# Functional and Phenotypic Characterization of the Humanized BLT Mouse Model

**A. K. Wege, M. W. Melkus, P. W. Denton, J. D. Estes, and J. V. Garcia(✉)**



**Abstract** T cells play a central role in the development of immune responses. Patients lacking T cells because of genetic defects such as DiGeorge or Nezelof syndromes and patients infected with the human immunodeficiency virus are highly susceptible to infections and cancers. The lack of adequate in vivo models of T cell neogenesis have hindered the development and clinical implementation of effective therapeutic modalities aimed at treating these and other clinically important maladies. Transplantation of severe combined immunodeficient (SCID) mice with human hematopoietic stem cells results in long-term engraftment and systemic reconstitution with human progenitor, B, and myeloid cells, but curiously, human T cells are rarely present in any tissue. While the implantation of SCID mice with human fetal thymus and liver (SCID-hu thy/liv mice) allows the

J. Victor Garcia
Department of Internal Medicine, Division of Infectious Diseases Y9.206, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75390-9113, USA
victor.garcia@utsouthwestern.edu

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development of abundant thymocytes that are localized in the human organoid implant, there is minimal systemic repopulation with human T cells. However, we have recently shown that transplantation of autologous human hematopoietic fetal liver $CD34^+$ cells into the nonobese diabetic (NOD)/SCID mouse background previously implanted with fetal thymic and liver tissues results in long-term, systemic human T cell homeostasis. In addition to human T cells, these mice have systemic repopulation with human B cells, monocytes/macrophages, and dendritic cells (DC). Importantly, in these mice the T cells developed in the human thymic implant are capable of being activated by human antigen-presenting cells and mount potent human MHC-restricted T cell immune responses.

**Abbreviations** APC: antigen-presenting cell; BLT: Bone marrow/liver/thymus; DC: dendritic cell; EBV: Epstein-Barr virus; EGFP: enhanced green fluorescent protein; HIV: human immunodeficiency virus; IL-7: interleukin 7; LCL: lymphoblastoid cell line; MHC: major histocompatibility complex; SCID: severe combined immunodeficiency; TCR: T cell receptor; TSST-1: toxic shock syndrome toxin 1

# 1 Introduction

## *1.1 Definition of the Problem Addressed*

Lack of T cells in humans due to primary or secondary immune deficiencies as in patients with genetic defects such as DiGeorge or Nezelof syndromes or in patients infected with the human immunodeficiency virus (HIV) results in heightened susceptibility to a variety of infections and cancers (Markert et al. 1999; Shearer et al. 1978). The development of effective therapeutic modalities to treat these types of diseases has been hindered, in part, by the lack of adequate in vivo models of human T cell neogenesis. Small animal models of human hematopoiesis have been extensively used as surrogates to study a variety of important aspects of human transplantation, immune function, bacterial and viral infection, tumorigenesis, gene transfer, and in vivo repopulating potential of embryonic and somatic stem cells (Akkina et al. 1994; Aldrovandi et al. 1993; Islas-Ohlmayer et al. 2004; Lapidot et al. 1992, 1997; 1992; McCune et al. 1988; Palucka et al. 2003). Despite their enormous success in a multitude of applications, each of these systems has significant limitations that curtail its usefulness. The development of improved systems with long-term systemic reconstitution with a full complement of human hematopoietic cells is obtained and would provide unique opportunities to study a multitude of timely and relevant issues that cannot be addressed with current models.

## *1.2 Human/Mouse Xenograft Models to Study Human Hematopoiesis*

Immunodeficient mouse models, in general, have been extensively used to study the in vivo function of human hematopoietic stem cell engraftment and reconstitution, important aspects of gene transfer and gene therapy, the replication of human viruses, and the role of human cytokines in hematopoietic homeostasis and, more recently, to study the ontogeny and function of different components of the human immune system, including dendritic cells (DC) (Cravens et al. 2005; Greiner et al. 1998; Islas-Ohlmayer et al. 2004; Lapidot et al. 1992, 1997; Palucka et al. 2003). Perhaps the two in vivo systems most widely used to study different aspects of human hematopoeisis are one in which human $CD34^+$ cells are used to repopulate SCID mice (Greiner et al. 1998) and another in which a small piece of fetal liver or fetal bone is coimplanted with fetal thymic tissue underneath the skin or kidney capsule (SCID-hu thy/liv) (McCune et al. 1988). In the first example, transplantation of preconditioned SCID mice with human $CD34^+$ cells results in relatively low levels of systemic reconstitution of different mouse tissues with human hematopoietic cells (Greiner et al. 1998). However, one limiting aspect of this system is the fact that B cells are the most abundant cell type in these mice, accounting for up to 90% human reconstitution in all tissues, and virtually no human T cells can be detected in any of the mouse hematopoietic organs including the mouse thymus. In addition, SCID mice show relatively low levels of reconstitution with human cells in general.

One important improvement to the original transplantation model using SCID mice was the use of other immunodeficient strains like NOD/SCID mice, as recipients for transplantation. Transplantation of NOD/SCID mice with human $CD34^+$ cells results in dramatically higher levels of systemic repopulation with human cells. However, despite the higher levels of human reconstitution, T cells generally fail to develop in this system (Greiner et al. 1998; Islas-Ohlmayer et al. 2004). Nevertheless, this model has proven to be extremely useful to study a variety of important aspects of hematopoeisis, the pathogenesis of human-specific virus infection, and the ontogeny and function of the human immune system in vivo (Bente et al. 2005; Cravens et al. 2005; Islas-Ohlmayer et al. 2004; Miyoshi et al. 1999; Palucka et al. 2003).

In contrast to the lack of human T cells in SCID or NOD/SCID mice transplanted with purified $CD34^+$ cells, in the SCID-hu thy/liv mice there is an abundance of human thymocytes. However, virtually all human cells are confined to the thymic organoid that develops after implantation (Aldrovandi et al. 1993; McCune et al. 1988; Vandekerckhove et al. 1991), except for the spleen, where low levels (<1%) of human T cells (and rarely human B cells) can be found. Thus, SCID-hu thy/liv mice do not have significant systemic repopulation with human T cells and are virtually devoid of human B cells, monocytes/macrophages, and dendritic cells. Despite these limitations the SCID-hu thy/liv system has been instrumental as a surrogate model to study hematopoietic stem cell function, thymopoeisis, HIV infection, and pathogenesis (Akkina et al. 1994; Aldrovandi et al. 1993; Amado et al. 1999;

Bonyhadi et al. 1993; Brooks et al. 2001; Jenkins et al. 1998; Kitchen et al. 2000; Kollmann et al. 1995; Napolitano et al. 2003; Okamoto et al. 2002; Su 1997).

Multilineage human reconstitution including T cells in newly developed strains of immunodeficient mice transplanted as neonates with human $CD34^+$ cells from different sources has been reported (Gimeno et al. 2004; Ishikawa et al. 2005; Shultz et al. 2005; Traggiai et al. 2004). The requirements for T cell development in these different strains vary, but for the most part, injection of neonatal mice seems to result in production of T cells, whereas transplantation of adult mice of the same strains results in low to undetectable levels of human T cells that in one instance could be increased by repeated administration of a human IL-7 analog (Shultz et al. 2005). The fact that T cells only arise in mice transplanted as neonates indicates the need for a developing system conducive to expansion of the T cell compartment. The single common denominator in all these recently described systems is the fact that the selection of the human T cell repertoire is believed to take place in the context of the mouse MHC in the mouse thymus (Ishikawa et al. 2005; Ito et al. 2002; Shultz et al. 2005; Traggiai et al. 2004; Yahata et al. 2002). In these mouse models, positive selection presumably occurs when human thymocyte TCRs interact with mouse MHC molecules loaded with mouse self-peptide on murine thymic epithelial cells.

## *1.3 Generation of the Bone Marrow/Liver/Thymus Humanized Mouse Model*

There is a great interest in generating humanized mice in which human T cells develop in the context of a human thymic environment in order to understand human T cell ontogeny and human MHC-restricted T cell responses in vivo. When autologous or allogeneic human $CD34^+$ progenitor cells are provided with an appropriate thymic microenvironment, such as being injected directly into the thymic organoid that develops in the SCID-hu thy/liv model, they can mature into naive single-positive human T cells (Akkina et al. 1994; An et al. 1997). We therefore asked whether $CD34^+$ cells introduced via bone marrow transplantation could systemically reconstitute the mouse and sustain thymopoiesis in the implanted human thymic tissue. In order to maximize the likelihood of seeding the transplanted thymus with human T cell progenitors originating from the human stem cells present in the mouse bone marrow, we used NOD/SCID mice instead of SCID mice, because NOD/SCID mice support significantly higher levels of reconstitution after transplantation with human $CD34^+$ cells because of lower endogenous mouse NK cell activity (Greiner et al. 1998).

Allogeneic cord blood $CD34^+$ cells were originally used in this system because of their accessibility and high in vivo repopulating potential (Vormoor et al. 1994). However, regardless of their source, transplantation with allogeneic $CD34^+$ cells failed to sustain the implanted thymic tissue and resulted in clearance of the bone marrow graft in this model. Therefore, autologous fetal liver $CD34^+$ cells were used to determine whether they could contribute to the overall levels of human reconstitution when transplanted after implantation of MHC-matched human fetal thymus and liver.

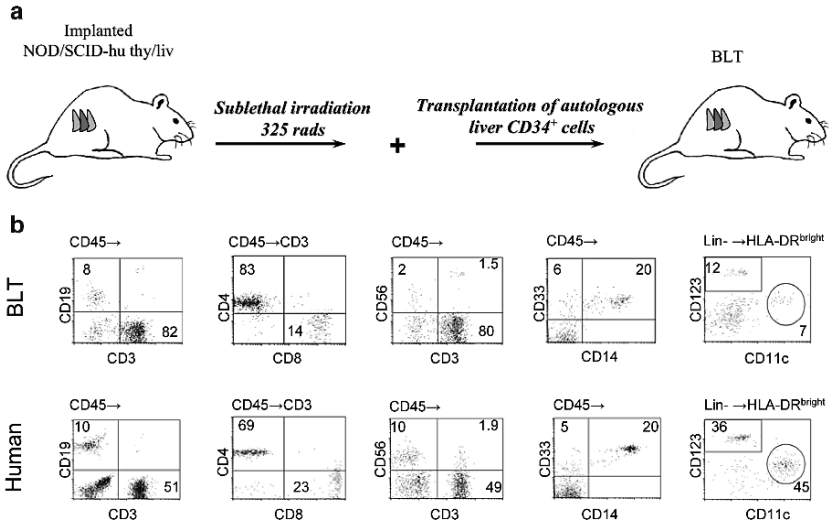



**Fig. 1** Reconstitution of NOD/SCID-hu thy/liv mice transplanted with autologous human hematopoietic stem cells. **a** Schematic illustration of the different steps involved in generating NOD/SCID-hu BLT mice. **b** flow cytometry analysis of peripheral blood from one representative BLT mouse and a healthy human control. Samples were analyzed by gating on CD45 and then for the specific antigen (i.e., CD45→CD3→CD4 or CD8, CD45→CD19). The percentages of the individual human cell subsets in each sample are indicated by the *numbers* in each of the quadrants. Human DC were defined as lineage-negative and HLA-DR$^{bright}$ cells expressing either CD123 or CD11c (i.e., lin$^{-}$→HLA-DR$^{bright}$→CD123 or CD11c)

In essence, NOD/SCID mice were implanted with human fetal thymic and liver tissues, preconditioned with a sublethal dose of gamma radiation, and transplanted with autologous human CD34$^{+}$ cells obtained from a portion of the same fetal liver used for the implant (Fig. 1a). To emphasize the importance of the reconstitution of the mouse bone marrow with human hematopoietic stem cells to repopulate the previously implanted fetal liver and thymic tissue, we have adopted the designation NOD/SCID-hu BLT mice (or BLT mice) to distinguish them from the SCID-hu thy/liv model.

## 2 Human Hematopoietic Reconstitution in BLT Mice

### *2.1 Peripheral Blood Reconstitution of BLT Mice with Human Hematopoietic Cells*

Human cells represent a minor proportion of cells in the blood of mice coimplanted with human thymus and liver alone (Akkina et al. 1994; Aldrovandi et al. 1993; Amado et al. 1999; McCune et al. 1988, 1989). However, previously implanted NOD/SCID-hu thy/liv mice that received a bone marrow transplant with autologous CD34$^{+}$ cells had readily detectable levels of human hematopoietic cells in their

peripheral blood (Fig. 1b) (Melkus et al. 2006). When peripheral blood cells were characterized for different human hematopoietic lineages, B cells, monocyte/macrophages, dendritic cells, and specifically T cells were present in all reconstituted mice by 8 weeks and sustained for up to 26 weeks after transplant (Fig. 1b) (Melkus et al. 2006). These results demonstrated that the peripheral blood of bone marrow-transplanted NOD/SCID-hu thy/liv mice with purified autologous $CD34^+$ cells results in significant levels of multilineage peripheral blood reconstitution with human hematopoietic cells including T cells.

### *2.2 Systemic Reconstitution of BLT Mice with Human Hematopoietic Cells*

Since human hematopoietic cells were readily found in the peripheral blood of BLT mice, the presence of human cells in primary and secondary lymphoid tissues was determined. Overall levels of reconstitution with human cells in different mouse tissues are summarized in Fig. 2a. In contrast to SCID-hu thy/liv mice, in BLT mice bone marrow, lymph nodes, spleen, thymic organoid, liver, lung, and gut demonstrated substantial levels of human $CD45^+$ cells (Fig. 2a). Therefore in BLT mice primary and secondary lymphoid organs as well as no-lymphoid tissues important in immune regulation and mucosal immunity are reconstituted with human hematopoietic cells. On analysis of these tissues, we noted the presence of human T cells and their subpopulations, B cells (Fig. 2b), monocyte/macrophages, and dendritic cells (Melkus et al. 2006). With respect to the human T cell subsets present in the peripheral blood, the mean of $CD4^+$ cells was 70% and the mean of $CD8^+$ T cells was 20% (Fig. 2c). The differentiation state of the human T cells in the peripheral blood of BLT mice was analyzed by determining the expression of CD45RA and CD27. Approximately 62% of the T cells in the periphery exhibited a naive phenotype, with 32% exhibiting a central memory phenotype (Fig. 2c, bottom). As expected for animals kept under sterile conditions, BLT mice had a higher percentage of naive T cells when compared to healthy human controls. In summary, these data demonstrated that BLT mice develop a remarkable state of sustained systemic multilineage reconstitution with human hematopoietic cells that can persist for months after transplantation.

## 3 T Cell Development in Humanized BLT Mice

### *3.1 Human Thymopoeisis in the BLT Humanized Mouse Model*

One of the distinctive features of BLT mice is the fact that human T cells are generated in the context of a human thymus (Melkus et al. 2006). The relative proportion of double- and single-positive thymocytes of the human thymic organoid in BLT

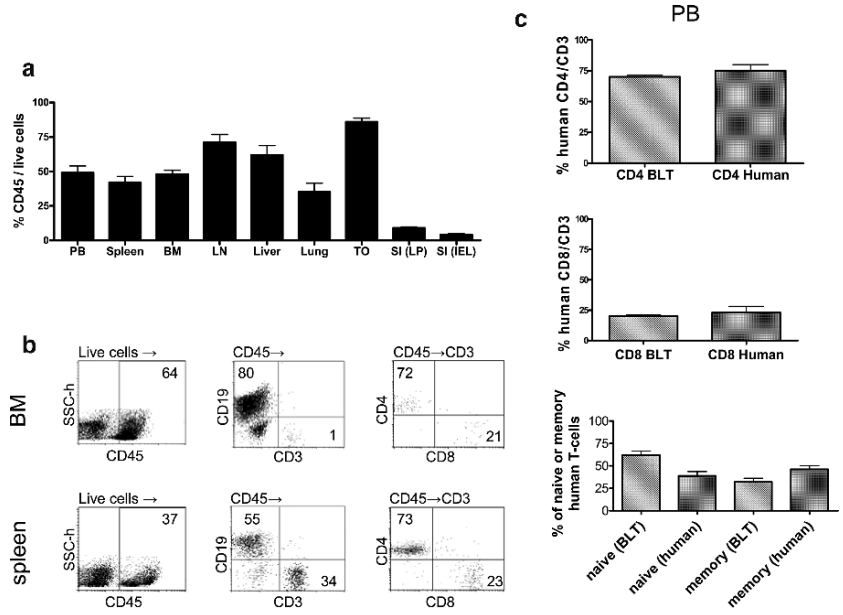



**Fig. 2** Analysis of human hematopoietic reconstitution of different tissues in BLT mice. **a** Systemic human reconstitution in peripheral blood (*PB*; $n=20$), spleen ($n=24$), bone marrow (*BM*; $n=24$), lymph nodes (*LN*; $n=14$), liver ($n=5$), lung ($n=7$), thymic organoid (*TO*; $n=24$), small intestine lamina propria [*SI (LP)*; $n=24$], and small intestine intraepithelial lymphocytes [*SI (IEL)*; $n=24$] of BLT mice. Human reconstitution in each tissue was determined by flow cytometry gating on live cells expressing human CD45. **b** Analysis of the B and T cell subsets in the bone marrow (*BM*) and spleen of a representative BLT mouse. Samples were analyzed by gating on CD45 and then for the specific antigen (i.e., CD45→CD3→CD4 or CD8, CD45→CD19). **c** Comparison of the percentage of $CD4^+$ and $CD8^+$ T cell subsets and naive vs. memory cells in the peripheral blood of BLT mice ($n=55$) vs. healthy human controls ($n=4$)

mice is similar to that observed in human fetal and child thymi. Despite the radiation treatment received by the implanted thymic organoid in the BLT mice, these animals maintained relatively constant levels of $CD4^+CD8^+$ T cells in the human thymic organoid over their life span, demonstrating long-term sustained thymopoiesis (Fig. 3a, b). In addition, human T cells in BLT mice were found to express a diverse repertoire of Vβ T cell receptors (TCR) (Fig. 3d).

Progenitor seeding of the implanted human thy/liv organoid from transplanted bone marrow stem cell progenitors and their contribution in sustaining thymopoiesis by generating de novo T cells in the BLT mouse model was demonstrated by using a lentivirus-based vector expressing enhanced green fluorescent protein (EGFP). In essence, autologous fetal liver $CD34^+$ cells were first transduced with an EGFP-expressing lentivirus-based vector. Transduced cells were then used to reconstitute NOD/SCID-hu thy/liv mice (Melkus et al. 2006). Human cells expressing CD45 and EGFP were found in bone marrow, spleen, and thymic organoid (all the tissues examined). Analysis of

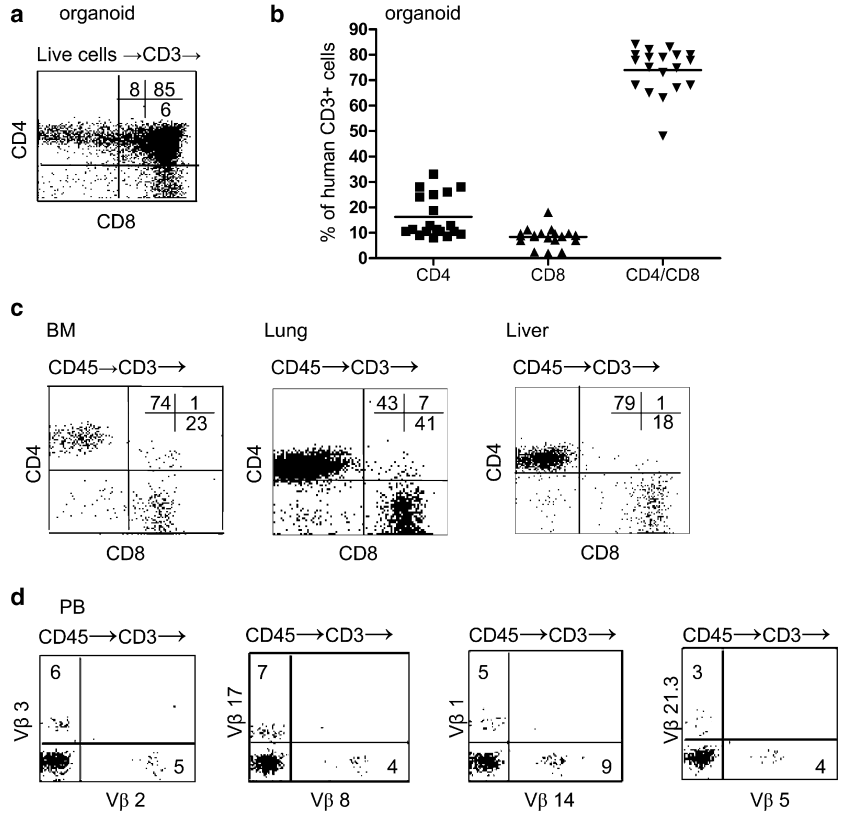



**Fig. 3** Human thymopoiesis in BLT mice. **a** Flow cytometry analysis for CD4 and CD8 expression in the thymic organoid of a representative BLT mouse. **b** Analysis of thymic reconstitution of single- and double-positive thymocytes (*lines* represent medians). **c** Analysis of T cell subsets in BM, lung, and liver of one representative BLT mouse. **d** Analysis of selected T cell receptors in peripheral blood human T cells of BLT mice

EGFP expression in different hematopoietic lineages, as well as T cell subsets, further demonstrated that the transplanted $CD34^+$ cells contributed to the overall levels of engraftment and reconstitution of all human lineages examined. Specifically, they contributed to the seeding of the thymic organoid with human T cell progenitors, thus contributing to de novo thymopoiesis and overall T cell homeostasis.

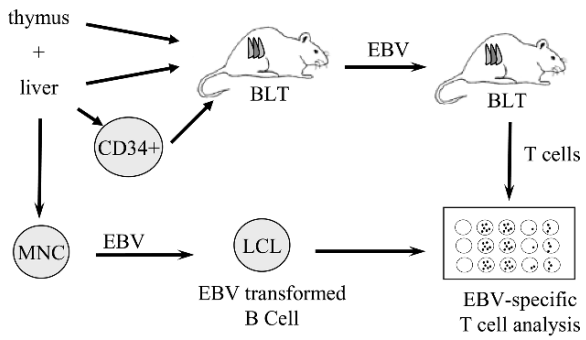
## *3.2 Human MHC-Restricted T Cell Response to EBV in BLT Mice*

In the BLT mouse model human T cells develop properly in an autologous human thymic environment. Therefore it was important to determine whether these human T cells recognize antigens in the context of human MHC to generate

specific T cell responses. This was accomplished with a clinically relevant approach, namely, the development of a T cell response to Epstein-Barr Virus (EBV). In parallel to the implantation of the fetal liver/thymic tissue and the isolation of autologous $CD34^+$ cells, fetal liver B cells were used to establish autologous lymphoblastoid cell lines (LCLs) to serve as antigen-presenting cells (APC) (Fig. 4a). Autologous BLT mice were then infected with EBV. Peripheral blood T cells from infected animals demonstrated a dramatic increase in the percentage of $CD45RA^-CD27^+$ central memory T cells (Melkus et al. 2006). This was reflective of the pattern of expansion of T cells in the peripheral blood of patients during acute EBV infection (Roos et al. 2000).

When human T cells isolated from different organs were cocultured with the previously established autologous antigen-presenting LCLs, the human T cells produced significant levels of γ-interferon (Fig. 4b). γ-Interferon production was inhibited when LCLs were pretreated with anti-human MHC class I and/or class II

**a**




**b**

| Tissue | ELISPOT results | | |
|---|---|---|---|
| | T-cells + LCLs | T-cells + LCLs+ αMHCI | T-cells + LCLs+ αMHCI & II |
| Spleen | ++++ | ++ | + |
| Liver | ++++ | ++ | - |
| Lung | ++++ | + | - |

**Fig. 4** Characterization of the human MHC-restricted immune response to EBV in BLT mice. **a** Diagram showing the different steps used to induce an EBV-specific immune response and analyzing for EBV-specific γ-interferon production. **b** Results of ELISPOT analysis for the presence of EBV-specific T cells producing γ-interferon from spleen, liver. and lung. Also shown are the inhibitory effects of anti-human MHC I alone or in combination with anti-human MHC II antibodies. Symbols: −, <10 spots; +, 10-20 spots; ++, 30-60 spots; ++++, 100-180 spots per $10^6$ human T cells

antibodies, demonstrating that human T cells developed in BLT mice produce human MHC-restricted responses to EBV and highlighting the dynamic interaction between human T and B cells in this model.

### *3.3 In Vivo T Cell Response to Toxic Shock Syndrome Toxin-1 in BLT Mice*

T cell responses in vivo are governed by their interaction with APC. As demonstrated by the experiments described above, in BLT mice human B cells can present viral antigens to human T cells, resulting in a specific cellular immune response. It was then important to demonstrate that human APC are also capable of inducing in vivo a specific human T cell response. For this purpose, toxic shock syndrome toxin-1 (TSST-1) was chosen as a stimulus because of its clinical relevance in human disease and because the immunological response to TSST-1 in humans has been well characterized (Dinges et al. 2000). TSST-1 specifically activates and induces TCR $V\beta2^+$ T cells to proliferate through cross-linking of the TCR on T cells and MHC II on APC (Makida et al. 1996). Consistent with the specificity of TSST-1 for T cells expressing the Vβ2 TCR, a dramatic expansion of this specific subset of human T cells was observed in the periphery of BLT mice. Furthermore, an increase in the number of human $V\beta2^+$ T cells was also observed in all animals after TSST-1 administration (Fig. 5a).

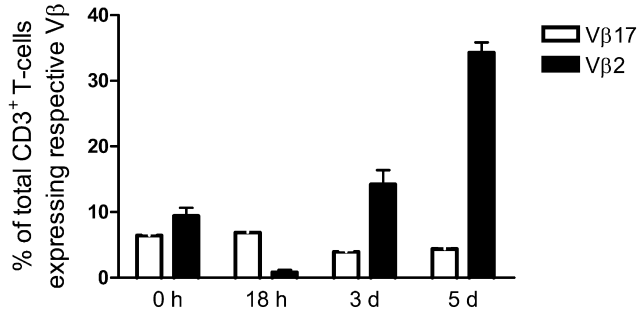
Massive cytokine production resulting from exposure to TSST-1 is a key factor in the pathogenesis of toxic shock syndrome in humans (Kum et al. 2001). The plasma levels of human cytokines in control and TSST-1-treated BLT mice showed significant increases in the systemic levels of human INF-γ, IL-10, IL-6, IL-8, and TNFα (Fig. 5b). This cytokine profile in response to TSST-1 in BLT mice resembles that seen in humans (Kum et al. 2001), further demonstrating that human T cells within BLT mice are capable of exerting effector function after TCR stimulation by APC.

## 4 Functional Characterization of Human Dendritic Cells in Humanized BLT Mice

### *4.1 Human Dendritic Cells in Humanized BLT Mice*

Dendritic cells (DC) play important roles in health and disease and are generated in the bone marrow from $CD34^+$ cells. NOD/SCID mice reconstituted with human $CD34^+$ cells have been shown to have a full repertoire of human DC. In fact, the ontogeny of human DC in NOD/SCID mice transplanted with human $CD34^+$ cells

**a**




**b**

| Cytokine | Control (n=4) | TSST-1 | |
|---|---|---|---|
| | | 1h after (n=4) | 18h after (n=6) |
| IFN γ | 50.1 +/- 25.1 | 106.1 +/- 35.9 | 479.9 +/- 143.0 |
| Il-10 | 24.4 +/- 1.9 | 85.1 +/- 26.8 | 257.6 +/- 71.8 |
| TNF α | 13.4 +/- 1.2 | 197.7 +/- 109.9 | 23.5 +/- 2.9 |
| Il-8 | 31.0 +/- 2.6 | 46.1 +/- 6.2 | 128.1 +/- 36.1 |
| Il-6 | 33.8 +/- 13.1 | 107.2 +/-18.2 | 335.3 +/- 92.7 |

**Fig. 5** Innate immune response to TSST-1 in BLT mice. **a** Increase in the percentage of Vβ2$^+$ T cells at different times after TSST-1 injection. Note the lack of response by the Vβ17$^+$ human T cells that served as internal control for these experiments. **b** Cytokine production (±SEA) in response to TSST-1 in the plasma of BLT mice before and 1 h and 18 h after TSST-1 administration

was shown to be parallel what is seen in humans in all aspects (Cravens et al. 2005). For their analysis in humanized BLT mice, DC were defined as lineage-negative, HLA-DR$^{bright}$ CD11c$^+$ or CD123$^+$. In BLT mice, DC are systemically distributed. DC were found in peripheral blood, bone marrow, lymph nodes, thymic organoid, spleen, liver, lung, and gut.

## *4.2 In Vivo Analysis of the Human DC Response to TSST-1 in BLT Mice*

Superantigens induce T cell responses by bridging the T cell receptor and the MHC II molecules expressed on the surface of APC (Dinges et al. 2000; Karp et al. 1990). Whereas the T cell response has been extensively studied in humans, the

response by APC has not been so carefully described. The extent and the kinetics of the phenotypic changes to human DC in response to systemic administration of TSST-1 in vivo regarding activation and maturation antigens in BLT mice provided some highly relevant and novel information. Before TSST-1 administration, $CD123^+$ and $CD11c^+$ DC in bone marrow of BLT mice have an immature resting phenotype characterized by either low levels or the absence of human CD40, CD80, CD86, and CD83 surface expression (Melkus et al. 2006). Similarly, $CD123^+$ and $CD11c^+$ DC in the spleen of unmanipulated BLT mice have an immature resting phenotype (Fig. 6).

TSST-1 administration did not significantly alter the phenotype of human $CD123^+$ DC in the bone marrow or spleen of BLT mice. TSST-1 administration did not alter the phenotype of bone marrow $CD11c^+$ DC, either. In contrast, a dramatic (but transient) upregulation of activation and maturation markers was observed in the $CD11c^+$ DC present in the spleen of BLT mice treated with TSST-1 (Fig. 6). Administration of TSST-1 to a set of transplanted mice with a full complement of human DC but devoid of human T cells (because they were not implanted with human fetal thymic and liver tissue before transplant) did not result in upregulation of DC activation and/or maturation markers, demonstrating

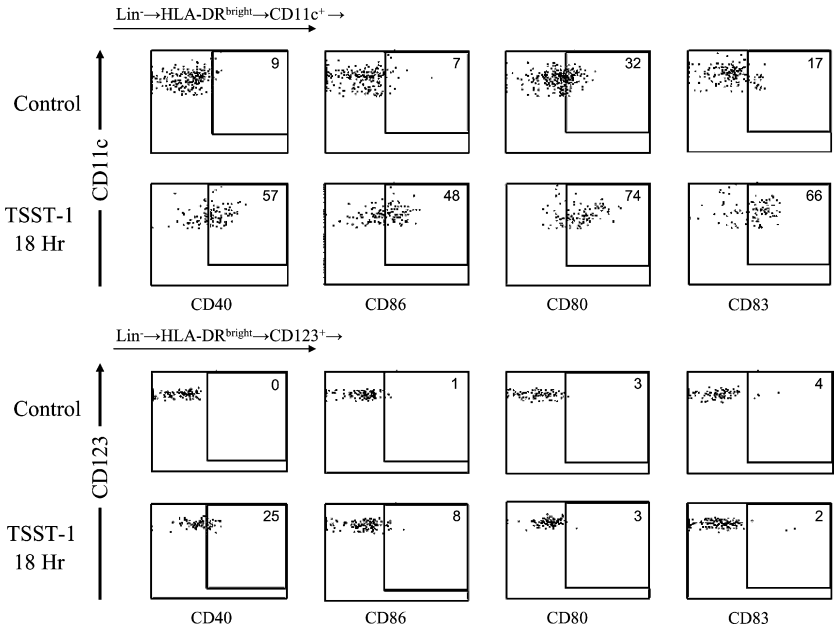



**Fig. 6** In vivo response of human dendritic cells isolated from the spleen of BLT mice to TSST-1. ***Top*** Upregulation of activation and maturation markers on $CD11c^+$ DC. ***Bottom*** Analysis of activation and maturation marker expression on $CD123^+$ DC. Human DC were defined as lineage-negative and HLA-$DR^{bright}$ cells expressing either CD123 or CD11c (i.e., lin→HLA-$DR^{bright}$→ $CD123^+$ or $CD11c^+$)

that in combination with the T cells present in the BLT mice, human CD11c$^+$ DC respond to TSST-1 by upregulating activation and maturation markers in spleen but not bone marrow.

## 5 Summary and Conclusions

Hematological abnormalities that result in immunodeficiency are generally associated with a multitude of complications and relatively poor prognosis. For example, DiGeorge syndrome is a rare congenital disorder in which the thymus fails to develop (Markert et al. 1999). Thus, DiGeorge syndrome patients develop all aspects of a functional immune system except that they are T cell deficient, making them susceptible to numerous opportunistic infections. Transplantation of allogeneic thymic tissue into patients with DiGeorge syndrome has been shown to restore T cell production and restore significant immune function (Markert et al. 1999). This advance was made possible by knowledge derived from in vivo experimentation in mice without other available systems that could serve to bridge the transition between experimentation in mice and the clinical implementation of this knowledge in humans (Weissman and Shizuru 1999). During the past two decades, the development and implementation of experimental models to study human hematopoietic and immune system dysfunction have been greatly facilitated by the availability of several immune-deficient mouse strains capable of accepting human grafts (Greiner et al. 1998; Ishikawa et al. 2005; Ito et al. 2002; Shultz et al. 2005; Traggiai et al. 2004; Yahata et al. 2002). Whereas none of these models fully recapitulates all aspects of human hematopoeisis, individually they all have served in the study of a variety of aspects regarding hematopoietic stem cell function as well as immune function.

Transplantation of SCID or NOD/SCID mice with human hematopoietic stem cells recapitulates several important aspects of DiGeorge and Nezelof syndromes, namely, thymic atrophy and the development of multilineage human hematopoeisis in the absence of T cells (Markert et al. 1999; Shearer et al. 1978). The molecular basis for the T cell lineage restriction in these mice is currently unknown. In SCID-hu thy/liv mice there is long-term sustained thymopoiesis in the graft without systemic peripheral reconstitution, preventing a wider utilization of this otherwise very useful model (Aldrovandi et al. 1993; McCune et al. 1988; Vandekerckhove et al. 1991).

This is in contrast to SCID or NOD/SCID mice transplanted with human hematopoietic stem cells. In transplanted mice there is systemic reconstitution with hematopoietic cells, but T cells are not generated (Greiner et al. 1998; Islas-Ohlmayer et al. 2004). The absence of human T cells in transplanted SCID or NOD/SCID mice was hypothesized as being due to the lack of an appropriate microenvironment for human T cell development. To test this hypothesis we implanted NOD/SCID mice with human fetal thymus and liver, preconditioned them with a sublethal dose of radiation, and transplanted them

with autologous CD34+ human hematopoietic cells (BLT mice). Transplantation of human CD34+ cells into previously implanted mice resulted in systemic repopulation with multilineage human hematopoietic cells including T cells (Melkus et al. 2006).

Phenotypic analysis of human cells in the peripheral blood of BLT mice matches that of cells from normal humans. In peripheral blood, spleen, lung, liver, and bone marrow of BLT mice human single-positive CD4+ and CD8+ T cells, B cells, monocytes, macrophages, and both CD123+ and CD11c+ DC are present, indicating multilineage hematopoietic reconstitution in the different primary and secondary lymphoid organs as well as important tertiary lymphoid tissues. In the implanted human thymic organoid double-negative, double-positive, and single-positive human thymocytes are present, in contrast to secondary lymphoid organs and other nonlymphoid organs like the gut, liver, and lung, where for the most part only single-positive T cells are present.

In contrast to other humanized animal models, in BLT mice thymocytes undergo positive and negative selection in the human thymic organoid in the context of autologous MHC restriction and not in the mouse thymus. In fact, no human T cells are detected in the mouse thymic tissue of fully reconstituted BLT mice. These observations indicate a preferential trafficking of human hematopoietic cells into the human thymic tissue in BLT mice.

By infecting BLT mice with EBV, a specific MHC I- and MHC II-restricted human T cell immune response was demonstrated to occur. Analyses of secondary lymphoid tissue like the spleen, sites of immune regulation like the liver, and mucosal sites like the lung all showed significant T cell responses. Studies of EBV infection in animals lacking human T cells has shown tumor development (Islas-Ohlmayer et al. 2004). This was not noted in BLT mice, perhaps the result of protection by a T cell immune response.

Superantigens such as TSST-1 have been shown to be the causative agents of a variety of human diseases (Dinges et al. 2000). TSST-1 triggers an excessive cellular immune response that can lead to systemic release of cytokines, the expansion of Vβ2+ T cells, and lethal toxic shock. In addition, it has been suggested that bacterial and viral superantigens in general may play a major role in generating a break in immune tolerance and increased epitope spreading in autoimmune diseases (Soos et al. 2002). A powerful aspect of the BLT model was demonstrated by showing that the developed human immune system can be dissected to determine the role of not only human T cells but also of human DC during an immune response.

The BLT model allowed for genetically identical mice to be generated with a complete human immune system or, in nonimplanted mice, an immune system devoid of human T cells. This attribute facilitates studies of how human T cells interact with other immune cells, specifically DC, and how these interactions might result in preventing or inducing disease in vivo. Two important aspects of the in vivo response to TSST-1 are worth emphasizing. First, no significant phenotypic changes were noted in CD123+ DC in BLT mice. Second, administration of TSST-1 to animals devoid of human T cells (i.e., reconstituted with human CD34+ cells but

not implanted with human thy/liv tissues) did not result in the production of detectable levels of human cytokines or any changes in the phenotype of human DC. These results demonstrate the potential of this system to investigate the cellular interactions of the human innate immune response to superantigens and to evaluate novel therapeutic modalities aimed at preventing the devastating effects of toxic shock syndrome and other superantigens in general.

In summary, BLT mice represent a novel model of human hematopoiesis that incorporates the best attributes of two well-established systems: the SCID-hu thy/liv mouse and the NOD/SCID-hu bone marrow transplant mouse. BLT mice develop de novo T cells within a human thymic environment and generate a systemic human hematopoietic system, which is maintained long term in all tissues examined and is capable of mounting a human MHC-restricted T cell immune response. Practical models of human hematopoiesis are essential to bridge the gaps in our understanding of human immune system response and maturation. No animal model will completely recapitulate all aspects of human hematopoiesis or the human immune system. However, new and improved models that closely recapitulate key aspects of human hematopoiesis will serve to provide insight into fundamental aspects of human stem cell engraftment and reconstitution, immune system development, new strategies to study human pathogenesis, and novel therapeutic approaches to alleviate or cure human diseases.

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