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Review

Fate and functions of human adult lymphoid cells in immunodeficient mice

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Summary. Laboratory models enabling to study *in vivo* human leukocyte functions have been developed. Most of the models consist of human immunocytes transferred to mice homozygous for the *scid* mutation. Mice with additional immunodeficient-prone genetic background or with immunodeficiency-induced conditioning have also been used. Human grafts mainly consisted of human immune cells in suspension injected intraperitoneally, or in pieces of human organs containing immunocytes implanted subcutaneously. Cells in suspension could be easily manipulated *in vitro* before transfer to the animal, but disseminated within the mouse body. In opposition, human cells mostly remained within implantation areas of animals given human organ pieces. This favorizes cell interactions and helps for cell recovery after their *in vivo* passage. Moreover, the diversity of antibodies in animals transplanted with human lymphoid organ pieces appeared broader than that of mice transferred with lymphocytes in suspension. Spontaneous recall antibody and autoantibody productions have been generally observed in animals transferred with cells from donors with such antibodies. *In vivo* boosting of recall antibody by antigen has been most successful, but such a manipulation inconstantly boosted autoantibodies. Primary human T and B cell responses were difficult to obtain in xenochimeric animals, and success has been generally obtained by optimizing human immune response parameters, such as antigen presentation.

Key words: SCID mice, Human lymphoid cells, Immune response

Introduction

Investigations of *in vivo* mechanisms underlying differentiation and functions of human hematolymphoid cells in humans are limited by human ethical or technical constraints. Therefore, there is an interest for developing animal models supporting *in vivo* human cell survival and functions. The *in vivo* fate of human hematolymphoid cells has been mainly studied in xenochimeric models consisting of immunodeficient animals transplanted with human cells. In particular, human peripheral blood leukocytes (PBL) as well as mature lymphoid cells from adult human tissues have been transferred to immunodeficient mice without being rejected. This review focuses on the fate and characteristics of mature human lymphoid cells in xenochimeric immunodeficient mouse models, and on their function towards primary and secondary immunizations.

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Mouse recipients of human cells

Genetic background of mouse recipient

Various types of immunodeficient mice have been utilized as recipients of human graft. First, athymic nude mice, which are deficient for T cell-mediated immune responses, were populated with human PBL, with lymphocytes infiltrating various human organs, and with organ pieces containing immunocytes with variable success. In general, human lymphocytes were short-lived in the animals. In particular, nude mice have been implanted with human thyroid xenografts. In this environment, human thyrocytes survived, but human intrathyroidal infiltrating lymphocytes did not (Usadel et al., 1987; Morita et al., 1993; Kawai et al., 1998). The disappearance of human lymphocytes can be attributed to the presence of mouse natural killer (NK) cells and B lymphocytes mediating lysis of human lymphocytes.

Rag-1- or Rag-2-deficient mice have been generated by knocking-out the recombination-activating gene-1 (rag-1) or -2 (rag-2), respectively. These mice lack mature T and B cells (Mombaerts et al., 1992; Shinkai et al., 1992). Rag-2 knock-out mice have been successfully engrafted with human liver cells or thyroid tissue (Martin et al., 1994; Brown et al., 2000). However, engraftment of human lymphocytes, including human

PBL, was limited in Rag-1- or Rag-2-deficient mice as judged by low production of human Ig in mouse sera and of human B and T cells in xenochimeric animals (Martin et al., 1994; Steinsvik et al., 1995; Berney et al., 2001b). Engraftment of human PBL can be optimized using Rag-2-deficient mice in which the common cytokine receptor gamma chain has been knocked-out. Mice with this latter knock-out have no mouse NK cells, and animals with both gene deletions are better recipients of human T cells than animals solely deficient in Rag-2, attested by higher levels of human $CD45^+$ and human $CD4^+$T cells in their spleen (Goldman et al., 1998).

Mice homozygous for the severe combined immune deficient (*scid*) mutation (SCID mice) lack both humoral and cell-mediated immunity due to the absence of mature B and T cells. These mice are better recipients for human cells, including PBL, than Rag-2-deficient mice and have been extensively used for xenotransplantation (Steinsvik et al., 1995). The strain background on top of which the *scid* mutation is introduced influences engraftment. For example, C.B-17/SCID mice reject less PBL graft than C3H/SCID mice do (Marcus et al., 1996). This observation could be secondary to C.B-17/SCID mice having lower NK cell activity than C3H/SCID mice (Nonoyama et al., 1993a). Indeed, mouse NK cell presence has been linked with human PBL rejection, and SCID mice have generally higher NK cell activity than wild-type mice (Greiner et al., 1998), probably reflecting a compensatory effect following the absence of an adaptive immune system. To diminish NK cell activity in SCID mice, the *scid* mutation has been back-crossed onto mouse strains with deficient NK cell activity, such as mice homozygous for the beige (*bg*) mutation. SCID-*bg*/*bg* mice have an acceptance rate of human skin grafts superior to that of the grafts on SCID mice (Takizawa et al., 1997). SCID-bg/bg also readily accept human PBL (Berney et al., 2001a), but, in contrast to skin grafting, the level of PBL engraftment is relatively low and similar to that in populated SCID mice when the mutations were implanted on a C57BL/6 background (Christianson et al., 1996).

NOD mice are partially deficient in NK cell, myeloid cell, and macrophage functions (Shultz et al., 1995). By backcrossing the *scid* mutation onto NOD mice, NOD/SCID mice have been generated that exhibit not only an impaired adaptive immune system, but also a marked reduction of NK cell and of myeloid cell/macrophage activities (Shultz et al., 1995). These characteristics are additive and NOD/SCID mice accept human lymphoid cells more readily than SCID mice do (Christianson et al., 1997b). More recently, NOD/SCID/ß2-/- mice have been generated by backcrossing the ß2 microglobulin null allele (ß2-/-) onto NOD/SCID background. ß2 microglobulin knock out prevents cell surface MHC class I expression and abolishes the residual low NK cell activity of NOD/SCID mice (Raulet, 1994; Christianson et al., 1997b). Thus, NOD/SCID/ß2-/- mice have essentially undetectable levels of NK cell activity, and are better recipients of human $CD4^+$T cells than NOD/SCID animals (Christianson et al., 1997b). Mice with a NOD/SCID background have a high incidence of thymic lymphomas (Prochazka et al., 1992). This reduces the mean life-span of NOD/SCID mice to 8 months (Shultz et al., 1995) and that of NOD/SCID/ß2-/- mice to 6 months (Christianson et al., 1997b), and constitutes a limitation in their use for long-term PBL repopulating capacity studies.

Somatic manipulations of mouse recipients

Mouse recipients have been conditioned prior to cell transfer to improve human cell engraftment. In particular, SCID and NOD/SCID mice received sublethal doses of total body irradiation and/or were injected with antibodies directed toward specific mouse NK cell surface molecules. Injection of NK-depleting anti-asialo GM1 or N.K.1.1 antibodies did not significantly increase human cell survival in SCID mice, while a dose of 2.5 to 3 Gy radiation slightly increased human cell engraftment (Duchosal et al., 1992b; Shpitz et al., 1994; Christianson et al., 1996). However, combination of irradiation and anti-asialo GM1 antibody injection significantly improved engraftment of human T, B and NK cells compared to either treatment alone (Shpitz et al., 1994). SCID mice were also treated, prior to cell transfer, with a rat antibody (TM-ß1) directed against the ß-chain of the murine IL-2 receptor. This latter manipulation significantly increased the survival of transplanted human cells when evaluated 4 weeks after PBL transfer (Tournoy et al., 1998).

Another approach aiming at improving human cell engraftment consisted in transferring human bone marrow cells or human PBL with SCID mouse bone marrow to lethally irradiated immunocompetent mice. The “trimera” mouse obtained readily accepted human cells (Lubin et al., 1991). When human PBL were used and injected intraperitoneally (I.P.), human T and B cells were detectable in the peritoneum of the animals for about two months and also migrated to mouse organs where they were detectable for two to three months (Lubin et al., 1994).

Fate of human cells in xenochimeric models

Transfer of human cells in immunodeficient mice is a powerful technology to study the engraftment capacity and functions of human mature immune cells in an *in vivo* environment. Various results have been obtained depending on the route of implantation and on the source of human cells used.

Human cells in suspension have been mostly injected I.P. or intravenously (I.V.), while human organ pieces have been primarily implanted in the subcutaneous tissue or in the peritoneal cavity. Human cell engraftment has been generally evaluated by measuring human Ig levels in mouse serum, or by

evidencing human CD45+ cells in mouse organs, in implants or in blood. Intravenously-injected cells engraft preferentially in bone marrow while I.P. injection rather leads to peritoneum, spleen, and in some cases thymus localization (Rice et al., 2000). When compared to I.P. injection, I.V. injection results in a weaker cell engraftment when determined by human Ig production (Mosier et al., 1988; Martino et al., 1993a). For this reason, most investigators inject cell suspension in the mouse peritoneal cavity. The threshold for observing successful lymphoid cell transfer following I.P. injection has been evaluated by several authors, and it appears that suspensions containing at least 10^7 human peripheral blood mononuclear cells or tonsillar cells are necessary for observing repeatedly good engraftment. A complication under the form of human B cell lymphoproliferative disease may arise in animal recipients of immunocytes from donors with previous contact with the Epstein-Barr virus (EBV). We convey the reader to extensive reviews on this subject that will not be detailed here (Mosier et al., 1992; Fuzzati-Armentero and Duchosal, 1998; Johannessen and Crawford, 1999).

Fate of human peripheral blood cells injected in mice

Human PBL injected I.P. in immunodeficient mice engraft and after three weeks most of human cells are recovered in peritoneal fluid where human CD45+ cells represent about 60% of the whole cell content. With time, human cells disperse within the whole mouse body and can be found in almost every mouse organ (see Fig. 1). Human cells are then notably highly concentrated in the thymus and to a lesser extent in spleen, liver and lung, and are also present in bone marrow and peripheral blood (Nadal et al., 1991; Abedi et al., 1992; Duchosal et al., 1992b; Shpitz et al., 1994). Some authors also reported human cell presence in kidney (Fig. 1) (Abedi et al., 1992; Duchosal et al., 1992b). In general, no human cells were detected in intestine, heart or skin. At immunohistochemistry analysis, we found that the majority of human cells infiltrating SCID mouse lymphoid and non-lymphoid organs were T cells, and that few B cells were present (Duchosal et al., 1992b). This observation was confirmed by FACS analyses of xenochimeric mouse spleen cells one month post PBL transfer, where human cells predominantly contained T cells, whereas B cells and NK cells were less represented (Shpitz et al., 1994). Macrophages were marginally detected (Garcia et al., 1997). The above-described cell subset distribution within the mouse correlated well with cell compartment proportion within the PBL graft. Indeed human T cells predominate within peripheral blood cells, and macrophages represent a low percentage of PBL, further reduced during purification procedures. The dispersion in all mouse organs of mature human cells with low proliferation potential may hamper their detection using cellular (FACS, immunohistochemistry), and molecular biology (PCR) techniques. This may explain the lack of recognition of human B cells within main lymphoid mouse organs while human Ig were still detected in serum from some animals (Saxon et al., 1991; Murphy et al., 1992).

Injection of purified B or T cells separately demonstrated that B cells alone are not able to engraft in mouse (Ueno et al., 1992; Nonoyama et al., 1993b), and that the presence of T cells is necessary for human B cell engraftment. The essential role of CD4+ T cells for observing neonatal B cell engraftment was demonstrated by co-injection of B cells and of purified sub-population of T cells. CD4+ T cells allowed B cell engraftment, as demonstrated by detectable human Ig in the animals, while T cells depleted of their CD4+cell content did not (Hasui et al., 1994). A more general influence of CD4+T cells on adult PBL engraftment has recently been demonstrated by the observation that PBL depleted of CD4+ cells were not detectable after their transfer in the animals, whereas CD8+- or CD14+-depleted PBL were readily demonstrable (Duchosal et al., 2001). It should be mentioned that CD4+ T cells promote both PBL engraftment and graft-versus-host reaction in xenochimeric models including SCID mouse models (Huppes et al., 1994; Tary-Lehmann et al., 1994; Sandhu et al., 1995).

Fate of human lymphoid cell suspensions from organs injected in mice

Cell suspensions from human lymphoid organs such as tonsil, spleen or thymus have been transferred to SCID mice. In particular, tonsil cells in suspension, I.P. injected, engrafted into the mouse and disseminated within the whole animal body similarly to PBL. In such populated animals, cell subset analysis showed that: a) B cells (detected by secretion of human Ig) were detectable in peritoneal cavity, liver and lung, and to a lesser extend in blood, spleen and bone marrow; and b) T cells were present in spleen (Nagasawa et al., 1996). Using such a model, Nadal et al. failed to evidence human cells in lymph node and intestine (Nadal et al., 1991).

Human splenocytes were also successfully engrafted in SCID mice after I.P. injection (Alegre et al., 1994). Three weeks after cell transfer, most of the human CD45+ cells were localized in the peritoneal cavity. Human cells were also highly represented in lymph node, blood and spleen, whereas few human CD45+ cells were detected in the thymus. Cell subset analysis demonstrated that most of the human cells present in the peritoneal cavity and in the various mouse organs were CD3+ T lymphocytes. The proportion of CD19+ B cells was reduced when compared to that of the human inoculum, and only a few CD14+ monocytes engrafted in murine compartments.

The fate of human thymocytes injected into SCID mice appears variable. No human CD45+ cells were detected by FACS in mouse organs and peritoneum 120 days after I.P. injection of up to 10^8 thymocytes from a healthy donor, indicating that cell suspension from

human thymus does not readily engraft in SCID mice (Tary-Lehmann and Saxon, 1992). In opposition, Yoshikawa et al. I.P. injected mice with 1.6 to $4x10^7$ thymocytes from patients with myasthenia gravis. The authors could evidence $CD3^+$T cells and $CD20^+$B cells by immunostaining in the peritoneal cavity of the mice up to 260 days after cell transfer (Yoshikawa, 1998).

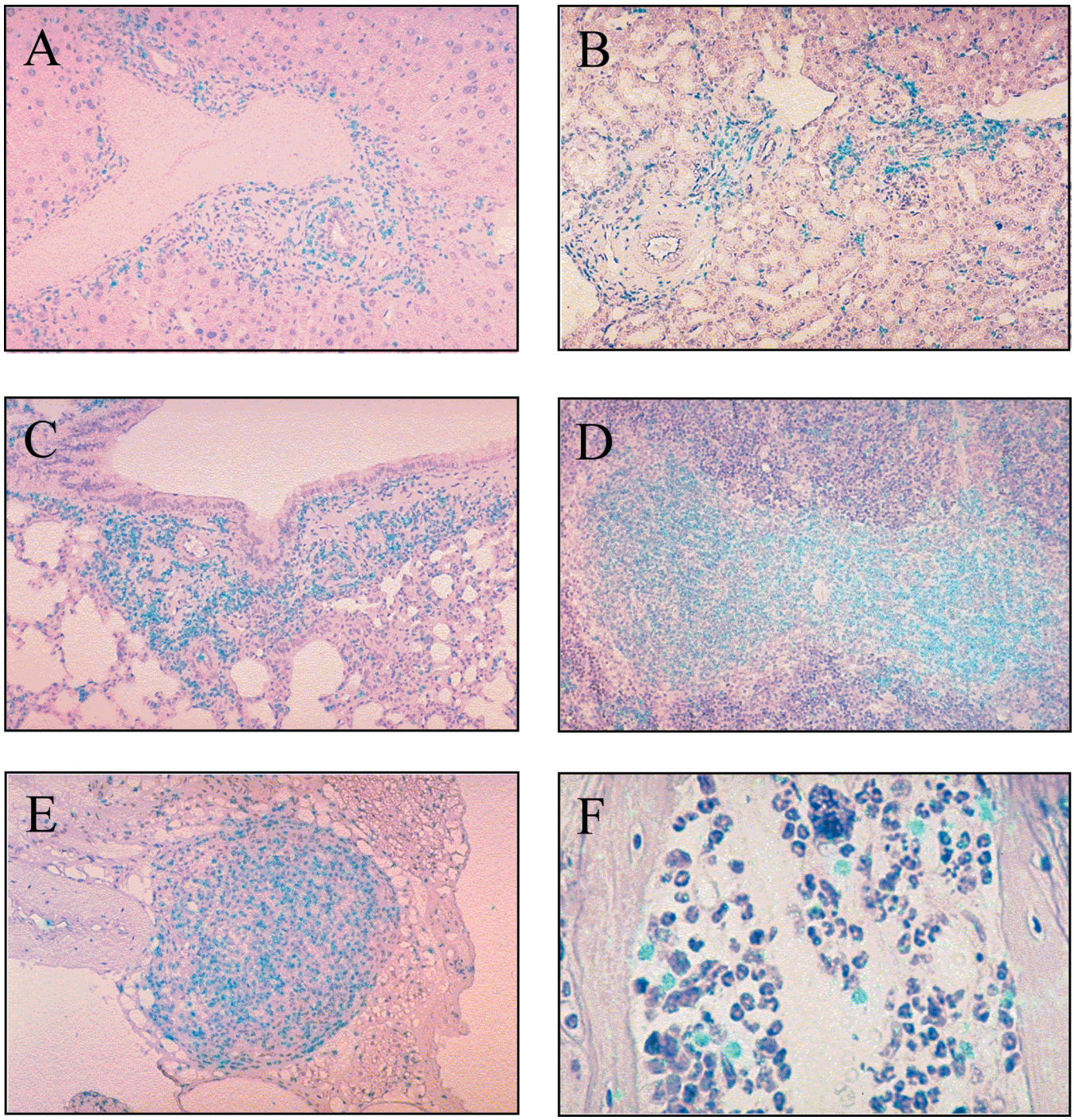

Fig. 1. Photomicrographs of sections of organs from PBL-populated SCID mice. SCID mice were I.P. injected with human PBL and sacrificed one month after transplantation. Human cells are evidenced by in situ hybridization with a human-specific ALU-repeat family probe (turquoise dots) and sections are Periodic acid-Schiff counterstained. **A.** Liver. x 10. **B.** Kidney. x 10. **C.** Lung. x 10. **D.** Spleen. x 10. **E.** Lymph node. x 10. **F.** Bone marrow. x 20

Fate of pieces of human non-lymphoid organ implanted in mice

Pieces from several human non-lymphoid organs have been implanted in immunodeficient mice to study their functions and in some cases their immune-based associated pathologies. In particular, human synovial tissues have been implanted either subcutaneously or under the renal capsule of SCID mice and of SCID-*bg*/*bg* mice. The human graft persisted over 300 days (Geiler et al., 1994). Four to six weeks after implantation, synovial grafts were vascularized by human and mouse vessels (Proudman et al., 1999; Wahid et al., 2000) and histological appearance of engrafted synovium was similar to that of fresh synovium (Rendt et al., 1993; Proudman et al., 1999). In particular, xenografts of healthy synovium contained only a small number of human fibroblasts and infrequent human $CD14^+$ macrophages, whereas human macrophages, B cells ($CD20^+$) and T cells ($CD4^+$or $CD8^+$) persisted for at least four weeks after engraftment with pieces from rheumatoid arthritis (RA) patients (Rendt et al., 1993; Proudman et al., 1999). In these models, the clustering of human immune cells within the implant is indicated by the observation that few human $CD45^+$ cells were found within mouse PBL, and no human cells were detected in mouse liver, spleen or thymus.

Human thyroid pieces from healthy donors or from patients with thyroiditis such as Hashimoto's thyroiditis and Graves' disease (GD) have been subcutaneously implanted in SCID mice (Akasu et al., 1993; Morita et al., 1993; De Riu et al., 1994; Resetkova et al., 1996; Fisfalen et al., 1997; Yoshikawa et al., 1997). In general, thyroid tissue architecture persisted in the mouse, and some intrathyroidal lymphocytes migrated to the mouse spleen (Akasu et al., 1993). Six weeks after engraftment, the level of human IgG was low in mice implanted with thyroid pieces from healthy donors, while levels of human IgG were elevated in mice implanted with GD thyroid. Animals implanted with thyroid pieces from GD patients had autoantibodies to thyroglobulin and to thyroperoxydase in their sera similarly to GD patients (Yoshikawa et al., 1997; Kawai et al., 1998).

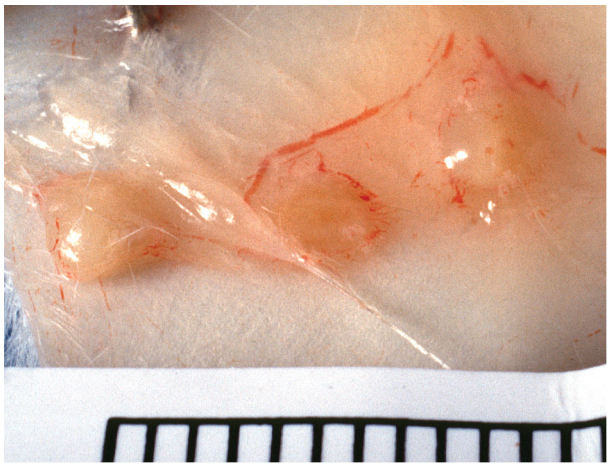

Fig. 2. Photograph of three tonsil implants in a SCID mouse subcutaneous area. One SCID mouse was subcutaneously implanted with tonsil pieces and the xenochimeric mouse was sacrificed 30 days thereafter. Scale: each graduation corresponds to one millimeter.

SCID mice have been implanted with human skin pieces from healthy donors. Such models were utilized to study graft rejection and angiogenesis (for review: Boehncke, 1999; Nickoloff, 2000). Two to three weeks after transplantation, skin grafts healed and supported physiological keratinocyte differentiation for at least two months (Murray et al., 1994; Nickoloff et al., 1995). In this model, human microvessels were anastomosed to murine microvessels and grafts were adequately perfused (Murray et al., 1994). Skin grafts from patients with various dermatological diseases such as psoriasis, pemphigus or alopecia have been implanted and several characteristics of the diseases were reproduced. In particular, histological and immunohistological features associated with psoriasis, notably the infiltration of human $CD4^+$ and $CD8^+$ T cells, were present for up to 16 weeks within skin grafts derived from psoriasis plaques (Nickoloff et al., 1995; Raychaudhuri et al., 2001).

Fate of human primary lymphoid organ pieces implanted in mice

Lymphoid organ pieces have been implanted in immunodeficient mice, in particular postnatal human thymus, as well as thymus from patients with myasthenia gravis (Barry et al., 1991; Schonbeck et al., 1993). When examined three months after implantation, the graft originating from healthy thymus was increased in size compared to the original implant size, and was vascularized. This latter process was boosted by mouse host NK cell depletion using anti-asialo GM1 antibody. Human thymocytes persisted in the thymic graft, but no human cells were detected in mouse PBL, spleen or thymus (Barry et al., 1991).

Fate of human secondary lymphoid organ pieces implanted in mice

Pieces from various human secondary lymphoid organs have been implanted in SCID mice. The function of such implanted organs has been subsequently compared to that of suspension of cells from similar origin injected I.P. In particular, SCID mice have been subcutaneously implanted with human adult tonsil pieces distributed in five subcutaneous areas (Duchosal et al., 2000). Such xenochimeric mice had more than three times higher levels of human Ig one month after implantation than mice injected with three times more cells in suspension. High levels of human Ig in the sera of such implanted animals persisted over 9 months. The proportion of subcutaneous areas containing implants decreased with time, and implants were recovered in all

implantation areas in 42% of mice 1 month after surgery (one implant area depicted in Fig. 2). In some cases, human tonsil pieces persisted for over a year. Human cells were observed only in implants and not in mouse organs, which indicated that live cells did not disseminate in the mouse. Immunocytochemical analysis showed that human B cells, T cells and macrophages were present in human implants, even when the architecture of the implant adopted a more diffuse appearance compared to that of the original tonsil follicle (Duchosal et al., 2000; Blades et al., 2002). Blades et al. subsequently analyzed lymph-node pieces implanted in mouse dorsal area. They demonstrated that implants were vascularized by mouse subdermal vessels anastomosed to implant vessels, and that stromal cell-derived factor 1 (SDF-1) could induce human PBL migration to the implant (Blades et al., 2002).

In summary, it appears that suspension of cells as well as pieces of several human organs engraft in SCID mice. Human cell suspension, injected I.V. or I.P., tends to spread in the animal body, while implantation of organ pieces leads to the persistence of human cells within the implant areas. This latter mouse model facilitates the recovery of implanted cells after their *in vivo* passage for further analyses and optimizes interactions between the cells participating in immune response.

Functions of human cells in xenochimeric mice

Human B cells in non-immunized xenochimeric mice

B cell function in xenochimeric mice has usually been evaluated by measuring human Ig presence in mouse serum. Human IgG became detectable few days after PBL injection and serum levels increased to reach a peak about 60 days after cell transfer. At this time and in our hands, mean level of human IgG in mouse serum reached up to 4.4 mg/ml. IgG level generally decreased gradually after 60 days post cell transfer but was still detectable 2 years after engraftment. IgM levels usually peaked between 60 and 90 days post PBL transfer, similarly to IgG, and decreased thereafter (Duchosal et al., 1992b). Mice injected with PBL from donor with previous contact with the EBV tend to have much higher levels of human IgM than those given cells from EBV-negative donors (Abedi et al., 1992; Duchosal et al., 1992b). IgA has also been detected in populated animals, with mean levels 60-90 days post cell transfer between 0.1 and 87 μg/ml (Abedi et al., 1992). No production of human IgE is observed in SCID mice by injecting PBL from healthy donors. However, human IgE are detected by using PBL from allergic patients (Ito et al., 1992; Spiegelberg et al., 1994). It should be noted that a great variability has been observed from mouse to mouse for every Ig type (Abedi et al., 1992; Duchosal et al., 1992b). This variability is partly dependent on the Ig concentration in the serum of the donor, but variability between animals similarly populated also exists.

Human Ig half-life in SCID mice has been measured by injecting monoclonal human Ig or human serum in mice and by evaluating the decay of the respective human Ig class in mouse serum. The half-lives of human Ig in SCID mice are generally shorter than that of similar proteins in humans, but studies reported a large variability in T 1/2 values (Table 1). This may result from the various Ig preparations utilized, and also by the levels of Ig obtained in sera of injected animals known for influencing Ig catabolism. In addition, clearance of human IgG1 appears much more rapid in NOD/SCID/ß2-/- mice than in NOD/SCID mice (Christianson et al., 1997b), an observation that may be secondary to the absence of an "IgG protective [FcRn] receptor" whose expression is dependent upon that of ß2 microglobulin (Ghetie et al., 1996; Junghans et al., 1996; Christianson et al., 1997a).

To improve human cell clustering, Depraetere et al. (2001) injected human PBL directly into the mouse spleen. By using this procedure, they observed significantly higher serum levels of human IgG and IgM when compared to those of I.P. injected animals. We used another strategy by implanting lymphoid organs subcutaneously in mice. We observed that IgG levels one month after implantation were significantly higher in mice implanted with tonsil pieces (1.9±0.3 mg/ml, n=3) than in mice I.P. injected with $5x10^7$ tonsil cell suspension (0.3±0.09 mg/ml, n=10) (Duchosal et al., 2000). These observations supported a high engraftment level of the human B cell population and probably its expansion by using one of the above-reported transplantation routes.

The clonality of human B cells producing Ig in xenochimeric animals has been assessed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and isoelectric focusing (IEF) analyses. Polyclonal human Ig production was observed 3 to 4 weeks after transfer of human PBL by I.P. injection in SCID mice. After this time, a gradual shift to oligoclonality led to a restriction of the B cell repertoire (Saxon et al., 1991; Abedi et al., 1992; Tissot et al., 1996). Interestingly, mice implanted with tonsil pieces had human Ig polyclonality persisting over time, and little clonal restriction was observed (Layer et al., 2000).

Table 1. Human immunoglobulin half-lives.

	IgG	IgM	IgA	IgE
Human Ig half-life in SCID mice	4-12 days	0.6-4.3 days	1 day	1 day
Human Ig half-life in humans	21-25 days	5 days	6-7 days	2.3 days

From: Hassan et al., 1991; Abedi et al., 1992; Duchosal et al., 1992c; Donjon et al., 1993; Steinsvik et al., 1997.

Human T cells in non-immunized xenochimeric mice

Human PBL engraft in SCID mice following their I.P. injection in the animal. Human cells in the mouse were predominantly $CD3^+$ T cells, with most of them bearing the $CD4^+CD8^-$ or the $CD8^+CD4^-$ phenotype and only a few of them being $CD4^+CD8^+$ T cells (Tary-Lehmann et al., 1992; Garcia et al., 1997). Moreover, most T cells expressed markers characteristic of activated ($HLA\text{-}DR^+$) and memory ($CD45RO^+$) phenotypes. CTL function present in the donor may be passively transferred to SCID mice by immunocytes. In particular, animals given immune cells from an HIV-negative donor previously immunized by an HIV subunit vaccine inducing strong CTL response were protected from an HIV challenge (Sarin et al., 1995). Interestingly, mice injected with splenocytes had T cells responding to mitogens and to alloantigens three weeks after cell transfer. Such xenochimeric animals were also capable of rejecting human skin allograft (Alegre et al., 1994). However, three months after PBL injection, T cells appeared to be anergic since they did not proliferate in vitro after CD3 stimulation and did not produce cytokines like IL-2 or IFN-γ (Tary-Lehmann et al., 1994). PBL-injected animals also depicted a restricted human Vß T cell repertoire when analyzed 6 to 8 weeks after PBL injection (Mancia et al., 1994; Garcia et al., 1997). In opposition, the repertoire of T cells in mice implanted for 2 months with human tonsil pieces was polyclonal and similar to that of the original human tonsil (Duchosal et al., 2000).

Responses of human B and T cells towards immunization

Immunodeficient mice given PBL or suspensions of lymphocytes from donors with (auto)antigen-specific antibodies may have similar (auto)antibodies in their serum, albeit generally at lower levels than those found in the donors (Duchosal et al., 1992a; Leader et al., 1992; Mosier et al., 1993). Such antibodies were functional, as demonstrated by the resistance of SCID mice to infection with HIV when animals were populated with PBL from HIV-gp160 immunized donors (Mosier et al., 1993). These observations correlate with those of polyclonal human Ig presence in sera of SCID mice early after receiving PBL (Tissot et al., 1996), indicating that a large repertoire of B cell-producing antibodies was transferred and that cells producing antibodies with a particular antigen specificity were likely to be present in the mouse. The presence and level of (auto)antibodies with particular (auto)antigenic specificity within a xenochimeric mouse depends on the frequency of (auto)antigen-specific B cells within the transplanted human lymphoid cell population. This may explain why animals receiving PBL from donors with very low levels of IgG anti-tetanus toxin (TT) had no or low levels of antibodies in their sera, whereas animals given PBL from donors with high IgG anti-TT levels had uniformly high levels of such antibodies. The differential frequency of (auto)antigen-producing B cells between various human body compartments can also explain the differential expression of such (auto)antibodies between animals given cells from various organs. This may be particularly true for rheumatoid arthritis (RA), an autoimmune disease characterized by rheumatoid factor (RF) presence and articular inflammation. High levels of RF in the animals accompanied the transfer of intrasynovial lymphocytes from RA patients whereas such autoantibodies were not detectable following transfer of PBL from patients (Tighe et al., 1990).

Secondary immune responses in xenochimeric mice

Antigen-specific antibodies have been elicited or boosted following antigen challenges with a large success in immunodeficient mice given lymphocytes from immune donors (Mosier et al., 1988; Carlsson et al., 1992; Duchosal et al., 1992a). Various antigens from several sources have been successfully used, including TT (Carlsson et al., 1992; Duchosal et al., 1992a; Daniel and Krammer, 1994), preparation of mite antigen from whole mite body (Chiang et al., 1995), fusion protein purified from respiratory syncitial virus (Chamat et al., 1999), recombinant [bacterial membrane, hepatitis B core antigen (HBc), hepatitis B surface antigen] proteins (Duchosal et al., 1992a; Abiko et al., 1997; Bocher et al., 2001; Depraetere et al., 2001), Haemophilus influenzae b polysaccharide (Lucas et al., 1992), alloantigens such as HLA and red blood cell antigens (Leader et al., 1992; Lazarus et al., 1997), and DNA vectors encoding protein (Bocher et al., 2001). Recall humoral protective immune response to Haemophilus influenzae type b polysaccharide also occurred when animals were vaccinated with an anti-idiotypic $F(ab')_2$ fragment coupled to a fragment to CD3 (Reason et al., 1994). Human monoclonal Fab fragments and antibodies have been generated from several of the above boosted animals, demonstrating that xenochimeric models can bypass the need of boosting humans for the generation of such monoclonal antibodies (Carlsson et al., 1992; Duchosal et al., 1992a; Neil and Sammons, 1992; Eren et al., 1998; Chamat et al., 1999; Depraetere et al., 2001). Several of the above-mentioned recall antibody responses were elicited by thymus-dependent antigens. The direct evidence of a T cell response in such processes has been more difficult to evidence, but was successful, notably for TT (Mosier et al., 1988; Somasundaram et al., 1995).

We investigated humoral anti-TT immune response of human cells in SCID mice transplanted with immunocytes from 11 TT immune donors. Three to four weeks after engraftment, TT-specific IgG (mean ± SEM) were detectable in 16 non-boosted mice (622 ± 107 mUI/ml) injected with human PBL from three donors. Fifteen additional SCID mice given donor-matched PBL and boosted at cell transfer had a 95-fold increase in

specific serum antibodies (58994±13451 mUI/ml). In the model, boosting performed several weeks after cell transfer led to poor or no immune response (Markham and Donnenberg, 1992, and unpublished data). In another set of experiments, IgG anti-TT was also present in 14 mice implanted with tonsil pieces from four donors (189±98 mUI/ml). Donor-matched animals (n=14) boosted with TT at the day of implantation had a 410-fold increase in levels of TT-specific IgG (77630±10117 mUI/ml). The human antibody response in tonsil-implanted mice was long-lasting (more than 8 months) (Duchosal et al., 2000), and could be elicited later after grafting. This was demonstrated by a 58-fold increase in TT-specific IgG levels in animals antigen-boosted 2 weeks after implantation (four experiments, 8572±3001 mUI/ml, n=9, immunized mice; versus 147±55 mUI/ml, n=9, non-immunized mice). This latter model was also characterized by a large diversity of TT-specific antibodies when analyzed by two-dimensional electrophoresis, which was intermediate between that of hu-PBL-SCID animals and of boosted humans (Layer et al., 2000), and by a long-lasting antibody response (>9 months) (Duchosal et al., 2000). Nevertheless, almost all models of immunodeficient mice populated with human lymphoid cells appear to display inter-individual variability with regard to antibody levels, even when animals receive similar numbers of cells from the same origin. This observation is compatible with an "*in vivo* limiting dilution" phenomenon. Such populated mice, particularly those given PBL, indeed receive few B cells with particular antigenic specificities, as demonstrated by an electrophoretically-restricted pattern of antigen-specific antibodies at immunoelectrophoresis and at two-dimensional electrophoresis analysis of boosted animals (Bazin et al., 1996; Layer et al., 2000).

The interaction between B and T cells in the model was first indicated by the observation that animals receiving PBL depleted in $CD4^+$ T cells did not have human Ig. Analysis of such animals failed to demonstrate human PBL, indicating that T cells provide help for PBL engraftment (Duchosal et al., 2001). The T cell help for antigen-specific antibody production in the animal has been precised. In particular, it appears that such antibody presence is dependent on $CD45RO^+$ T cells (Martensson et al., 1994) and on interactions between B and T cells through the ligation of costimulatory molecules present on the cell surface. One of the most important costimulatory signals is mediated by CD40 on B cells and CD40 ligand (CD154) on activated T cells. Using an anti-CD154 antibody blocking CD40-CD154 interaction, Foy et al. inhibited recall humoral responses in immunized xenochimeric SCID mice (Foy et al., 1998). Similarly, blockade of the CD40-CD154 costimulatory pathway using soluble CD154 inhibited secondary anti-HLA human antibody response (Lazarus et al., 1999). These observations indicated that human immune response in SCID mice involved interactions between human B and T cells through very similar mechanisms to those acting in humans (Chen et al., 1995; Murphy et al., 1999).

Total IgE was low or not observed in sera of mice injected with PBL from non-allergic patients (Chiang et al., 1995; Gagnon et al., 1995; Tonnel et al., 1995), but could be detected when PBL from allergic patients was used (Ito et al., 1992; Spiegelberg et al., 1994; Chiang et al., 1995; Tonnel et al., 1995). Antigen-specific IgE were nevertheless inconsistently detected in these latter animals, and amounts of IgE were highly variable between xenochimeric animals, and partly dependent on IgE levels in donor sera. IgE appeared or IgE levels increased following mouse immunization with allergen (Chiang et al., 1995; Gagnon et al., 1995; Tonnel et al., 1995). In addition, IgE production in animals given cells from atopic donors could be boosted 10- to 100-fold by adding IL-4 (Spiegelberg et al., 1994), and decreased by an antagonist to IL-4 and IL-13 receptors (Carballido et al., 1995). Thus, SCID mice reconstituted with PBL from allergic patients can be used as a model to study regulation of human IgE production by antigens and by immunomodulators.

Primary immune response in xenochimeric mice

Primary immune and autoimmune responses have been difficult to generate in xenochimeric mice, with rare success in animals given solely PBL (Mazingue et al., 1991). In our hands such primary responses were inconsistent and, when present, at very low levels even when PBL were primed both *in vitro* before transfer and *in vivo* after their engraftment in the animals (Duchosal et al., 1992a). Interestingly, Brams et al. (1998) obtained in xenochimeric animals high levels of horse ferritin-specific antibodies using human splenocytes both primed *in vitro* with the addition of IL-2 and boosted *in vivo*, which suggests that either the cell origin or the *in vitro* conditions for immunocyte priming with the neo-antigen may help for observing high antibody responses to such antigen. The level of graft expression also appeared to influence the success of obtaining a primary response in xenochimeric animals. Some authors suppressed mouse (NK) cell functions by using anti-asialo GM1 with or without irradiation to enhance human graft expression. They were able to observe primary antibody responses to KLH (keyhole limpet hemocyanin), carbohydrate or peptidic antigens conjugated to KLH, or preparation of parasitic proteins following immunizations (Kudo et al., 1993; Sandhu et al., 1994). The trimera mouse model was also accompanied by high human PBL engraftment, and the model has been successfully utilized to generate primary responses, both humoral to KLH and to HIV nef protein (Marcus et al., 1995), and cellular (CTL) to HIV nef protein, HBc, and allogeneic human cells (Segall et al., 1996; Bocher et al., 2001). Using SCID mice implanted with tonsil pieces, we observed low primary antibody response to HBc antigen in few animals (Duchosal et al., 1992a). The failure to obtain a significant response in a model where immune cell engraftment is optimized indicates that the main

limitation in obtaining primary response is indeed poorly dependent on engraftment level.

Dendritic cells (DC) are essential for naïve antigen presentation, and human DC functions have been optimized in xenochimeric models. As an example, primary T cell response has been induced *in vivo* in SCID mice populated with PBL from HIV-negative donors, and grafted with autologous human skin. Injection of IL-2 and of a canary pox virus vector expressing HIV-gp160 into the human skin graft induced locally DC appearance and human T cell recruitment. T cell lines from skin-infiltrating T cells were subsequently derived with a specificity to gp160 (Delhem et al., 1998). Neutralizing human antibodies to HIV have also been elicited in SCID mice given PBL from healthy donors and DC differentiated from monocytes by type I interferon and GM-CSF and pulsed with inactivated HIV or with a recombinant protein containing an epitope from the membrane gp41 (Santini et al., 2000; Marusic et al., 2001). Similarly, trimera mice injected with antigen (HBc, and Borrelia burgdorferi antigen) -loaded DC were able to mount a primary Th cell response (Bocher et al., 2001). Ifversen et al. (1995) successfully utilized chimeric molecules consisting of various B cell epitopes (from the hapten DNP, a peptide from HIV-1 glycoprotein gp120, a melanoma-associated ganglioside, or from an ovine mucin) associated with helper TT epitopes. Bombil et al. utilized a similar association to generate de-novo antibodies to canine albumin. In these cases, TT-specific T cell epitopes from the memory compartment provided the necessary T cell help for primary antibody production. A different approach has been reported by Westerink et al. (1997) who postulated that a lack of B cell differentiation and antibody production in the model could be overcome by the addition of human IL-12 and IL-2. Using such a strategy, the authors were able to mount a primary antibody response towards meningococcal polysaccharide, predominantly of the IgG isotype.

Collectively, these results demonstrate that primary immune response has not been reliably obtained in xenochimeric mouse models. Several factors, including notably the low number of human B cells transplanted, anergy, impaired human immune cell cooperation, and deficient antigen presentation, may explain this difficulty. These data indicate that optimization of transferred human immune system must be performed to reliably observe naïve antibody responses.

Production of autoantibodies in xenochimeric mice

If immunodeficient mice exhibit relatively easily serum human autoantibodies following transfer of autoimmune human B cells (Krams et al., 1989; Duchosal et al., 1990; Martino et al., 1993b), boosting of such autoantibody production in the animals has been difficult (Davies et al., 1991; Martin et al., 1992, and data not published), but successful in some cases (Petersen et al., 1993). Generation of de novo significant levels of IgG autoantibodies has also been induced in SCID mice injected with PBL by using an autoantigen (prostate-specific antigen, PSA) conjugated to mouse IgG2a. Such a protein complex binds efficiently to dendritic cells (DC) and transfer of autologous, protein-pulsed DC induced IgG anti-PSA production in the SCID mice given female PBL, whereas PSA on adjuvant failed to do so (Coccia and Brams, 1998).

De novo production of IgG autoantibodies in SCID mice injected with PBL may also depend on the autoantigen itself. This has been exemplified by several observations including: a) the successful immunization of transplanted human PBL with human CD2 (Uchibayashi et al., 1995), and b) the constantly successful purification of neutralizing anti-Factor VIII IgG autoantibodies in xenochimeric animals given PBL from either an hemophilic A patient or from healthy donors (Vanzieleghem et al., 2000). Immunization of such xenochimeric animals with human Factor VIII did not boost nor tolerized anti-factor VIII antibody production.

Conclusions and perspectives

Several types of xenochimeric models where human cells are transferred to genetically-determined and/or induced immunodeficient animals have been established. In general, high animal immunosuppression correlates with robust human graft expression and function. Nevertheless, strong mouse immunosuppression can be accompanied by a high animal mortality rate that can limit the long-term analyses of human cells *in vivo*.

Human cells in suspension can be *in vitro* manipulated before transfer to the animal. In particular cell subpopulations can be purified and immune cells can be pre-stimulated with the relevant cytokine or antigen. Cell suspensions injected in the animal disseminate within the whole body, reducing human cell-cell interactions and increasing contact between murine and human cells. The consequences are a limitation of the function of human immune cells, and the propensity to develop graft-versus-host disease within the animal. Implantation of organ pieces preserves human cell clustering within the animal. This leads to conserved human cell-cell interaction capabilities, and highly populated xenochimeric animals that have no sign of graft-versus-host disease. This latter model is accompanied by high diversity of immunoglobulin and of recall antibody responses that persist for months. Xenochimeric models have proven to be useful for generations of human monoclonal antibodies, but antibodies to neo-antigens has been more difficult to obtain, with a higher success rate when antigen-presentation functions within the animal have been optimized.

Immunodeficient mice have also proven to be good recipients of human non-lymphoid cells (Boehncke, 1999; Yonou et al., 2001) and of human haematopoietic

stem cells (McCune et al., 1988; Dick, 1996; Greiner et al., 1998; Kollet et al., 2000) similarly to that of lymphoid cells. Combination of tissue/cell implantations from two or three of the above-mentioned categories has already been reported (Carballido et al., 2000; Raychaudhuri et al., 2001; Yamanaka et al., 2001) and will be expanded in the future. It is expected that such models containing both human immune and non-immune cells will be useful for studying human immune cell trafficking and fate, and for evidencing the influences of organ microenvironment on hematolymphoid cells fate or the effects of such cells on the surrounding organ.

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