

REVIEW

The severe combined immunodeficient (SCID) mouse as a model for the study of autoimmune diseases

A. O. VLADUTIU *Departments of Pathology, Microbiology, and Medicine, SUNY at Buffalo School of Medicine and Biomedical Sciences, and The Buffalo General Hospital, Buffalo, NY, USA*

(Accepted for publication 29 April 1993)

SUMMARY

There are no readily available *in vivo* models to study immune cells from humans with autoimmune diseases. SCID mice, which virtually lack both T and B lymphocytes and accept xenogeneic cells, have been used during the last 5 years to provide a milieu for lymphocytes isolated from individuals with various autoimmune diseases, or for lymphocytes from mice that have a systemic lupus erythematosus-like syndrome. Whilst human autoantibodies to organ antigens have been demonstrated in most SCID mice engrafted with human lymphocytes from the peripheral blood or the target organ, inflammation of the mouse target organ has not generally been observed. This review critically analyses experiments in this area reported so far. Some pitfalls of the SCID mouse model of human autoimmune diseases are mentioned, and future experiments to study mouse and human autoimmunity with this model are proposed.

Keywords autoimmunity SCID mice xenografts lymphocytes

INTRODUCTION

Autoimmunity is an important pathogenetic mechanism for many common disorders in man. Indeed, diseases with an autoimmune component represent an appreciable percentage of human diseases that impose an increasing burden on medical services all over the world. In addition to clinical importance, the study of autoimmune diseases has contributed to the understanding of major concepts of immunology such as tolerance to self. There are still many questions unanswered concerning human autoimmunity, and many aspects of autoimmune diseases are not fully understood. For instance, the pathogenetic mechanisms of many human autoimmune diseases are still unclear, e.g. the contribution of antibody-initiated and T lymphocyte-initiated inflammation and tissue damage, and the relationship between these two mechanisms. Immunoregulatory processes, for example the role of interleukins, are not well understood. The role of the genetic background of the individual in the initiation and perpetuation of the autoimmune reaction has to be further clarified. For human diseases in particular, many putative autoantigens and their so-called pathogenic epitopes that might drive the autoimmune response are incompletely characterized. This might explain why the

treatment of autoimmune diseases still has a long way to go, especially with regard to autoantigen-specific treatment (immunotherapy).

Do T cell receptors (TCR) have a limited diversity in some autoimmune diseases? Is there a role for the expression of class II MHC antigens on various cells in triggering and/or perpetuating the autoimmune reaction? Are CD5⁺ B lymphocytes, stress proteins and superantigens involved in human autoimmunity? Are antigenic mimicry and retroviruses implicated in this process? To answer these and other questions, new means of studying autoimmune diseases are required.

For several decades experimental models of autoimmune diseases have been employed to study mechanisms of the autoimmune response. The use of inbred strains of animals is particularly helpful, since humans are not a homogeneous population and their diseases are not easily accessible to experimentation. Animal models for human autoimmune diseases could also help the investigation of treatment modalities for these diseases. It is noteworthy that some experimental autoimmune diseases were described before their counterparts were recognized in humans, e.g. experimental autoimmune thyroiditis (EAT) [1]. The genetic control of autoimmunity was firmly shown in animals [2] before being demonstrated in humans. Some induced or spontaneous autoimmune diseases in animals are fairly good models for several human autoimmune diseases, e.g. the spontaneous disease of (NZB × NZW) F₁ mice resembles human systemic lupus erythematosus (SLE), EAT

resembles Hashimoto's thyroiditis, and the relapsing form of experimental autoimmune encephalomyelitis (EAE) has similarities with multiple sclerosis. A myasthenia gravis-like disease could also be induced in animals by immunization with acetylcholine receptor (AChR).

SCID mice, which virtually lack both cellular and humoral immunity, can be reconstituted with xenogeneic (e.g. human) cells. The question arose as to whether human autoimmune diseases could be 'transferred' to SCID mice by engrafting them with immune cells from patients with these diseases. In the last 5 years lymphocytes from individuals with several autoimmune diseases were injected in SCID mice, thus allowing the study of pathogenetic mechanisms and treatment modalities in these diseases. Furthermore, lymphoid cells from mice that spontaneously develop a systemic SLE-like disease were engrafted to SCID mice. This review will focus on the experiments performed so far to study autoimmunity in these mice, and will analyse various aspects of these experiments. The pitfalls of the SCID mouse model of human autoimmune diseases and the potential importance of this model will be reviewed, and future experiments with SCID mice will be suggested. A general discussion on SCID mice will not be undertaken, since this topic was recently reviewed [3,4].

SCID MICE

SCID mice are a mutant of the mouse strain C.B-17, an *IgH^b* congenic partner of the BALB/c (*H-2^d*) mouse. The mutation in SCID mice is autosomal recessive and was mapped to the centromeric region of chromosome 16, near the mahoganoid coat colour. Although the SCID mutation affects all cell lineage, its effect is manifested in lymphoid cells. Lymphoid organs of SCID mice are small and contain only a few lymphocytes. Myeloid, erythroid, antigen-presenting cell (APC), splenic colony stem cell, and NK cell development and function are not influenced by the SCID mutation. There is a defect in a DNA break repair enzyme and a VDJ recombinase-associated defect that affects somatic rearrangement of antigen receptor genes in T lymphocytes and immunoglobulin genes in B lymphocytes. The development of T lymphocytes is stopped at the CD4⁻, CD8⁻ immature thymocyte stage and the maturation of B lymphocytes is arrested at the pro-B lymphocyte.

SCID mice fail to produce antigen-specific antibody responses *in vivo* and, therefore, have very low or undetectable levels of immunoglobulins in serum. Sometimes, functional antigen receptor rearrangements do occur through 'illegitimate' recombination [5]. A few T and B lymphocyte clones survive and can expand *in vivo* after exposure to antigens [6]. This phenomenon, that was referred to as 'leakiness' by Bosma *et al.*, is observed in about 15% of young SCID mice, and increases in frequency with age and environmental exposure, especially in mice that are not kept under strict aseptic conditions [6]. NK activity appears to have a role in regulating differentiation of rare T and B lymphocyte precursors in SCID mice [6].

SCID mice were reconstituted with various human cells, e.g. fetal liver cells, thymocytes, and peripheral blood lymphocytes (PBL). Complete lymphoid reconstitution requires pretreatment of adult recipients with sublethal whole-body irradiation [7]. It appears that the intraperitoneal route offers some advantages when compared with the intravenous route for reconstitution with human (hu)-PBL. The first tissue to which

hu-PBL T lymphocytes migrate after i.p. injection is mouse abdominal lymph nodes [8]. A critical amount of hu-PBL must be injected intraperitoneally (about 15 million cells) to obtain consistent reconstitution (e.g. production of human immunoglobulins) because presumably only a fraction of injected human cells persist, collaboration between relatively rare human cells is necessary for successful reconstitution, and perhaps a large number of human cells are required to overcome host resistance [4]. Preincubation of human PBL with antigen before injection to SCID mice markedly enhances the ability of these cells to produce antibodies to the respective antigen in mice [9].

Virtually all of the immunoglobulin secretion in hu-PBL-SCID mice is T-dependent. The low frequency of hu-PBL-grafted SCID mice that respond to primary immunization may indicate generally impaired hu-PBL function *in vivo*. It has been suggested that T lymphocytes are converted from 'naive' to 'memory' phenotypes, perhaps as a result of *in vivo* stimulation by murine xenoantigens [10]. Human T lymphocyte unresponsiveness can occur because of the absence of autologous APC that provide costimulatory signals required for T lymphocyte activation. The human T lymphocytes from hu-PBL engrafted to SCID mice do not produce graft-versus-host disease (GVHD) according to some researchers [11], but may show evidence of GVHD according to other investigators [12,13]. Leakiness is associated with GVHD and sporadic GVHD might be explained by variations in natural killer (NK) activation. Xenoreactivity of engrafted T lymphocytes could be present when autologous human APC are available [13].

SCID MICE AND AUTOIMMUNITY

Human and mouse autoimmune diseases

SCID mice were used to provide a milieu for lymphocytes collected from individuals with several autoimmune diseases (e.g. Hashimoto's thyroiditis, Graves' disease, myasthenia gravis, SLE, etc.), lymphocytes isolated from (NZB × NZW) F₁ and MRL mice, and from mice with SLE-like disease that occurs after immunization with a human MoAb expressing a common idiotype, 16/6 Id [14]. For some of these human diseases an autoimmune pathogenesis is certain (e.g. Hashimoto's thyroiditis or myasthenia gravis), whereas others are only presumed to be autoimmune diseases (e.g. multiple sclerosis, rheumatoid arthritis). In some of the diseases autoantibodies are of paramount importance (e.g. anti-receptor antibodies in myasthenia gravis and Graves' disease), in others the autoantibodies might play an important role (e.g. SLE, primary biliary cirrhosis) and finally, in some diseases (e.g. multiple sclerosis) T lymphocytes rather than autoantibodies are important in the pathogenesis. The triggering autoantigens are known for a few autoimmune diseases, but for most the autoantigens are still not determined. Cells or tissues from human and mouse autoimmune diseases that were transferred to SCID mice are given in Table 1. Sjögren's syndrome [15] was not included in Table 1 because it is more related to lymphoproliferative than to autoimmune diseases. Only human autoantibodies were consistently shown in chimeric hu-SCID mice, and organ damage has not been found or was mild in these mice (see below).

Table 1. Autoimmune disease cells engrafted to SCID mice

Disease (human)	Autoantigens	Mechanisms of tissue damage	Cells or tissue engrafted to SCID mice	Development of		
				Autoantibodies (human origin)	Tissue damage (in mice)	Reference
Autoimmune thyroid diseases (Graves' disease, Hashimoto's thyroiditis)	TPO*, TG TSH receptor	Autoantibodies, T cells	Thyroid tissue with lymphocytic infiltration, infiltrating lymphocytes PBL LNC	Anti-TPO, anti-TG, anti-TSH receptor	No	[16,17,19,22,24,28]
Myasthenia gravis	AChR	Autoantibodies	Thymus tissue, thymus cells, PBL	Anti-AChR	IgG deposits at neuromuscular junction	[23]
SLE	?	Autoantibodies?	PBL, spleen cells	ANA, anti-ssDNA, anti-SS-A, anti-SS-B, anti-RNP	Glomerular deposits of immunoglobulins, mild glomerular changes	[25,27,37,38]
Primary biliary cirrhosis	E2 (pyruvate dehydrogenase)	T lymphocytes, autoantibodies?	PBL	AMA	Yes?	[12]
Rheumatoid arthritis	?	T lymphocytes?	Synovial tissue, synovial cells	RF, ANA	No	[11,20,21]
Multiple sclerosis	Myelin proteins?	T lymphocytes	CSF cells		Yes	[29]
Scleroderma	?	T lymphocytes?	PBL		?	[13]
SLE-like disease in mice	?	Autoantibodies?	PBL, spleen cells	ANA, anti-Sm, anti-DNA	Yes (kidney)	[14,18,25]
Experimental autoimmune encephalomyelitis (EAE) in mice	PLF 139-151	T lymphocytes	T lymphocytes		Yes	[31]

AChR, Acetylcholine receptor; AMA, anti-mitochondrial antibodies; ANA, antinuclear antibodies; CSF, cerebrospinal fluid; LNC, lymph node cells; MBP, myelin basic protein; PBL, peripheral blood lymphocytes; RF, rheumatoid factor; SLE, systemic lupus erythematosus; TG, thyroglobulin; TPO, thyroid peroxidase; TSH, thyrotropin.

Cells engrafted to SCID mice

In many experiments hu-PBL, purified by Ficoll gradient centrifugation, were engrafted to SCID mice but were not further separated into lymphocyte subpopulations, and it was assumed that the cells were mostly T lymphocytes, although B lymphocytes were also present. Few experiments dealing with transfer of purified lymphocyte subpopulations or of mixtures of known amounts of defined lymphocyte subpopulations have been reported [16,17]. Macht *et al.* [17] injected in SCID mice populations of PBL depleted of T or B lymphocytes, or depleted of either CD4⁺ or CD8⁺ lymphocytes. Reininger *et al.* [18] transferred to SCID mice a line of pre-B lymphocytes, isolated from fetal liver cells of (NZB × NZW) F₁ mice, whereas Macht *et al.* [19] injected lymphocytes isolated from lymph nodes that drain the target organ (thyroid). These cells included memory B lymphocytes and activated lymphoblastoid B cells or plasma cells. In several experiments, lymphocytes that infiltrate the human target organ, e.g. thyroid [19], were separated and engrafted to SCID mice, resulting in higher amounts of autoantibodies in these mice compared with transfer of hu-PBL from similar patients. Lymphocytes isolated from synovial fluid and synovial-infiltrating cells of patients with rheumatoid arthritis produced higher levels of rheumatoid factor (RF) in recipient SCID mice than PBL from the same patients [11,20,21]. Although T lymphocytes isolated from target tissues are selected for the autoantigen, they still need APC for their function. However, deliberate addition of syngeneic irradiated APC to hu-SCID mice did not increase the titre of autoantibodies [11]. Adams *et al.* [20], Rendt *et al.* [21], and Morita *et al.* [22] grafted target tissue infiltrated with lymphocytes (e.g. thyroid from patients with autoimmune thyroiditis and synovium from patients with rheumatoid arthritis) and found that these cells produced higher amounts of human autoantibodies than PBL in SCID mice. Perhaps the grafted tissues provided more APC (e.g. epithelial cells with inappropriately increased expression of class II MHC antigens). Schönbeck *et al.* [23] have shown that thymus fragments from patients with myasthenia gravis produced much higher levels of antibodies to AChR than the lymphocytes isolated from thymus in SCID mice. Thymuses from patients with myasthenia gravis contain many autoantibody-producing B lymphocytes, and it seems that the thymus microenvironment (both cortex and medulla) is necessary for the lymphocytes to produce autoantibodies [23].

Tissue (e.g. rheumatoid synovium) infiltrated with lymphocytes obtained from patients with autoimmune diseases was usually grafted into SCID mice under the kidney capsule [19,21,23], a common place for xenografts, but Morita *et al.* [22] and Akasu *et al.* [24] successfully grafted thyroid tissue into each groin. Therefore, it is not clear whether the site of the graft is important for the survival of human lymphocytes in SCID mice.

There are only few reports on the transfer to SCID mice of lymphoid cells from strains of mice that develop autoimmune diseases spontaneously [18,25]. Segal *et al.* [14] injected SCID mice intravenously with splenocytes from BALB/c mice immunized with a human anti-DNA MoAb expressing a common idotype, 16/6 Id. It appears that the i.v. route was at least as efficacious in producing reconstitution as the i.p. route of cell inoculation.

Human immunoglobulins can be easily and rather consistently detected in SCID mice injected with hu-PBL, up to 2 years after transfer [26], therefore human diseases in which autoanti-

bodies are a major feature could be studied in the hu-PBL-SCID mouse model (e.g. to investigate the amount of autoantibodies in relation to the total amount of human immunoglobulins, their persistence in the circulation, etc.). This is not the case for those autoimmune diseases in which a T lymphocyte response is the major pathogenetic pathway, because cooperation between human APC and T lymphocytes is essential and, except for B lymphocytes, there are very few APC in hu-PBL grafted to SCID mice. Organ-specific antibodies produced in hu-PBL-SCID chimeric mice react with human antigens, but it is not clear whether these human autoantibodies also react with mouse antigens.

Autoantibodies

A proof of the transferred human lymphocytes' functionality, which should also be one of the goals of the experiments just described, is the production of a disease in SCID mice similar to the autoimmune disease in human donors. Since in many human autoimmune diseases autoantibodies can be readily detected, it is not too surprising that antibodies to human antigens, namely, anti-thyroid peroxidase (TPO), anti-thyroglobulin (TG), anti-AChR, anti-thyrotropin (TSH) receptor, several antinuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), and RF were found in many SCID mice that received lymphocytes from individuals with the respective autoimmune disease. Indeed, patients with autoimmune diseases resemble recently immunized donors by having B memory cells in their PBL able to produce autoantibodies *in vitro* or after transfer in SCID mice. Macht *et al.* [17] suggested that memory B lymphocytes from individuals with autoimmune diseases, once transferred to SCID mice, are activated in response to cytokines produced during the reaction of human lymphocytes to xenogeneic mouse antigens, and specific autoantibody production could be increased by the presence of antigen-laden cells. The amount of human autoantibodies in SCID mice was in some cases as high as or higher than that in the donors [22,27], especially when calculating their concentration relative to the total human IgG (i.e. the 'specific activity') [22]. However, in many instances the amount of human autoantibodies was small, and this has been thought to be the major reason for the virtual lack of inflammatory reaction in the SCID mouse target organ for most models of human autoimmune disease, except for multiple sclerosis, where the role of autoantibodies, if any, is not important. Some autoantibodies, e.g. anti-TPO, could be detected in the three major classes of immunoglobulins, mainly in the IgG [24]. When thyroid-infiltrating lymphocytes from patients with autoimmune thyroiditis were transferred to SCID mice, the same subclass distribution of autoantibodies was found in mice as in the patients [19]. In almost all instances the duration of human autoantibodies in the circulation of SCID mice was relatively short, e.g. for TG antibodies with the earliest onset at 7 days and maximum level between 3 and 4 weeks post-transplant [19]. Some autoantibodies (e.g. AMA and ANA) were detected up to 7 months post-engraftment [12,27], and anti-human TPO have been shown in SCID mice 119 days after grafting hu-PBL [28]. It is not clear whether (auto)antibodies to certain antigens persist longer than those to other antigens and, if this is the case, whether the cross-reactivity between mouse and human antigens is of importance in this respect. Autoantibodies peaked earlier than total IgG and declined before the decline of total IgG [17]. The short duration of human

autoantibodies in the circulation of SCID mice can be explained in part by the short life of human B lymphocytes in SCID mice; after several weeks virtually only T lymphocytes are detected in the spleen of SCID mice (B. Albini, personal communication). Furthermore, it appears that B lymphocytes that produce autoantibodies are even shorter lived than other B lymphocytes.

Tissue damage

The question arises of whether it is possible consistently to induce lesions in the equivalent target organ of SCID mice, similar to lesions in target organs of human autoimmune diseases, i.e. can these diseases be reproduced unequivocally in SCID mice? With the exception of lymphocytes taken from cerebrospinal fluid (CSF) of patients with multiple sclerosis, which induced central nervous system (CNS) inflammation when transferred intracisternally to SCID mice, and lymphocytes from patients with primary biliary cirrhosis, lymphocytes from patients with other autoimmune diseases did not produce inflammation of the mouse organs. Inflammatory lesions in the corresponding mouse target organs were not observed even when lymphocytes infiltrating the human target organ [19] or tissue infiltrated with lymphocytes (e.g. thyroid) [22,24] were grafted to SCID mice. Mononuclear cells isolated from CSF of patients with multiple sclerosis at the exacerbation stage but not at the remission stage, when infused intracisternally into SCID mice produced, after 4–6 weeks post-transfer scattered, discrete lesions (tissue necrosis and infiltration with inflammatory cells of presumably host mouse-origin, including macrophages and a small number of granulocytes) in the white matter of the brain stem and spinal cord. Reactive astrocytic gliosis as well as regions of demyelination occurred around necrotic foci [29]. Although the type of CSF cells transferred was not determined in these experiments (presumably mostly T lymphocytes), it is noteworthy that as few as 500 cells were sufficient to produce minimal CNS lesions, whereas 3000 cells consistently induced white matter lesions [29]. These human cells were hardly seen around the lesions, in contrast to patients with multiple sclerosis. Perhaps lymphocytes should be collected from patients with autoimmune diseases in the active stage (though this stage might be difficult to ascertain) to induce changes in the corresponding target organ of SCID mice. These mice engrafted with hu-PBL from patients with primary biliary cirrhosis had infiltrations with CD3⁺, CD4⁺ and CD8⁺ human lymphocytes, hu-Ig⁺ cells, and plasma cells around portal areas. There were also degenerative changes in or destruction of bile ducts, but similar pathology was found in some SCID mice reconstituted with normal hu-PBL, and it is apparent that these histopathological changes were due to GVHD [10]. An increase in dermal collagen in more than 80% of skin surface area was shown in all SCID mice that received hu-PBL from patients with scleroderma, but also in 1/4 of recipients of hu-PBL from normal donors [12]; however, this is a pathologic feature of human chronic GVHD [30].

Human autoantibodies to AChR were found deposited at neuromuscular junctions in SCID mice engrafted with thymus tissue from patients with myasthenia gravis [23]. Some SCID mice injected with PBL from patients with SLE showed kidney deposits of human IgG and, surprisingly, mouse C3, in a mesangial and capillary loop pattern, but the histopathological examination of kidneys showed only minimal mesangial proliferation and there was no proteinuria, or increased blood

creatinine even when lymphocytes were collected from patients with active SLE [27]. When pre-B lymphocytes isolated from (NZB × NZW) F₁ mice were injected into SCID mice, proteinuria in the latter mice was noted concurrent with deposition of IgG on the glomerular basement membrane [18]. Massive glomerular deposits of immunoglobulins were observed in SCID mice recipients of non-purified spleen cells from mice immunized with 16/6 Id, and a small number of glomerular deposits were seen in the kidneys of SCID mice engrafted with normal BALB/c spleen cells and immunized and boosted with 16/6 Id [14]. SCID mice injected with splenocytes from donor mice with SLE-like disease (produced by immunization with 16/6 Id) when boosted with the same antigen, showed on histopathological examination of the kidneys, mild to moderate thickening of the glomerular basement membrane, moderate mesangial proliferation and mild fibrotic changes [14]. Jones *et al.* [31] very recently reported (in an abstract) active induction of EAE in SCID-SJL mouse chimeras (produced by transplanting SJL fetal liver haematopoietic cells and fetal thymus tissue) immunized with encephalitogenic peptide. When these chimera did not have transplanted thymus implants, active EAE could not be induced, but EAE could be passively transferred with encephalitogenic peptide-specific T lymphocytes from SJL mice. In the spinal cord of the chimeras severe inflammation and demyelination were noted.

Changes in the function of target organs have been shown in some SCID mice engrafted with lymphocytes from patients with some autoimmune diseases. Transient hyperthyroxinaemia, peaking at 2 weeks after transfer of lymphocytes from patients with Graves' disease, was observed by some groups of investigators [22], but not by others [16,28]. SCID mice injected intracisternally with CSF cells from patients with active multiple sclerosis developed paresis and ataxia [29]. In two SCID mice injected with PBL from patients with active myasthenia gravis we noted pronounced slowness resembling mouse myasthenia (A. Vladutiu *et al.*, unpublished).

Grafting of human target tissue to SCID mice at the time of human lymphocyte transfer (or earlier) could be performed to study the mechanisms and evolution of tissue damage in human autoimmune diseases. As just mentioned, the role of autoantibodies is not known for many human autoimmune diseases (e.g. Hashimoto's thyroiditis or rheumatoid arthritis). Furthermore, it is not clear whether human autoantibodies cross-react with mouse autoantigens. Shared TG epitopes were shown for mouse and man, and human TG could induce autoimmune thyroiditis in high responder (susceptible) strains of mice [32], but the cross-reactivity of many other human autoantibodies with corresponding mouse autoantigens is not known. It has not been shown that effector T lymphocytes of human origin can be thyroidogenic in mice, nor that other T lymphocytes are activated by mouse epitopes. Akasu *et al.* [24] reported that human thyroid tissue collected from patients with Hashimoto's thyroiditis and grafted to SCID mice showed an increase in the severity of the lymphocytic infiltration several weeks after grafting, when compared with the histological picture of the tissue just after collection. This finding could suggest stimulation of human lymphocytes by mouse thyroid antigens. It is noteworthy that Macht *et al.* [19] found mostly anti-TG, whereas Davies *et al.* [16] found mainly anti-TPO in SCID mice engrafted with lymphocytes from patients with Hashimoto's thyroiditis. Volpé *et al.* [33] detected thyroid-stimulating anti-

bodies more readily than anti-TG and anti-TPO. Cross-reactivity between human anti-AChR and SCID mouse AChR has been reported [23]. It is conceivable that some mouse autoantigens, for example fragments of myelin-basic protein (MBP) or other CNS antigens, are more related to human autoantigens. Indeed, T lymphocytes from CSF of patients with multiple sclerosis seem to be stimulated *in vivo* by mouse CNS antigens in SCID mice [29]. These antigens are not necessarily MBP, because even in inbred strains of mice there are definite differences between the epitopes of MBP that are recognized by encephalitogenic T lymphocytes. There might be a need for continuous autoantigen presentation to stimulate and amplify the T lymphocyte response, and perhaps for certain antigens human B lymphocytes (from PBL) may not function effectively as APC. Even if mouse cross-reacting antigens are released in the blood of SCID mice and can be presented by mouse APC, these cells will be non-functional for human T lymphocytes. The MHC restriction between APC and CD4⁺ T lymphocytes could partly explain the lack of SCID mouse target-organ damage after injection of hu-PBL. Indeed, injection of human T lymphocytes sometime after the first transfer of hu-PBL did not help to induce target organ lesions in SCID mice [28]. As just mentioned, SCID mice have the genetic background of BALB/c mice, which are resistant (low responder) to some mouse autoimmune diseases, e.g. EAT and EAE. Although this resistance is largely due to the class II MHC antigens present on APC, there could be an influence of MHC on the target organ [34], and this might explain the failure to produce thyroiditis in SCID mice.

PITFALLS AND IMPORTANCE OF THE SCID MOUSE MODEL OF AUTOIMMUNITY

The SCID mouse model of autoimmune diseases has several pitfalls. It is obvious that these mice do not provide appropriate target organs for human lymphocytes, nor do they provide the proper milieu (e.g. lymphokines and cell adhesion molecules) for the function of human T lymphocytes. There are reports of GVHD that occurs after injection of hu-PBL [12], although most investigators did not find GVHD after this injection. Unsterile conditions (only a few reports mentioned the prophylactic use of antibiotics [16]) can lead to activation of NK cells, leakiness of SCID mice, and GVHD. In some reported experiments SCID mice were not checked for leakiness before and especially after transfer of human lymphocytes. About 70% of SCID mice became leaky after transplantation of thymus tissue from patients with myasthenia gravis [23], and it is not clear whether this was due to infections *per se* and/or to activation of immature mouse B lymphocytes by human lymphokines released by cells in the thymus tissue. If humans from whom lymphocytes are collected have been infected with Epstein-Barr virus (EBV), B cell lymphomas occur later in a large percentage of SCID mice that received PBL from these individuals, especially after i.p. but not i.v. transfer of lymphocytes [35]. Lymphomas of the grafted human thyroid were observed in 1/5 of the SCID mice that were engrafted with thyroid tissue from patients with Hashimoto's thyroiditis who had antibodies to EBV [33]. A variability of reconstitution of mice with hu-PBL via the i.p. route has been repeatedly shown. Even when lymphocytes obtained from the same patient with an autoimmune disease were used to inject several SCID mice, the results

(e.g. amount of human immunoglobulins in the circulation) were not similar [17,19], in part due to the unpredictability of hu-PBL homing and the influence of the microenvironment in each mouse. Many of the transferred hu-PBL remain in the peritoneum [16] and homing of the unimmunized cells in SCID mice may be different than that of immunized cells [14]. Regulatory lymphocytes may also be responsible for the variability of autoantibodies [19]. Macht *et al.* [17] showed that SCID mice engrafted with PBL depleted of CD8⁺ T lymphocytes from humans with Hashimoto's thyroiditis had enhanced autoantibody (anti-TG and anti-TPO) production in comparison with SCID mice repopulated with PBL. It is possible that there is a selection and growth of a small number of B cell clones in hu-SCID mice [35]. The restricted clonality of engrafted B lymphocytes was heterogeneous among different tissues and V_H diversity diminished with time following engraftment [36].

There seems to be a defect in B lymphocytes transferred from humans with autoimmune diseases. For example, SCID mice engrafted with hu-PBL from patients with autoimmune thyroiditis or engrafted with rheumatoid synovium produced less human IgG and IgM than SCID mice injected with hu-PBL from normal individuals [11,16,37]. There is a gradual decrease of human autoantibodies over time even in mice with increasing levels of total human immunoglobulins [17]. The relatively short period during which autoantibodies can be detected in the circulation could be due to [3] the short life span of autoreactive B lymphocyte clones; lack of continuous B lymphocyte stimulation in SCID mice due to the absence of human antigens (and lack of cross-reactivity between some mouse and human autoantigens); progressive loss of human T lymphocytes and rather quick reversal of CD4/CD8 ratio [16], e.g. from 3/1 1 month after engraftments to 1/2 at 2 months [27]. It is noteworthy that B lymphocytes alone, taken from patients with SLE, did not result in production of ANA, but transfer of PBL (presumably with T lymphocytes) from the same patients or from normal individuals led to the production of ANA [37]. Similarly, SCID mice repopulated with PBL, depleted of T lymphocytes or CD4⁺ lymphocytes, from patients with Hashimoto's thyroiditis had low or undetectable autoantibodies to TG and TPO in contrast to recipients of PBL [17]. These experiments show the importance of CD4⁺ T lymphocytes for the secretion of autoantibodies by B lymphocytes.

Despite the limitations of the SCID mouse model for human autoimmune diseases, this model is important, more so for human than for animal autoimmune diseases, because there are no comparable models to study these human diseases. To this end SCID mice are better than nude or irradiated mice; in the former the humoral immunity is not much impaired and in the latter there is damage to non-lymphatic tissue, and some lymphoid cells may be radio-resistant. The SCID mouse model has importance for studying general mechanisms of autoimmunity as well as importance for investigating specific autoimmune diseases. For example, in these mice it is possible to study the effect on human or mouse B lymphocytes (determined, for example, by measuring the amount and duration of human autoantibodies in the blood) of T lymphocyte subpopulations with different functions, from patients with autoimmune diseases or from autoimmune-prone and normal mice [17,18]. Perhaps a marker for the pathogenic human T cell clone can be found [3]. Analysis of the phenotypic manifestation of genetic defects that leads to autoimmune disease in the different cell

lineages of the immune system can also be performed. SCID mice can be used to determine cross-reactivity between human and mouse antigens or cytokines. These mice can also be used to study perturbation of immune regulation, since they can provide an ambience without suppressor mechanisms.

The hu-SCID mouse model of SLE can help elucidate the role of different cell types in the pathogenesis of SLE (by transferring to SCID mice different subpopulations of lymphocytes alone or in combination), and to ascertain the influence of the environment on these cells. Experiments with SCID mice suggested that T lymphocytes from patients with SLE are defective in supporting polyclonal immunoglobulin production [37]. Defects in B lymphocytes alone can lead to the development of renal disease, albeit at a slower rate, in SCID mice that had only high titres of mouse IgG or IgM ANA. It has also been shown that production of IgG2a and IgG3 autoantibodies in mice is a property of the B lineage, and that switching to these subclasses of immunoglobulins is a T lymphocyte-independent process. The sex of the environment (male or female SCID mice) did not influence the hypergammaglobulinaemia produced by transfer of lymphocytes from (NZB × NZW) F₁ mice to SCID mice [18].

The SCID mouse model can also be used to study regulatory mechanisms involved in the secretion of human antibodies to TG and TPO [17]. This model makes possible the study of the cellular interplay between antigen-producing, -presenting and -recognizing thymus cells in myasthenia gravis [23]. In the case of the SCID mouse model of multiple sclerosis, for example, studying the expression of cytokine genes in the lesions might identify cytokines responsible for the inflammatory process in the lesions, and can suggest a treatment for this disease [23].

Some findings that emerged from the study of autoimmune diseases in SCID mice are rather puzzling. For example, a booster immunization with autoantigen (e.g. human IgG or rat TPO, homologous to human TPO) had no effect on the titre of the respective human autoantibodies, i.e. there was no secondary immune response [11,16]. Human lymphocytes from patients with multiple sclerosis induced infiltrates with mouse cells, but not with donor cells in CNS of SCID mice. Thyroid tissue taken from patients with Hashimoto's thyroiditis showed an increased severity of lymphocytic infiltration after grafting to SCID mice [24].

CONCLUSIONS

In future experiments, mice with the *scid* mutation on a different genetic background (H-2) should be used to determine the role of MHC, if any, on induction of target organ lesions in hu-PBL-SCID mice. Primary immunization with human autoantigens (together with adjuvants) of SCID mice reconstituted with normal hu-PBL and human APC should be further attempted. Transplantation to SCID mice of normal human tissue against which the autoimmune response of T and B lymphocytes is directed should be performed to determine whether this tissue will be injured by the immune response mounted by human lymphocytes in the mouse environment. These grafts could be, for example, normal pancreas, thyroid, synovial tissue or intestinal mucosa. Once T lymphocytes migrate to the tissues, these cells can be isolated and characterized (i.e. with respect to their TCR), since they are a pool of organ-specific pathogenic T lymphocytes. The administration of certain human lympho-

cytes to SCID mice could lead to an increase in the amount of autoantibodies produced in these mice. Cloned T and B lymphocytes of human origin (e.g. B lymphocytes that produce AChR antibodies) or T cell lines isolated from the target organs of humans with autoimmune diseases could be transferred to SCID mice, and these lines may prove to be better than hu-PBL for studying human autoimmune diseases in these mice.

Other human autoimmune diseases could be studied in the SCID mouse model (e.g. insulin-dependent diabetes mellitus) by transferring PBL, lymphocyte-infiltrated tissue or lymphocyte lines from patients with these diseases. Mild x-irradiation (e.g. 2 Gy) before transfer, and treatment of SCID mice with human growth hormone or anti-asialo-GM-1 (to eliminate NK cells) [21] could lead to longer survival of human lymphocytes in SCID mice. They could be injected with T lymphocytes from humans with autoimmune diseases, together with epithelial cells (e.g. thyrocytes) from normal individuals cultured *in vitro* with factors known to increase expression of class II MHC antigens (e.g. interferon-gamma). Thus, it could be determined whether these epithelial cells can act *in vivo* as APC for mouse organ-specific antigens. Finally, lymphoid cells from mice with autoimmune diseases (induced as well as spontaneous, e.g. NOD mice) should be transferred to SCID mice. This could allow a better understanding of the pathogenesis of these diseases, especially when using SCID mice with several genetic backgrounds. Lymphocyte-infiltrated target organs (e.g. thyroids from mice with EAT) could be grafted to SCID mice, or infiltrated lymphocytes could be separated from organs of mice with autoimmune diseases and transferred to SCID mice. For mouse autoimmune diseases at least, the role of autoantibodies *versus* T lymphocytes can be studied by injecting SCID mice with different populations of mouse lymphocytes, collected at various time intervals after immunization of donor mice.

Although important questions in autoimmunity have not been answered by the use of the SCID mouse model of autoimmunity, it is conceivable that future experiments with these mice could unravel mechanisms characteristic for certain autoimmune diseases in humans. The treatment of some autoimmune diseases (e.g. use of anti-sense oligonucleotides or anti-TCR antibodies) could also benefit from valuable information from the SCID mouse model. Finally, the study of mouse autoimmune diseases with the use of SCID mice might be rewarding as well.

ACKNOWLEDGMENTS

I thank Dr Robert Volpé for providing me with preprints of his work, and Mrs Jacqueline Teitz for preparing the manuscript for publication.

REFERENCES

- Rose NR, Witebsky E. Studies on organ specificity V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *J Immunol* 1956; **76**:417-27.
- Vladutiu AO, Rose NR. Autoimmune murine thyroiditis: relationship to histocompatibility (H-2) type. *Science* 1971; **174**:1137-39.
- Barry TS, Haynes BF. *In vivo* models of human lymphopoiesis and autoimmunity in severe combined immune deficient mice. *J Clin Immunol* 1992; **12**:311-23.
- Mosier DE. Adoptive transfer of human lymphoid cells to severely immunodeficient mice: models for normal human immune function, autoimmunity, lymphomagenesis, and AIDS. *Adv Immunol* 1991; **50**:303-25.

- 5 Carroll AM, Hardy RR, Bosma MJ. Occurrence of mature B (IgM⁺, B220⁺) and T (CD3⁺) lymphocytes in scid mice. *J Immunol* 1989; **143**:1087-93.
- 6 Bosma GC, Fried M, Custer RP *et al.* Evidence of functional lymphocytes in some (leaky) scid mice. *J Exp Med* 1988; **167**: 1016-33.
- 7 Fullop GM, Phillips RA. Full reconstitution of the immune deficiency in scid mice with normal stem cells requires low-dose irradiation of the recipients. *J Immunol* 1986; **136**:4438-43.
- 8 Abedi MR, Christenson B, Islam KB *et al.* Immunoglobulin production in severe combined immunodeficient (SCID) mice reconstituted with human peripheral blood mononuclear cells. *Eur J Immunol* 1992; **22**:823-8.
- 9 Duchosal MA, Eming SA, Fisher P *et al.* Immunization of Hu-PBL-SCID mice and the rescue of human monoclonal Fab fragments through combinational libraries. *Nature* 1992; **355**:258-62.
- 10 Tary-Lehman M, Saxon A. Human mature T cells that are anergic *in vivo* prevail in SCID mice reconstituted with human peripheral blood. *J Exp Med* 1992; **175**:503-16.
- 11 Tighe H, Silverman G, Kozin F *et al.* Autoantibody production by severe combined immunodeficient mice reconstituted with synovial cells from rheumatoid arthritis patients. *Eur J Immunol* 1991; **20**:1843-8.
- 12 Krams SM, Dorshkind K, Gershwin E. Generation of biliary lesions after transfer of human lymphocytes into severe combined immunodeficient (scid) mice. *J Exp Med* 1989; **170**:1919-30.
- 13 Teitel A, Hines JJ, Gharavi AE *et al.* Reconstruction of SCID mice with mononuclear cells and skin from human scleroderma patients. *Arthritis Rheum* 1991; **34**:S44 (Abstr.).
- 14 Segal R, Globerson A, Zinger H *et al.* Induction of experimental systemic lupus erythematosus (SLE) in mice with severe combined immunodeficiency (SCID). *Clin Exp Immunol* 1992; **89**:239-43.
- 15 Fox RI, Pisa P, Pisa EK *et al.* Lymphoproliferative disease in SCID mice reconstituted with human Sjögren's syndrome lymphocytes. *J Clin Lab Anal* 1993; **7**:46-56.
- 16 Davies TF, Kimura H, Fong P *et al.* Thw SCID-hu mouse and thyroid autoimmunity: characterization of human thyroid autoantibody secretion. *Clin Immunol Immunopathol* 1991; **60**:319-30.
- 17 Macht LM, Corral RJ, Banga JP *et al.* Control of human thyroid autoantibody production in SCID mice. *Clin Exp Immunol* 1993; **91**:390-6.
- 18 Reiningger L, Radaszkiewicz T, Kosco M *et al.* Development of autoimmune disease in SCID mice populated with long-term 'in vitro' proliferating (NZB × NZW)F₁ pre-B cells. *J Exp Med* 1992; **176**:1343-53.
- 19 Macht L, Fukuma N, Leader K *et al.* Severe combined immunodeficient (SCID) mice: a model for investigating human thyroid autoantibody synthesis. *Clin Exp Immunol* 1991; **84**:34-42.
- 20 Adams CD, Zhou T, Mountz JD. Transplantation of human rheumatoid synovium into a SCID mouse as a model for disease activity. *Arthritis Rheum* 1990; **33**:S120 (Abstr.).
- 21 Rendt KE, Barry TS, Jones DM *et al.* An *in vivo* model of human rheumatoid arthritis (RA) using RA synovium engrafted into severe combined immunodeficient (SCID) mice. *FASEB J* 1991; **5**:1371 (Abstr.).
- 22 Morita T, Yoshikawa N, Resetkova E *et al.* Thyroid xenografts from autoimmune thyroid disease in SCID mice. *Thyroid* 1992; **2** (Suppl.1):S-47 (Abstr.).
- 23 Schönbeck S, Padberg F, Hohlfeld R *et al.* Transplantation of thymic autoimmune environment to severe combined immunodeficiency mice. A new model for myasthenia gravis. *J Clin Invest* 1992; **90**:245-50.
- 24 Akasu F, Morita T, Resetkova E *et al.* Reconstitution of severe combined immunodeficient (SCID) mice with intrathyroidal lymphocytes of thyroid xenografts from patients with Hashimoto's thyroiditis. *J Clin Endocrin Metab* (in press).
- 25 Ashany D, Hines JJ, Gharavi AE *et al.* Reconstitution of SCID mice with cells from human and murine lupus. *Arthritis Rheum* 1990; **33**:R35 (Abstr.).
- 26 Duchosal MA, Eming SA, McConahey PJ. The hu-PBL-SCID mouse model: long term human serologic evolution associated with the xenogeneic transfer of human peripheral blood leukocytes into SCID mice. *Cell Immunol* 1992; **139**:467-77.
- 27 Duchosal MA, McConahey PJ, Robinson CA *et al.* Transfer of human systemic lupus erythematosus in severe combined immunodeficient (SCID) mice. *J Exp Med* 1990; **172**:985-8.
- 28 Martin A, Kimura H, Thung S *et al.* Characteristics of long-term human thyroid peroxidase autoantibody secretion in *scid* mice transplanted with lymphocytes from patients with autoimmune thyroiditis. *Int Arch Allergy Immunol* 1992; **98**:317-23.
- 29 Saeki Y, Mima T, Sakoda S *et al.* Transfer of multiple sclerosis into severe combined immunodeficiency mice by mononuclear cells from cerebrospinal fluid of the patients. *Proc Natl Acad Sci USA* 1992; **89**:6157-61.
- 30 Parkman R. Graft versus host disease. *Ann Rev Med* 1991; **42**:189-97.
- 31 Jones RE, Whitham R, Bourdette D. Induction of EAE in SCID mouse-SJL mouse chimeras. *J Immunol* 1993; **150**:81A (Abstr.).
- 32 Kong Y-C. Shared thyroglobulin epitopes in autoimmune thyroiditis. In: Bigazzi PE, Wick G, Wicher K, eds. *Organ-specific autoimmunity*. New York: Marcel Dekker, 1990:229-40.
- 33 Volpé R, Kasuga Y, Akasu F *et al.* The use of severe combined immunodeficient mouse and the athymic 'nude' mouse as models for the study of human autoimmune thyroid disease. *Clin Immunol Immunopathol* 1993; **67**:93-99.
- 34 Okayasu I. The differences of susceptibility of the target thyroid gland to autoimmune thyroiditis and of antigenicity of thyroid gland for induction of experimental autoimmune thyroiditis. *Clin Immunol Immunopathol* 1986; **41**:392-8.
- 35 Duchosal MA, Eming SA, McConahey PJ *et al.* Characterization of hu-PBL-SCID mice with high human immunoglobulin serum levels and graft-versus-host disease. *Am J Pathol* 1992; **141**:1097-13.
- 36 Saxon A, Macy E, Dennis K *et al.* Limited B cell repertoire in severe combined immunodeficient mice engrafted with peripheral blood mononuclear cells derived from immunodeficient or normal humans. *J Clin Invest* 1991; **87**:658-65.
- 37 Geppert TD, Jasin HE. Immunoglobulin (Ig) and autoantibody production in mice with severe combined immunodeficiency (SCID) injected with lymphocytes from patients with systemic lupus erythematosus (SLE). *Arthritis Rheum* 1990; **33**:S99 (Abstr.).
- 38 Dickey WD, Scofield RH, Tateya D *et al.* Human autoantibody xenografts in severe combined immunodeficiency (SCID) mice. *FASEB J* 1990; **4**:A2102 (Abstr.).