Human T Cell Development and HIV Infection in Human Hemato-Lymphoid System Mice

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Abstract Advances in generation of mice that on human hematopoietic stem and progenitor cell transplantation develop and maintain human hemato-lymphoid cells have fueled an already thriving field of research. We focus here on human T cell development and HIV infection in Rag2$^{-/-}\gamma_c^{-/-}$ mice transplanted as newborns with human CD34$^+$ cord blood hematopoietic stem and progenitor cells.

Abbreviations CCR5: cc-chemokine receptor 5; CXCR: CXC-chemokine receptor; DC: dendritic cell; EBV: Epstein-Barr virus; HIV-1: human immunodeficiency virus type 1; IFN-γ: interferon-gamma; MHC: major histocompatibility complex; MLR: mixed lymphocyte reaction

1 Introduction

For good reasons knowledge of human physiology and pathology is largely gained by observation, cautious, informed consent, and safety-oriented *in vivo* experimentation and *in vitro* surrogate assays. By these approaches, rigorous scientific proof

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is often impossible. Thus, progress in clinical research is mostly slow. Most societies agree on animal research using worms, flies, and small vertebrates given appropriate ethical consideration. Because of their high similarity with human beings, easy access, and experimental feasibility, laboratory mice have become the main model for *in vivo* biomedical research reported in the vast majority of published work in high-ranking scientific journals. And for sure, the capturing slogan "They (laboratory animals) save more lives than 911 (the emergency call number)" is not an overstatement. However, although genome similarities are higher than naively expected, 65 million years of divergence in human and mouse evolution have shaped two species that differ substantially in size, life span, reproductive activity, and exposure to environmental challenges, as, for example, coevolving species-specific infectious agents. Thus, also regarding hematology and immunology, mice are not men, accounting for one of the reasons that great achievements in mice are quite often lost in translational research [14, 20].

Experimentation with human hemato-lymphoid system mice took off almost 20 years ago, paralleling increasing application of clinical hematopoietic stem cell transplantation for malignant disease and immunodeficiencies, as well as the rising HIV pandemic. Some basic requirements must be met for hematopoietic cell engraftment, differentiation, and function: Cells must find appropriate locations, must be supported by respective nurturing factors from the host environment, and must not be rejected. In cross-species transplantation, this requires immune deficiency of the recipient, as well as cross-reactivity of homing molecules, and differentiation and survival factors, if not produced in sufficient amounts by transplanted cells themselves.

As the detailed history and state of the art in this field were recently reviewed [8, 10, 11, 18] and major scientific contributions to the field are discussed in other chapters of this volume, we here briefly set the main focus of our work on T cell development and HIV infection using immunodeficient $Rag2^{-/-}\gamma_c^{-/-}$ mice (generated by M. Ito at the Central Institute for Experimental Animals, Japan) transplanted as newborns with human $CD34^+$ hematopoietic stem and progenitor cells.

2 Human T Cell Development and HIV Infection in Immunodeficient Mice Transplanted as Newborns with Human $CD34^+$ Hematopoietic Stem and Progenitor Cells

One of the major improvements achieved by using newborn NOD/SCID$\gamma_c^{-/-}$ and $Rag2^{-/-}\gamma_c^{-/-}$ mice as recipients for human hematopoietic stem and progenitor cell grafts is efficient intrathymic *de novo* development of human T cells [3, 5, 7, 23]. In contrast to newborn transplantation, T cell development in adult NOD/SCID$\gamma_c^{-/-}$ recipients is less efficient but can be enhanced by exogenously adding human IL-7 [6, 19]. Human T cells generated in the mouse thymus include in fairly physiological ratios $CD4^+$ and $CD8^+$ T cells with a broad Vβ distribution,

Foxp3$^+$ CD25$^+$ regulatory T cells, and γδ T cells. Mature T cells exit the thymus and home to secondary lymphoid organs. What is the MHC restriction and functionality of human T cells educated on a mouse thymic background? Data generated mostly in mice demonstrate that under normal developmental conditions positive selection preferentially occurs on cortical thymic epithelial cells, while both medullary epithelial cells and hematopoietic derived dendritic cells are involved in negative selection (e.g., reviewed in [15]). We did not observe human thymic epithelial cells in CD34$^+$ cell-transplanted mice in line with expectations of epithelial cell germ layer derivation, while dendritic cells of both mouse and human origin constituted the thymus. Thus, human developing thymocytes should in theory be positively selected on mouse epithelial cells, while negative selection might occur on both mouse and human MHC. If so, it would be reasonable to expect that T cells positively selected on mouse MHC would continue to preferentially interact in negative selection also with mouse MHC. However, under certain conditions hematopoietic offspring cells are likely involved in positive selection, as pointed out by several mouse-to-mouse transplantation studies [17, 26]. Moreover, species-specific differences in thymic selection might exist, and human thymocyte-thymocyte MHC class II interaction, and thus at least some CD4$^+$ T cell selection, might occur on human MHC [4, 9]. On T cell exit from the thymus, they depend on homeostatic factors, both MHC and cytokines for survival (e.g., reviewed in [22]). As in human hematopoietic stem and progenitor cell-transplanted mice human MHC is only present on hematopoietic but no other tissues, presentation of both "self" and "nonself" peptides in the context of both MHC class I and II will depend on the cellular tropism of these.

Given all the considerations mentioned above, what experimental data on reactivity of mouse background-generated human T cells has been reported? (1) Human T cells isolated from mouse lymph nodes and spleen proliferated vigorously in mixed lymphocyte reactions (MLRs) when stimulated with human allogeneic DCs, but weakly or not at all when stimulated with human autologous DCs. Proliferative response to mouse DCs was low; however, there was a small difference with stronger proliferation of T cells when stimulated with fully mismatched versus host mouse type DC [23]. (2) Cytotoxic activity against human allogeneic target cells could be blocked with human MHC class I or II antibodies, respectively [7]. (3) Some responses of human T cells in mice to *in vivo* infection of human B cells with Epstein-Barr virus (EBV) are observed; however, in some cases human T cells were not capable to control EBV-driven B cell proliferation [23]. (4) Human T cells specific for viral epitopes were only observed in the context of mouse MHC on infection of mice with influenza virus [8]. (5) When mature mouse-derived human T cells were transferred to nontransplanted Rag2$^{-/-}\gamma_c^{-/-}$ mice, we observed no relevant homeostatic cell expansion; similarly, high peripheral T cell turnover rates and lack of long-term T cell maintenance were observed by other groups [8], suggesting that peripheral homeostatic T cell maintenance might not function appropriately, an issue that still needs to be resolved by more experimentation.

In summary, thus far no firm conclusions can be drawn on the biology of T cell selection in this setting where the T cells are of human origin and the thymic stroma is of mouse origin. It can only be suggested that T cell tolerance, that is, possibly negative selection, for both autologous human and mouse MHC is achieved. Beyond that, insufficient T cell responses due to no or only weakly cross-species reactive costimulation and cytokine responses might account for additional difficulties in any human T cell response observed in this setting. In terms of appropriate T cell selection, the obvious solution is to replace mouse by human MHC components, creating at least for thymic selection a similar situation as in mice cotransplanted with same donor fetal thymic tissue [12, 13]. It will be of great interest to see whether human T cell development will progress in the absence of any tissue MHC, a model situation of allogeneic hematopoietic cell transplantation in human MHC deficiencies [16].

As in our human cord blood $CD34^+$ cell-transplanted mice human T cells developed and seeded secondary lymphoid organs, we, and concomitantly others, tested these mice and mice cotransplanted with human thymus as models for HIV infection [1, 2, 21, 24, 25]. We infected human hemato-lymphoid system mice intraperitoneally with either CCR5-tropic or CXCR4-tropic HIV-1 strains. Irrespective of coreceptor selectivity, HIV RNA plasma copies peaked at 2-4 weeks after infection, comparable to HIV infection in humans. Thereafter, viremia mostly stabilized at lower levels and was maintained for up to 190 days, the longest time followed. HIV generated in mice was functional, since supernatants of infected mouse-derived cell cultures propagated infection in primary human leukocytes. As in humans, developing human $CD4^+$ thymocytes in mice are mostly CXCR4-positive, but lack CCR5 expression, while peripheral $CD4^+$ T cells express CXCR4 and/or CCR5. Thymic HIV infection was detected on CXCR4-tropic infection, while secondary lymphoid organ infection occurred in both CXCR4- and CCR5-tropic virus-infected animals (Fig. 1). In both CXCR4- and CCR5-tropic strain-infected animals, productively HIV-infected cells, namely, HIV-$p24^+$ cells, were mostly $CD3^+$ and only occasionally non-T cells such as $CD68^+$ macrophages. In some mice with high numbers of productively infected cells, syncytium formation occurred in both spleen and lymph nodes, a process observed in brains and lymphoid tissue of HIV-infected individuals, likely associated with high viral replication, spreading, and $CD4^+$ cell loss. Overall, CXCR4-tropic HIV infection led to more rapid blood $CD4^+$ cell loss than CCR5-tropic infection, reminiscent of CXCR4-tropic emergence of HIV strains in late-stage human HIV disease. One of 25 infected mice mounted a HIV-specific IgG response detectable by standard clinical assays [1]. Furthermore, although based on limited data, we did not detect robust HIV-specific T cell responses, as determined by IFN-γ detection on *in vitro* restimulation, in line with other concomitant reports [24]. Thus, although specific immune responses are observed, they thus far lack robustness, that is, they are not predictable in frequency and levels, prohibiting at this stage, for example, efficient preclinical vaccine testing. However, as it stands, this model will be valuable to study virus-induced pathology and to evaluate new nonadaptive immunity-dependent approaches aiming to fight HIV.

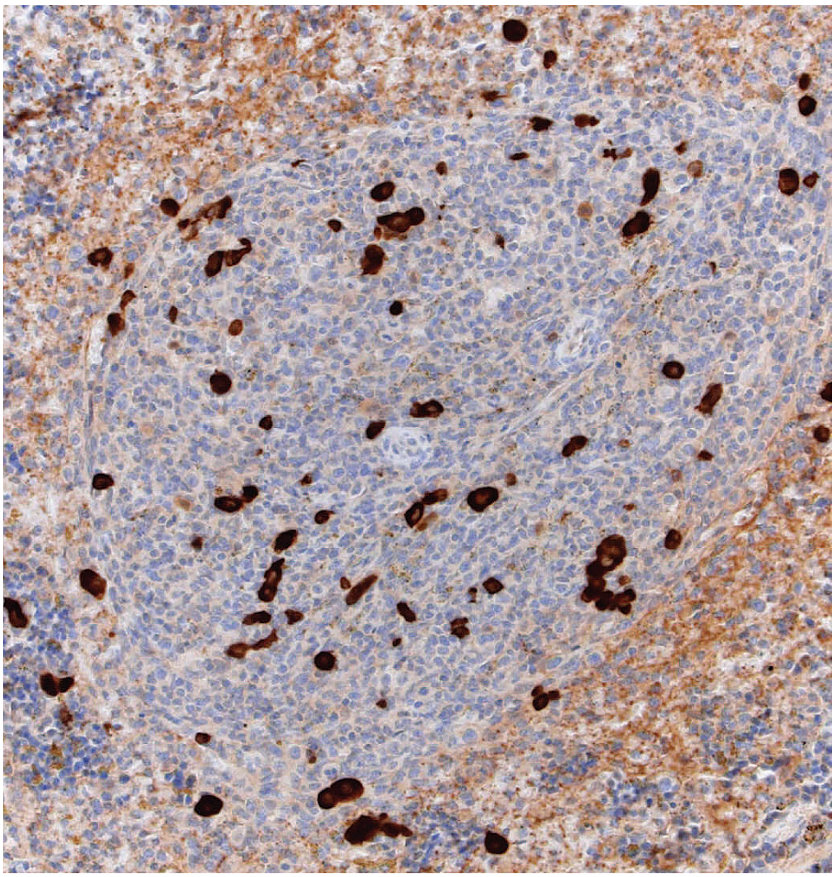

Fig. 1 Rag2$^{-/-}\gamma_c^{-/-}$ mice, transplanted as newborns with human CD34$^+$ cord blood cells and subsequently infected with HIV, develop lymphoid organ-disseminated, productive HIV infection. Histology shows HIV p24 staining preferentially localized in the white pulp area in the spleen of an HIV-infected mouse (18 days after infection with CCR5-tropic YU-2; Magnification 20x)

3 Conclusions

It can be anticipated that basic and preclinical *in vivo* human immunology and infectious disease research will greatly benefit from improved, easy-to-generate, and broadly available human hemato-lymphoid system mice over the coming years.

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References

1. Baenziger, S., R. Tussiwand, E. Schlaepfer, L. Mazzucchelli, M. Heikenwalder, M.O. Kurrer, S. Behnke, J. Frey, A. Oxenius, H. Joller, A. Aguzzi, M.G. Manz, and R.F. Speck. 2006. Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted Rag2−/−gamma c−/− mice. *Proc Natl Acad Sci USA* 103:15951-15956.
2. Berges, B.K., W.H. Wheat, B.E. Palmer, E. Connick, and R. Akkina. 2006. HIV-1 infection and CD4 T cell depletion in the humanized Rag2−/−gamma c−/− (RAG-hu) mouse model. *Retrovirology* 3:76.
3. Chicha, L., R. Tussiwand, E. Traggiai, L. Mazzucchelli, L. Bronz, J.C. Piffaretti, A. Lanzavecchia, and M.G. Manz. 2005. Human adaptive immune system Rag2−/−γc−/− Mice. *Ann NY Acad Sci* 1044:236-243.
4. Choi, E.Y., K.C. Jung, H.J. Park, D.H. Chung, J.S. Song, S.D. Yang, E. Simpson, and S.H. Park. 2005. Thymocyte-thymocyte interaction for efficient positive selection and maturation of CD4 T cells. *Immunity* 23:387-396.
5. Gimeno, R., K. Weijer, A. Voordouw, C.H. Uittenbogaart, N. Legrand, N.L. Alves, E. Wijnands, B. Blom, and H. Spits. 2004. Monitoring the effect of gene silencing by RNA interference in human CD34+ cells injected into newborn RAG2−/− gammac−/− mice: functional inactivation of p53 in developing T cells. *Blood* 104:3886-3893.
6. Hiramatsu, H., R. Nishikomori, T. Heike, M. Ito, K. Kobayashi, K. Katamura, and T. Nakahata. 2003. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. *Blood* 102:873-880.
7. Ishikawa, F., M. Yasukawa, B. Lyons, S. Yoshida, T. Miyamoto, G. Yoshimoto, T. Watanabe, K. Akashi, L.D. Shultz, and M. Harada. 2005. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chain^{null} mice. *Blood* 106:1565-1573.
8. Legrand, N., K. Weijer, and H. Spits. 2006. Experimental models to study development and function of the human immune system in vivo. *J Immunol* 176:2053-2058.
9. Li, W., M.G. Kim, T.S. Gourley, B.P. McCarthy, D.B. Sant'Angelo, and C.H. Chang. 2005. An alternate pathway for CD4 T cell development: thymocyte-expressed MHC class II selects a distinct T cell population. *Immunity* 23:375-386.
10. Macchiarini, F., M.G. Manz, A.K. Palucka, and L.D. Shultz. 2005. Humanized mice: are we there yet? *J Exp Med* 202:1307-1311.
11. Manz, M.G. 2007. Human-hemato-lymphoid-system mice: opportunities and challenges. *Immunity* 26:537-541.
12. McCune, J.M., R. Namikawa, H. Kaneshima, L.D. Shultz, M. Lieberman, and I.L. Weissman. 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 241:1632-1639.
13. Melkus, M.W., J.D. Estes, A. Padgett-Thomas, J. Gatlin, P.W. Denton, F.A. Othieno, A.K. Wege, A.T. Haase, and J.V. Garcia. 2006. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nat Med* 12:1316-1322.
14. Mestas, J., and C.C. Hughes. 2004. Of mice and not men: differences between mouse and human immunology. *J Immunol* 172:2731-2738.
15. Palmer, E. 2003. Negative selection-clearing out the bad apples from the T-cell repertoire. *Nat Rev Immunol* 3:383-391.
16. Reith, W., and B. Mach. 2001. The bare lymphocyte syndrome and the regulation of MHC expression. *Annu Rev Immunol* 19:331-373.
17. Shizuru, J.A., I.L. Weissman, R. Kernoff, M. Masek, and Y.C. Scheffold. 2000. Purified hematopoietic stem cell grafts induce tolerance to alloantigens and can mediate positive and negative T cell selection. *Proc Natl Acad Sci USA* 97:9555-9560.
18. Shultz, L.D., F. Ishikawa, and D.L. Greiner. 2007. Humanized mice in translational biomedical research. *Nat Rev Immunol* 7:118-130.
19. Shultz, L.D., B.L. Lyons, L.M. Burzenski, B. Gott, X. Chen, S. Chaleff, M. Kotb, S.D. Gillies, M. King, J. Mangada, D.L. Greiner, and R. Handgretinger. 2005. Human lymphoid and

myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* 174:6477-6489.
20. Steinman, R.M., and I. Mellman. 2004. Immunotherapy: bewitched, bothered, and bewildered no more. *Science* 305:197-200.
21. Sun, Z., P.W. Denton, J.D. Estes, F.A. Othieno, B.L. Wei, A.K. Wege, M.W. Melkus, A. Padgett-Thomas, M. Zupancic, A.T. Haase, and J.V. Garcia. 2007. Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1. *J Exp Med* 204:705-714.
22. Surh, C.D., and J. Sprent. 2005. Regulation of mature T cell homeostasis. *Semin Immunol* 17:183-191.
23. Traggiai, E., L. Chicha, L. Mazzucchelli, L. Bronz, J.C. Piffaretti, A. Lanzavecchia, and M.G. Manz. 2004. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* 304:104-107.
24. Watanabe, S., K. Terashima, S. Ohta, S. Horibata, M. Yajima, Y. Shiozawa, M.Z. Dewan, Z. Yu, M. Ito, T. Morio, N. Shimizu, M. Honda, and N. Yamamoto. 2007. Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. *Blood* 109:212-218.
25. Zhang, L., G.I. Kovalev, and L. Su. 2007. HIV-1 infection and pathogenesis in a novel humanized mouse model. *Blood* 109:2978-2981.
26. Zinkernagel, R.M., and A. Althage. 1999. On the role of thymic epithelium vs. bone marrow-derived cells in repertoire selection of T cells. *Proc Natl Acad Sci USA* 96:8092-8097.