Developmental dissociation of T cells from B, NK, and myeloid cells revealed by MHC class II–specific chimeric immune receptors bearing TCR-ζ or FcR-γ chain signaling domains

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The T-cell receptor ζ (TCR-ζ) and FcR-γ chains play a critical role in mediating signal transduction. We have previously described HIV glycoprotein 120 (gp120)–specific chimeric immune receptors (CIRs) in which the extracellular domain of CD4 is linked to the signaling domain of ζ (CD4ζ) or γ (CD4γ). Such CIRs are efficiently expressed following retroviral transduction of mature T cells and specifically redirect effector functions toward HIV-infected targets. In this report, we examine development of CD4ζ- or CD4γ-expressing T cells from retrovirally transduced hematopoietic stem cells following bone marrow transplantation. Although CD4ζ-expressing myeloid, NK, and B cells were efficiently reconstituted, parallel development of CD4γ-expressing T cells was blocked prior to the CD25+CD4+ prothymocyte stage. In contrast, T cells expressing a signaling-defective CIR were efficiently generated. When major histocompatibility complex (MHC) class II–deficient mice were used as transplant recipients, development of CD4γ-expressing T cells was restored. We conclude that CD4γ signaling generated following engagement of MHC class II selectively arrests T-lineage development.

Reference:

Brief report

Introduction

We have previously described HIV glycoprotein 120 (gp120)–specific chimeric immune receptors (CIRs) in which the cytoplasmic domain of either the T-cell receptor ζ (TCR-ζ) or the FcR-γ chain is linked to the extracellular and transmembrane domains of the human CD4 receptor. Mature CD4ζ-expressing T lymphocytes generated ex vivo by retroviral transduction are capable of highly efficient and specific cytolyis of both HIV-infected primary cells and HIVgp120-expressing tumors in vitro, and clinical trials involving adoptive transfer of autologous CD4ζ-transduced T cells in HIV-infected patients have been undertaken.

In vivo development of CIR-modified T cells from transplanted hematopoietic stem cells (HSCs) may have some advantages over adoptive transfer of ex vivo-transduced mature T cells, because the former bypasses the need for extensive ex vivo cell expansion and may improve T-cell function in vivo. Our previous studies have shown that severe combined immunodeficient (SCID) mice (which lack T and B cells) rapidly reconstitute CD4ζ-expressing myeloid and natural killer (NK) cells following transplantation with retrovirally transduced syngeneic bone marrow. Furthermore, such SCID mice that received CD4ζ transplants are protected from a lethal dose of HIVgp120-expressing leukemia cells. One of the many potential barriers to engraftment of a bone marrow transplantation (BMT) approach for generating functional CD4ζ T cells is the impact, if any, of retroviral-driven CIR expression early in hematopoiesis on T-cell development. The affinity of the human CD4 receptor for murine and human major histocompatibility complex class II (MHCII) is insufficient for activation of CD4ζ-expressing T cells. However, the consequences of such low-affinity interactions on lymphoid development have not been examined.

Study design

Vectors

Retroviral vectors expressing CD4del, CD4ζ, and CD4γ CIRs via an internal phosphoglycerate kinase (pgk) promoter have been described previously.

Mice

The MHCII-deficient (MHCII−) mice possess a disruption of the MHC class II Ab β gene (B6.129-Abbml1; Taconic, Germantown, MD) and...
therefore do not express surface MHCII in the C57BL/6 background. Wild-type C57BL/6 mice (Taconic) were used as MHCII+ recipients in experiments involving donor marrow from MHCII- mice.

Flow cytometry
Antibodies (Pharmingen, San Diego, CA) used to stain peripheral blood and thymocytes are described in the figure legends. Stained cells were analyzed on a FACScan cytometer (Becton Dickinson, San Jose, CA).

Results and discussion
Bone marrow was isolated from C3H mice and retrovirally transduced with CD4ζ, CD4γ, or a signaling-defective CIR (CD4del) as previously described.\textsuperscript{3,5} Surface CIR expression on transduced bone marrow cells before infusion is shown in Figure 1A.
Although the percentage of cells expressing CD4ζ and CD4γ was similar, the mean intensity of expression was considerably higher for the CD4ζ and CD4del receptors as seen in previous studies. The transduced bone marrow was subsequently transplanted into sublethally irradiated C3H mice via tail-vein injection.

Peripheral blood was isolated from reconstituted animals at approximately 6 weeks after transplantation and analyzed by 2-color flow cytometry using monoclonal antibodies against human CD4 and the following mouse cell lineage markers: GR-1 (granulocytes); B220 (B cells); 5E6 (NK cells), or CD3 (T cells; Figure 1B). CD4ζ, CD4γ, and CD4del were expressed on granulocytes and NK cells at similar frequencies, as previously described for SCID mice following BMT. Although similar levels of expression were observed in B cells for all 3 receptors, T cells expressing CD4ζ or CD4γ were not detected. In contrast, expression of the signaling-defective CIR, CD4del, was retained on this lineage.

The differential expression of CD4del and CD4ζ/γ in mature T lymphocytes prompted us to examine CIR expression during thymocyte development. Thymocytes were harvested from mice that had received either CD4ζ- or CD4del-transduced bone marrow 6 weeks before, and TN2, TN3, TN4, and TP (triple-positive)/H9256 that had received either CD4ζ- or CD4del-transduced bone marrow development. Thymocytes were harvested from mice in each case. ND indicates insufficient cells to analyze.

Figure 2. Development of CD4ζ/γ-expressing thymocytes and T cells is rescued in MHCII− mice. Bone marrow cells isolated from MHCII− mice were exposed to retroviral supernatant encoding CD4ζ, CD4γ, or CD4del (CD4Δ), and then infused into sublethally irradiated wild-type C57BL/6 (A) or MHCII−C57BL/6 (B) mice. Six weeks after transplantation, peripheral blood was isolated and analyzed by 2-color flow cytometry using PE-conjugated antihuman CD4 and FITC-conjugated anti-B220 or anti-CD3. The percentage of lineage positive cells expressing human CD4 is shown in the top left hand corner of each dot plot. Control cells stained with antihuman CD4-PE or isotype controls yielded indistinguishable results (data not shown). Results are representative of at least 8 additional mice. (C) Thymocytes were isolated from MHCII− mice in panel B, which had received CD4ζ- or CD4del (CD4Δ)−transduced bone marrow, and were analyzed by 4-color flow cytometry as described in the legend to Figure 1. The percentage of human CD4-expressing cells (solid line) in each TN and TP subset is indicated in each histogram. Shaded histograms represent cells from control animals that received transplants of unmodified bone marrow in each case. ND indicates insufficient cells to analyze.

To determine whether engagement of CD4ζ or CD4γ (CD4ζ/γ) by MHCII was indeed responsible for the failure of CD4ζ/γ-bearing thymocyte development, immune reconstitution was examined in mice lacking MHCII expression. Specifically, bone marrow derived from C57BL/6 mice lacking expression of MHCII (MHCII−) was transduced with CD4ζ, CD4γ, or CD4del, and transplanted into syngeneic (MHCII−) or congenic (MHCII+γ−) wild-type recipients. As before, peripheral blood was harvested from reconstituted mice approximately 6 weeks after transplantation, and B- and T-cell lineages examined for human CD4 expression by flow cytometry (Figure 2A). Analysis of peripheral blood isolated from MHCII+ or MHCII− recipients revealed expression of each of the 3 receptors on B cells (Figure 2A), myeloid cells, and NK cells (data not shown) as expected. Consistent with the experiment summarized in Figure 1 using C3H mice, T cells expressing the CD4del receptor, but not CD4ζ or CD4γ, were present in reconstituted MHCII+ recipients. In striking contrast, T cells from MHCII− recipients expressed either CD4ζ or CD4γ receptors at levels comparable to those of the CD4del receptor. Flow cytometric
analysis of thymocytes isolated from the MHCII− recipient mice in Figure 2B revealed that CD4ζ-expressing TN2, TN3, TN4, and TP thymocytes could now be detected at equivalent frequencies to the CD4del subsets (Figure 2C).

In summary, we have shown that development of CD4ζ/γ-bearing T cells from transplanted HSCs is arrested prior to the TN2 (CD44+CD25−) stage in normal mice. The developmental block is specific for the T lineage because myeloid, NK, and B cells are unaffected. In addition, we have shown that T-lineage arrest is dependent on the signaling domain of either ζ or γ (because CD4del-expressing T cells develop normally), and on expression of the CD4 ligand, MHCII. The process by which HSCs commit to the T-lymphoid lineage is poorly understood, particularly during adult steady-state hematopoiesis. Several studies suggest that TN1 cells (and in the thymus arise from, or are equivalent to, common lymphocyte precursors (CLPs) found in bone marrow,17,18 TN1 cells (and CLPs) have lost myeloid potential, but can still develop into lymphocytes (T, B, and NK cells).17,18 By the TN2 stage, however, the potential to develop into B and NK cells has also been lost. Our current hypothesis is that ζ/γ-mediated signal transduction occurs in response to CD4ζ/γ-MHCII engagement during the CLP/TN1→TN2 transition. The TN2→TN4 transition is mediated by pre-TCR signaling via the associated immunoreceptor tyrosine-based activation motif (ITAM)—bearing CD3ζ chains.19–23 Presumably, premature or aberrant ITAM-mediated signal transduction by CD4ζ/γ during the CLP/TN1→TN2 transition arrests T-lineage commitment or differentiation of committed T-cell precursors. This hypothesis is consistent with recent data from our laboratory showing that CD4ζ is expressed at high levels on TN thymocytes when under the transcriptional control of the CD2 enhancer instead of the constitutively active pgk promoter of the retroviral vector (manuscript in preparation). Despite the presence of only a single ITAM, CD4γ is as effective as CD4ζ in mediating this developmental defect. The FcR-γ chain is known to be expressed in normal DN thymocytes and has been implicated in the development of certain T-cell subsets.24,25 The mechanisms driving lineage commitment during the CLP/TN1→TN2 transition remain obscure. Studies are now underway to determine the specific step in this complex pathway at which CD4ζ/γ-induced arrest occurs, and to further dissect the process of T-lineage determination.

References

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