukemia; CR, complete remission; HD, Hodgkin's disease; IFN, interferon; LY, life year; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; PBSCT, peripheral blood stem cell transplantation; QALY, quality-adjusted life year.

trade-off at the same time. Use of the equations allows utility estimates to be based on patient self-reported data without the need for interviewers to administer standard gamble and time trade-off questions.

What makes cost-effectiveness and cost-utility analyses powerful is that diverse interventions may be compared and selected for their ability to provide maximal health benefit for money spent. For example, if health care dollars are limited, these methods allow some rational basis for recommending whether society should routinely provide coverage for a patient with acute myeloid leukemia (AML) in third complete remission scheduled for an unrelated donor HCT, or instead cover patients with congestive heart failure and severe diabetes who need heart transplants. Similarly, one can compare the health care gain of one high-cost treatment procedure, such as an autologous HCT for relapsed NHL, vs. the preventive strategy of providing statin therapy to several middle-age men. Based on the cost/effectiveness ratio for hemodialysis, a procedure covered separately by the federally funded Medicare program and thus available to all people, an acceptable cost/effectiveness ratio of <$50,000 per QALY has been proposed. A ratio >$100,000 is questionable because that money applied elsewhere may buy better health for the population. A ratio between $50,000 and $100,000 per QALY is in the gray zone, as we found for unrelated donor transplantation for stable phase chronic myeloid leukemia (CML) compared to interferon-alpha (IFN-α) therapy [16]. Some countries, such as Australia and Canada, require information on cost-effectiveness prior to drug approval. Table 32.3 shows examples of published cost-effectiveness and cost-utility studies in HCT and some comparisons in other fields [42–49]. League tables have been published to help put cost-effectiveness [50,51] and cost-utility ratios [52,53] into perspective. Online resources such as http://www.hsph.harvard.edu/organizations/hcra/cuadatabase contain comprehensive lists of cost analyses and patient utilities that may be downloaded [54].

How can one combine knowledge available from several different data sources to reach broader conclusions about HCT than are possible from any one study?

Registry studies

Several large transplant registries were established to provide national and international information on the outcomes of HCT. These include the International Bone Marrow Transplant Registry (IBMTR, http://www.ibmtr.org), the National Marrow Donor Program (NMDP, http://www.nmdp.org), the European Group for Blood and Marrow Transplantation Registry (EBMT Registry, http://www.ebmt.org) and Eurocord. These registries collect, computerize and make available data for analyses. While they suffer from limitations common to registries, including incomplete capture of all procedures, problems with data standardization, validation issues and difficulty obtaining detailed clinical information, they also provide the only means for determining the effectiveness of HCT as practiced outside of clinical trials and single institutions. Many important research questions can only be answered by registry studies because large patient numbers are required. Rarer diseases and clinical situations for which no single institution has adequate experience are also best approached by registry studies.

Decision analysis

There is rarely a definitive clinical trial or report that provides all the data necessary to settle a clinical question. People believe they can weigh complicated decisions fairly, but research shows that this *ad hoc* approach is subject to serious cognitive biases and frequently results in suboptimal decisions. Decision analysis uses computer modeling to determine the optimal treatment choice based on what is known about the probabilities and consequences of different treatment options [55]. In order to construct a computer model, an explicit description of the decision to be made and the likelihood of different outcomes emanating from each possible treatment choice are necessary. The analyst also decides which health states are relevant (e.g. dead, alive with disease, alive without disease, alive with chronic GVHD). Health states need to be broad enough to allow accurate estimation of the percentage of the population within them at any time but narrow enough to discriminate different clinical circumstances. The analyst also decides how often people can transition between different health states (cycle length) based on data available from clinical trials or observational studies.

Decision-analysis models are often depicted as "trees." A square represents the decision to be made (choice node), while branches off of circles (chance node) represent possible clinical consequences. A prudent analyst only includes clinical consequences material to the decision or else a decision tree can quickly become "too leafy" with small, perhaps inconsequential branches for which solid clinical data may be unavailable. Advanced programming capabilities allow the probabilities of

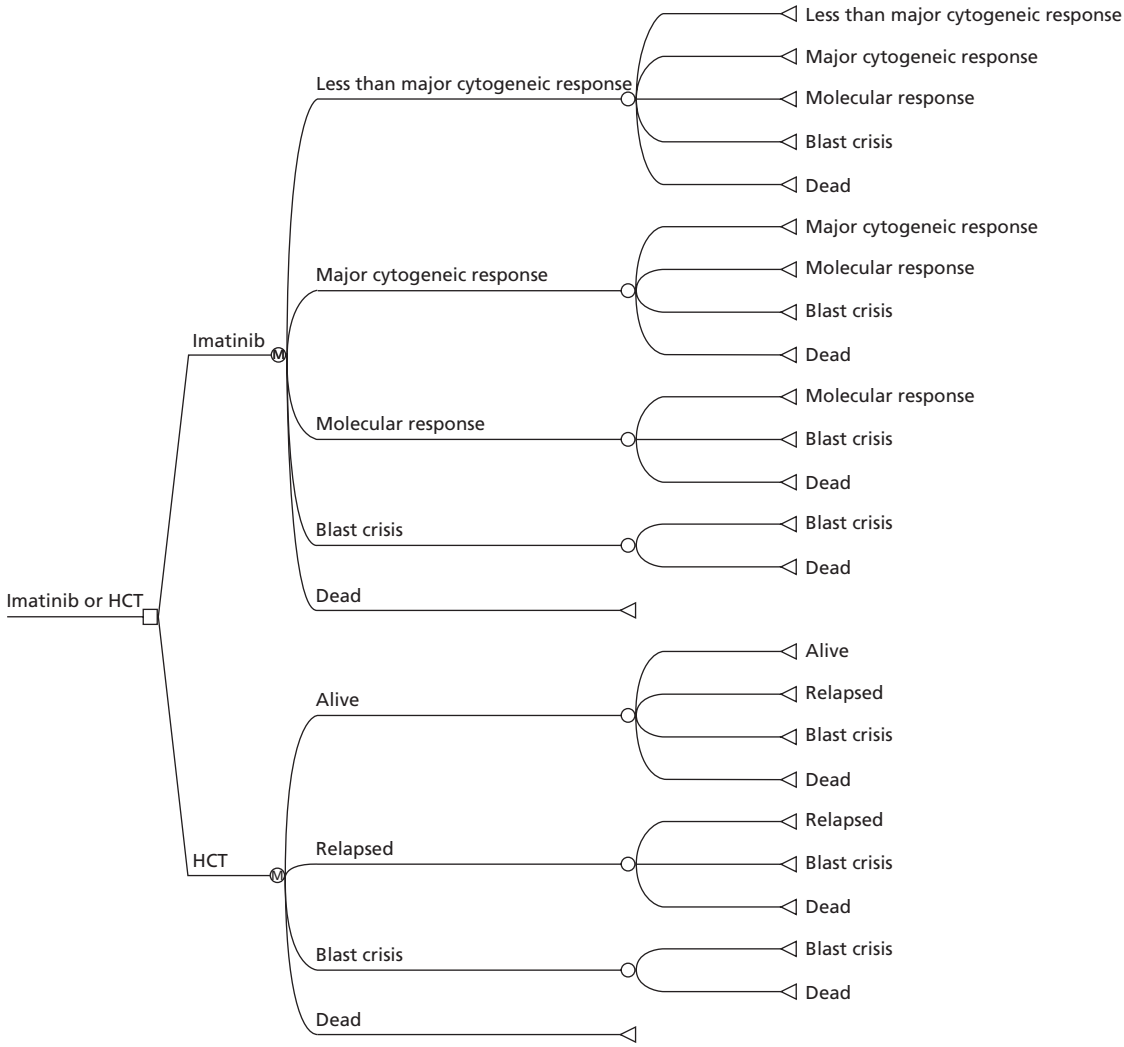


Fig. 32.2 Simplified structure of a decision analysis of imatinib mesylate (STI571, Gleevec®) vs. hematopoietic cell transplantation (HCT) for treatment of chronic myeloid leukemia (CML) in first chronic phase.

different outcomes to vary depending upon a patient's characteristics, time from diagnosis or prior clinical course, assuming such data are available. Figure 32.2 shows the structure of a simplified decision tree using the example of imatinib mesylate (STI571, Gleevec®) vs. allogeneic HCT for CML in the first chronic phase. Note that this simplified model does not account for combination therapy, crossover between imatinib mesylate and HCT, prognostic information based on response to imatinib mesylate or complications of HCT such as chronic GVHD.

The results of decision analysis provide a population-based approach to determine the optimal treatment choice. Treatment options are compared based on the area-under-the-survival-curve (life years-LYs) or the quality-adjusted area under the survival curve (QALY). In practical terms, a decision analysis may not distinguish between one individual surviving an extra 10 years and five individuals surviving an extra 2 years, although patients may view these outcomes differently. Such value judgements are incorporated into the model using discounting functions that value early survival greater than distant years of life. While a decision analysis obviously can not predict what will happen to any particular individual, if the information put into the model is correct, results should accurately reflect what happens to the population on which it is based [56].

Comparison of LYs or QALYs obtained from a decision analysis are not amenable to statistical testing in the classic sense, since *p*-values and confidence intervals (CIs) reflect the uncertainty in measurements and likelihood of chance findings. In decision analysis, it is up to the reader to compare the gains in LYs or QALYs and determine if one treatment is optimal. Obviously such comparison is easier when the survival benefit associated with one option is several years and the other offers several weeks. Published league tables can help put gains in life expectancy into perspective [57]. Sometimes decision analysis can identify key pieces of information that should influence treatment decisions and, conversely, point out which considerations should *not* affect a rational decision. When assumptions and estimates have to be made because sufficient clinical data are not available, sensitivity analysis helps determine whether results hinge on those estimates. If conclusions are the same despite drastically changing an assumption, then the analysis is "robust" and not dependent on that variable. Several assumptions may be tested at the same time to see if any combination of values would change conclusions.

Several decision analyses have been performed in HCT. For example, an analysis of autologous HCT vs. conventional combination therapy for a 50-year-old woman with progressively erosive, active rheumatoid arthritis after initial therapy suggests equivalent QALYs with either approach [58]. Similarly, a decision analysis of allogeneic bone marrow transplantation (BMT) vs. periodic blood transfusion for patients with sickle cell anaemia and elevated cerebral blood velocities suggested that

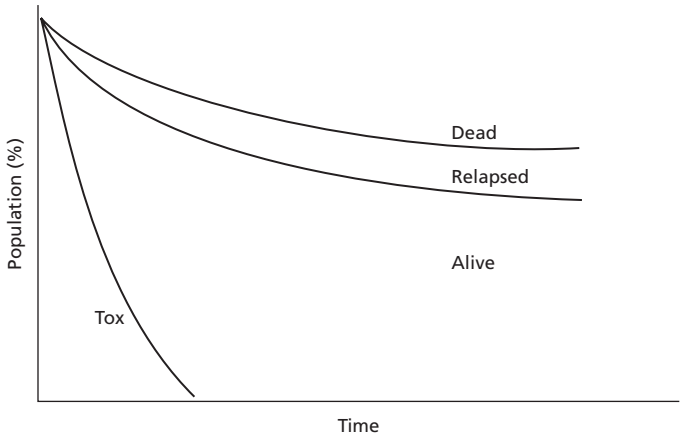


Fig. 32.3 Schematic of a Q-TWiST analysis. The population is divided into Dead, Relapsed, Alive (without disease or toxicity) and Tox (alive with toxicity from treatment).

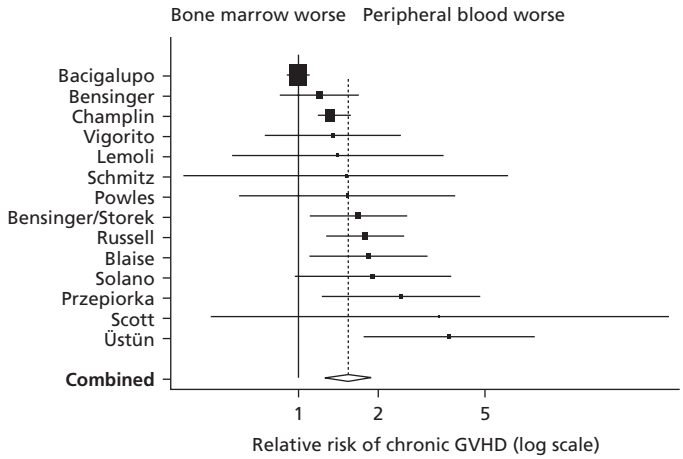


Fig. 32.4 Results of a meta-analysis of the risk of chronic graft-vs.-host disease (GVHD) associated with peripheral blood or bone marrow transplantation (BMT). Reproduced with permission of the American Society of Clinical Oncology from Cutler *et al*. [66].

either treatment approach was reasonable [59]. A decision analysis of unrelated donor BMT for chronic phase CML vs. IFN-α (prior to the approval of imatinib mesylate) suggested that early transplantation maximized quality-adjusted survival [60,61]. The widespread use of imatinib mesylate illustrates how decision analyses need to be updated as new treatments and data become available.

Q-TWiST

Q-TWiST stands for "*q*uality *t*ime *wi*thout *s*ymptoms of *t*oxicity." It is another method of integrating QOL and survival data [62,63]. Although concurrent data on QOL, survival and DFS may be obtained in a single clinical trial, information on symptoms and QOL are often derived independently. Figure 32.3 shows a schematic of a Q-TWiST analysis in which patients are divided up into four mutually exclusive categories: alive without disease or treatment toxicity, alive with symptoms of toxicity, alive in relapse and dead. Quality-adjustment is applied to the different health states, and the area-under-the-curves are aggregated. If there are two available treatments, the option that provides the greatest quality-adjusted survival is judged superior. This type of analysis has been used to suggest that autologous HCT is better than chemotherapy for aggressive NHL in first complete remission [64] and that allogeneic transplantation is better than chemotherapy or autologous transplantation for pediatric AML in first complete remission [65].

Meta-analysis

Meta-analysis is another technique that allows the results of several studies to be aggregated, increasing the power of the analysis and enhancing confidence in the results. This statistical technique is particularly useful in detecting treatment differences if negative studies are due to small sample size and lack of power. Study-level meta-analyses use individual studies as the unit of analysis. Patient-level meta-analyses actually retrieve and analyze data on individual patients, although each patient's participation in a particular study is incorporated into the analysis. Analyses can either use "fixed-effect" or "random-effect" models. In fixed-effect models, one true effect of the treatment is assumed and any differences in studies from that effect are considered part of variability. In random-effect models, each study estimate may be true because of differences in studies and the analysis allows for a range of "true" values. Tests for homogeneity/heterogeneity can address whether the reported effect sizes from different studies could vary due to chance, although these tests lack power and should only be minimally reassuring. Both randomized, controlled trials and observational studies are often included in meta-analyses, although effect sizes are usually smaller in randomized trials. Sensitivity analyses that exclude lower quality studies can be performed to increase confidence in the conclusions. The results of meta-analyses are displayed as point estimates and confidence intervals for each study with the width of the box reflecting relative study size. A diamond is used to depict the aggregate estimate.

All meta-analyses depend upon data available in the literature or otherwise attainable. Thus, publication bias may significantly affect results. A funnel plot is a graphic that can help determine if a meta-analysis is likely to suffer from publication bias. Effect size is plotted vs. study size. If all studies are published and available regardless of their conclusions, then the plot should result in a funnel shape with the apex centered on the true value. This pattern occurs because the ranges of effect sizes are wider for the smaller studies due to statistical factors, while larger studies should provide results closer to the actual truth. For example, Cutler and colleagues published a meta-analysis of acute and chronic GVHD and hematopoietic stem cell source ($n = 16$ studies) [66]. They found that rates of both acute and chronic GVHD were elevated when peripheral blood served as the source of the graft instead of marrow. Figure 32.4 shows the study results.

Evidence-based medicine

Critical reviews of the literature summarize the available evidence for or against certain practices, and they are often translated into practice guidelines. The methodology is very well established and several organizations, such as the Cochrane Collaboration, the Agency for Healthcare Research and Quality, Cancer Care of Ontario, the American Society of Clinical Oncology and the American Society of Hematology, have performed critical reviews. The American Society for Blood and Marrow Transplantation has produced evidence-based reviews of HCT for NHL and multiple myeloma (http://www.absmt.org). A typical grading system for critical reviews is shown in Table 32.4, and is based on the type, frequency and consistency of evidence [67]. One practical limitation of evidence-based reviews compared to consensus statements or clinical reviews is that the published evidence is strictly interpreted [68]. For example the American Society of Hematology guidelines on the management of CML deferred comment on the relative value of transplantation vs. nontransplantation strategies because randomized studies have not been conducted [69]. In contrast, editorials, book chapters and clinical reviews are not held to the same high standard of evidence, and thus some reasonable triage strategies have been suggested [61,70–73]. However, these algorithms predate the widespread availability of imatinib mesylate.

Table 32.4 Levels of evidence and grade of recommendations. Reproduced with permission from the American Society of Hematology [67].

Level	*Types of evidence*
I	Evidence obtained from meta-analysis of multiple, well-designed, controlled studies. Randomized trials with low false-positive and low false-negative errors (high power)
II	Evidence from at least one well-designed experimental study. Randomized trials with high false-positive and/or high false-negative errors (low power)
III	Evidence obtained from well-designed, quasi-experimental studies such as nonrandomized, controlled, single-group, prepost, cohort, time, or matched case–control series
IV	Evidence from well-designed, nonexperimental studies such as comparative and correlational descriptive and case studies
V	Evidence from case reports and clinical examples
Grade	*Grade of recommendation*
A	There is evidence of type I or consistent findings from multiple studies of types II, III or IV
B	There is evidence of types II, III, or IV, and findings are generally consistent
C	There is evidence of types II, III, or IV, but findings are inconsistent
D	There is little or no systematic empiric evidence

Many organizations have produced practice guidelines. A nonexhaustive list relevant to transplantable diseases includes the American Society of Clinical Oncology, American Society for Blood and Marrow Transplantation, National Comprehensive Cancer Network (NCCN, http://www.nccn.org), and the Physician Data Query (PDQ, http://www.nci.nhi.gov/cancer_information/pdq). However, relatively little research has evaluated the influence of practice guidelines on clinical practice and patient outcomes, and what has been published suggests less improvement than hoped [74–76].

What is the patient's experience with HCT?

Beyond the traditional biological endpoints of survival and relapse reported in HCT studies, outcomes research tries to measure and put into perspective other factors that determine whether an intervention is ultimately judged a success or failure. Many of these factors are subjective (not directly observable) and are referred to as "constructs." For example, measurement of health-related quality of life (HRQOL, the QOL related to health, disease and medical treatment), satisfaction and patient utilities are considered part of outcomes research. Measuring these endpoints relies heavily on survey research, the collection of data directly from patients.

Qualitative methods

The goal of qualitative studies is to capture the breadth of possible patient attitudes or experiences. One forum is a focus group, in which 8–10 people are lead by a moderator and discuss particular topics. Focus groups usually last about 2 h, and participants may be paid a nominal amount for participation. They are often audio- or videotaped, with an additional researcher taking notes. The interactive nature of the communication process allows topics to be probed and ideas developed under the influence of group dynamics, which may lead to unexpected insights about the topic under discussion. Focus groups are often used for formative research to explore patient attitudes and opinions prior to launching a formal study.

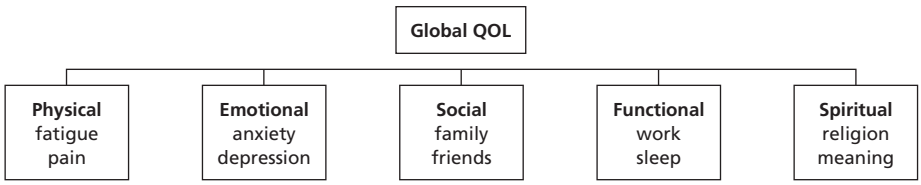
Qualitative information is also collected through interviews or open-ended survey questions. In contrast to quantitative studies in which generalizability is critical, the goal in qualitative studies is not to obtain a representative sample. In fact, "purposive" or targeted sampling can be performed in order to ensure the spectrum of possible patient experiences is represented. For example, if 80% of the population has a typical experience, 5% has a less typical experience and the remaining 15% all have unique experiences, the goal would be to interview 17 people, one from the majority, one from the minority and all 15 who had unique experiences. Usually, transcriptions are made of the interviews, and qualitative coding software is used to mark the transcripts for easier analysis. Transcripts are reviewed by a limited number of individuals who code them for themes, aggregate the concepts into broader groups if possible, and report on the range of patient experiences.

Qualitative methods have been used in several studies to evaluate aspects of recovery following HCT [77–80]. These studies revealed several themes that are not particularly well covered in standardized instruments; for example, strategies that patients use to compensate for limitations, multiple losses in all aspects of their lives and the greater appreciation for life brought about by the HCT experience.

Quality of life

Quality of life (QOL) is composed of diverse determinants including physical abilities, symptoms, social well-being, psychoemotional status and spiritual/existential experiences. It reflects how well people feel, what they can accomplish, how satisfied they are with their lives and whether their lives have meaning and purpose (for details regarding QOL after HCT see Chapter 39). Capturing these domains requires multidimensional instruments. QOL studies in HCT have generally sought to: (i) describe the long-term QOL, adaptation, and recuperation of patients; (ii) find predictors of better or worse QOL; and (iii) compare populations treated with different procedures. With the availability of numerous validated instruments, QOL is generally measured quantitatively with questionnaires. One commonly used instrument is the Medical Outcomes Study Short Form 36 (SF-36) a generic multidimensional 36 item instrument that has been used on thousands of patients and healthy people to measure physical and mental health status [81,82]. Other cancer-specific instruments, such as the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ) C30 (30 items) [83] and the Functional Assessment of Chronic Illness Therapies (27 items) [84], are designed for use in cancer populations. Both of these instruments also offer HCT-specific modules that can be added to the core form to capture issues specific to HCT. When scored according to psychometrically tested methods, they provide standardized QOL information that may be compared with other populations, and that describe the QOL and functioning of patients. Figure 32.5 shows an example of the physical functioning scales from these instruments. Of note, comparative studies have shown that despite similarly named subscales, instruments are actually measuring different constructs and it is difficult to compare studies unless they use identical instruments [85,86].

Many QOL surveys have been translated into other languages, an arduous process that first requires translation, then back-translation to see if



SF-36 (physical)
Does your health now limit you in:
- Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports
- Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf
- Lifting or carrying groceries
- Climbing several flights of stairs
- Climbing one flight of stairs
- Bending, kneeling, or stooping
- Walking more than a mile
- Walking several blocks
- Walking one block
- Bathing or dressing yourself

EORTC (physical)
- Do you have any trouble with strenuous activities, like carrying a heavy shopping bag or a suitcase?
- Do you have any trouble taking a long walk?
- Do you have any trouble taking a short walk outside of the house?
- Do you have to stay in bed or in a chair for most of the day?
- Do you need help with eating, dressing, washing yourself or using the toilet?

FACT (physical)
- I have a lack of energy
- I have nausea
- Because of my physical condition, I have trouble meeting the needs of my family
- I have pain
- I am bothered by side effects of treatment
- I feel ill
- I am forced to spend time in bed

Fig. 32.5 Dimensions of quality of life and examples of the physical domain from validated questionnaires.

meaning is preserved. It is a common mistake to assume that interpreters can administer English instruments to a non-English speaker. Instead, the validated version in the subject's native language should be used since interpreters may unintentionally change the meanings of items and responses.

Ideally, any QOL report should include information about the instruments used, reasons for missing data, a comparison of respondents and those who choose not to participate, and response rates at each assessment point to assist in interpretation of study quality. Missing data are a great problem in QOL and survey research for several reasons. First, 10–50% of data may be missing due to logistical problems and patient refusal to complete questionnaires. One barrier may be literacy, since studies have shown that approximately 25% of the US population is functionally illiterate [87]. Second, data are often not missing at random, but rather reflect poor health or other characteristics that could influence QOL. Many validated scales are long, and ill HCT patients are more likely to refuse to complete them. This consideration is called "respondent burden" and is an important part of planning any survey-based study.

Biostatistical methods for analyzing QOL data can be complicated because they must address issues of longitudinal data analysis, informatively missing data and other problems exacerbated by the nature of QOL data [88,89]. Repeated measures analysis and mixed models allow differences between populations and over time to be studied, but methods of reporting results might not be intuitive to physicians. Also, it is important to remember that QOL studies are often cross-sectional and represent only those patients surviving the procedure at a particular point [90,91].

Developing methods to place QOL differences into their clinical context is an active area of research. Although validated scales are psychometrically sound and allow comparison of treatment groups by statistical testing, the results of QOL studies are not intuitive to patients and physicians. For example, a survival difference of 10% is easily interpretable. But many find it harder to interpret a QOL difference of 50 vs. 35 on a given scale and place such an observation in its clinical context. There is no intuitive feeling for what a person with a score of 50 feels like compared to someone with a score of 35.

A group of QOL researchers began meeting in 2000 to address the issue of clinical significance for QOL measures. A "clinically meaningful difference" is defined as the difference in QOL that would prompt adoption of the intervention or a change in practice. Two approaches have been suggested: anchor-based and distribution-based. Anchor-based methods rely on patient-reported differences to determine clinically meaningful differences. For example, patients are asked a global change question such as, "Overall, is your QOL a lot better, a little better, somewhat better, somewhat worse, a little worse or a lot worse." This overall category is then compared to their QOL scores [92–94]. However, this method uses patient-perceived differences in QOL as the gold standard, raising the question of why we cannot just ask patients directly about changes in their QOL. The second approach is based on the statistical distribution of QOL scores. Generally, a difference of 0.5 standard deviation is considered to be clinically meaningful.

Results and conclusions of specific QOL studies are discussed in more depth in Chapter 39.

Patient satisfaction

Patient satisfaction has proven an elusive construct to measure in medicine. Although patient satisfaction with care and the results of treatments are undoubtedly important, most validated scales have shown a ceiling effect, defined as an inability to distinguish variations in satisfaction because most people are highly satisfied with their personal care. Patient satisfaction as a primary outcome of HCT studies awaits more responsive instruments that can distinguish gradations of satisfaction.

How are new tools to measure subjective or clinical endpoints developed?

Instrument development

Instrument (or survey) development is considered a facet of outcomes research because it establishes the validity of clinical tools to measure subjective endpoints. In order for an instrument to be useful, it needs to reflect what it purports to measure (validity), be an accurate measure (reliability), separate people into clinically meaningful groups (discrimination) and detect important changes (sensitivity). Instrument development from scratch is a demanding process. First, a list of relevant concepts should be created from prior literature, focus groups or other means of

formative research. Then, a draft scale is created. Attention should be given to the wording of specific questions and response items to allow sufficient variability to capture the range of clinical conditions. Then, a pilot study is conducted with cognitive interviewing to ensure that patients understand the questions and are selecting response options consistent with their intent. Finally, a larger study is performed to document validity, reliability and sensitivity. Many survey developers neglect the final step of confirming sensitivity to change. This is an important feature since many instruments are intended to describe the experiences of population subgroups, compare one population to another, or show changes over time.

Reliability refers to whether a measure is consistently reflecting the true status of a subject. Internal reliability is usually reported as a Cronbach's *alpha* with acceptable values greater than 0.7. Cronbach's *alpha* measures whether items are correlated with each other and measure the same underlying construct. Stability of measurements is reported as "test–retest reliability," the correlation between two measurements separated in time when an individual's status has not changed. The range is from 0 to 1.0, with higher values reflecting greater stability and values >0.5 generally considered acceptable. If test–retest reliability is <0.5 and the subject's clinical situation has not changed, the scale is probably susceptible to influences unrelated to the clinical status of the person.

Validity refers to whether an instrument is truly reflecting what it is supposed to measure, and is usually expressed as correlation coefficients or effect sizes. Content validity refers to how well the scale measures the different aspects of the construct. Convergent validity is demonstrated when the scale correlates highly with other scales measuring similar constructs, while discriminant validity means there is little correlation with scales measuring unrelated concepts. Discrimination refers to the ability of the scale to separate people into clinically meaningful groups, while sensitivity to change means that as a person's clinical situation changes, they should score differently on the instrument.

Several surveys have been developed specifically for HCT. For example, McQuellon and colleagues developed and validated a HCT module for the Functional Assessment of Chronic Illness Therapies (FACT) that assesses additional transplant-specific symptoms [84]. A leukemia module for the EORTC QLQ C30 has been used in a study comparing chemotherapy to autologous and allogeneic transplantation for AML [83,95]. Lee and colleagues have developed a chronic GVHD symptom scale [96]. This scale was designed to be self-administered and brief (5 min) and to follow patients with chronic GVHD over time to detect improvement or worsening in their symptoms. It includes questions about bothersome eye, mouth, lung, skin, nutrition, emotional and energy symptoms. Comparison with the SF-36 and FACT-BMT showed adequate convergent and discriminant validity, discrimination between patients with self-assessed mild, moderate or severe chronic GVHD, and sensitivity to change using an anchor-based method of assessment.

Scale development

Clinical syndromes such as acute and chronic GVHD have been notoriously difficult to measure, yet they are important endpoints in almost every allogeneic HCT report (for details see Chapter 50). The greatest challenge arises from the heterogeneous clinical manifestations complicating standardization of severity grading. Martin *et al*. [97] showed that interobserver differences in acute GVHD grading from medical records were substantial, and suggested a more objective way of coding this complication. However, his approach has not been widely adopted. A second barrier to scale development is the need to validate the scale against a gold standard. Since a gold standard does not exist for GVHD severity, developers have used survival or chronic GVHD mortality as objective endpoints.

In acute GVHD, several grading systems have been proposed including the Glucksberg scale [98], the modified Glucksberg scale [99], the Consensus Conference grading system [100] and the IBMTR index [101]. Application of these grading systems first requires ascertainment of performance status and staging of skin, liver and gastrointestinal involvement, followed by aggregation into five grades (0–IV or 0, A, B, C, D). With the exception of the IBMTR index, all the grading systems were developed by observation and consensus. The IBMTR used one large set of patients undergoing human leukocyte antigen (HLA)-matched sibling BMT for acute or chronic leukemia ("training set," $n = 2129$ given cyclosporine and methotrexate) to develop the index, then validated it in an independent dataset ("testing set," $n = 752$ receiving T-cell depletion) using survival as the primary endpoint [101]. Additional validation studies in separate cohorts have yielded conflicting results [102,103].

Several scales have also been proposed for grading the severity of chronic GVHD. Akpek and colleagues identified three dichotomous variables (extensive skin involvement, thrombocytopenia and progressive onset) that could be combined to distinguish three groups at different risks of chronic GVHD-specific mortality [104]. A multicenter validation study has been performed to show that the scale successfully predicts survival in four independent cohorts (G. Akpek, manuscript submitted). Lee and colleagues took a similar approach to devising a chronic GVHD severity score using IBMTR and NMDP data [105]. The resulting grading scheme is complicated and difficult to use in clinical practice, but does predict survival and treatment-related mortality.

How can the practice of HCT be improved through health services research?

Access

Studies seeking to understand the nonmedical barriers to appropriate health care are termed "access" studies. They generally focus on socioeconomic, political, cultural and other nonbiological factors. For example, ethnic and racial minorities have long been under-represented in HCT statistics for unclear reasons. In addition, survival varies by racial group even after controlling for disease and transplant characteristics (F.R. Loberiza, manuscript submitted). While similar observations have been made in other areas of medicine, issues of access and social determinants of outcome are only starting to be evaluated in HCT. Kollman and colleagues at the NMDP studied reasons why only a third of promising initial searches proceed to transplantation [106]. They identified death of the patient, worsening of the patient's health and length of the search process as major barriers accounting for up to a quarter of failures to proceed to transplantation. The importance of financial issues could not be adequately evaluated in this study, but 41% of coordinators listed insurance coverage as a potential barrier at the time of initial search. Importantly, 34% of white- compared to 13% of African-Americans went on to transplantation. In another study, a survey of 589 African-Americans was conducted to examine barriers to participation in an unrelated donor program. The cost of donation, limited opportunities to donate and lack of knowledge about the life-saving potential of HCT from an unrelated donor were found to be important barriers to donation. Importantly, with introduction of an educational program, the African-American donor pool at the Medical College of Virginia increased substantially [107].

Unfortunately, one of the best data sources for access studies is not relevant for HCT. The linked SEER-Medicare database provides cancer-specific information and inpatient and outpatient billing data on approximately 14% of the US population, but is limited to patients aged 65 or older [11]. Oncology studies using this and other databases suggest that African-Americans are less likely than white Americans to receive screening exams for cancer, to be diagnosed with cancer in its early stages and to receive adjuvant therapy and aggressive care [108–114]. African-Americans are also less likely to undergo some procedures than white Americans, such as renal transplantation, even after correcting for

clinical characteristics [115] and patient preferences [116]. However, once access to treatment is controlled, survival and DFS is similar between African-American and white American patients, suggesting that the biological response to treatment is comparable [117,118].

Quality of care

HCT has been relatively spared from scrutiny about quality of care. Significant practice variation has been tolerated and, in fact, encouraged, as a means of testing different approaches that could eventually improve the field of transplantation. The relatively low volume of procedures per center and the inevitable case mix differences have made it nearly impossible to provide standardized center statistics, although the NMDP is required to report center-specific disease activity and risk-adjusted survival information. Nevertheless, a volume-outcome and experience-outcome relationship is probably operating in HCT similar to what has been observed in other technologically sophisticated procedures such as solid organ transplantation and complicated surgical procedures [119–122]. A time-series study of costs during autologous HCT for NHL suggested that technological advances and learning curve effects (institutional familiarity with a procedure tends to improve outcomes) probably both contribute to falling costs, although the study was not designed to compare clinical outcomes [123]. There is every reason to believe that greater attention to institutional and programmatic factors contributing to patient outcome may identify ways to improve care and decrease costs [122]. For example, a study documented significant variation in vaccination practices following HCT [124], and recommendations for infectious disease prophylaxis have subsequently been published [125].

Summary

Outcomes and health services research seeks to answer questions that are relevant as a procedure matures beyond the experimental phase: What are the costs of providing these services? Is society getting its money's worth? How do you pull together disparate sources of data (now that they are available)? What do patients experience with the procedure? Can we develop new or better tools to measure results of treatments? Is the procedure equally available to all people and of the highest possible quality? Answers to these questions are moving targets as HCT evolves. Nevertheless, for people afflicted with diseases treated by HCT and societies trying to control health care spending, decisions have to be made today. Outcomes research tries to provide the necessary data so that personal and societal decisions can be based on the best information available.

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