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Cutting Edge: Two Distinct Mechanisms Lead to Impaired T Cell Homeostasis in Janus Kinase 3- and CTLA-4-Deficient Mice

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Cytokine receptor signaling and costimulatory receptor signaling play distinct roles in T cell activation. Nonetheless, deficiencies in either of these pathways lead to seemingly similar phenotypes of impaired T cell homeostasis. A dramatic expansion of CD4 + peripheral T cells with an activated phenotype has been observed in both Janus kinase (Jak) 3-deficient and CTLA-4-deficient mice. Despite these similarities, the mechanisms driving T cell expansion may be distinct. To address this possibility, we examined the TCR repertoire of peripheral T cells in Jak3 −/− and CTLA-4 −/− mice using complementarity-determining region 3 spectratype analysis. Interestingly, a restricted and highly biased TCR repertoire was observed in the Jak3 −/− T cells, strongly supporting a role for foreign Ag in the activation and expansion of these cells. In contrast, CTLA-4 −/− T cells had a diverse and unbiased TCR repertoire, suggestive of a universal, Ag-independent mechanism of activation and expansion. These findings provide insight into the diverse mechanisms controlling T cell homeostasis. The Journal of Immunology, 2001, 166: 727–730.

T cell homeostasis is a tightly regulated process. The peripheral immune system maintains a constant number of total T cells, and also conserves the relative balance between naïve T cells, with a diverse TCR repertoire, and memory T cell clones generated during Ag-specific T cell responses (1, 2). The system allows for the dramatic expansion and subsequent contraction of the Ag-specific T cell pool during an immune response (3, 4). In recent years, mouse lines with specific genetic deficiencies have provided some insights into the regulation of T cell homeostasis; however, this process is still poorly understood.

Two such models of disregulated T cell homeostasis are mice deficient in the tyrosine kinase, Janus kinase (Jak) 3, and mice lacking the T cell costimulatory molecule, CTLA-4 (CD152). Mice deficient for either Jak3 or CTLA-4 present a similar phenotype characterized by an apparently polyclonal expansion of the peripheral T cells. However, it is not known whether similar mechanism(s) are responsible for this expansion. Understanding the basis of the phenotype observed in these animals would provide important insights into the roles of Jak3 and CTLA-4 in T cell homeostasis.

Jak3 is preferentially expressed in hematopoietic cells, where it is associated with the cytokine receptor common γ-chain, a component of the receptors for IL-2, -4, -7, -9, and -15 (5). Deficiencies in Jak3 lead to severe combined immunodeficiency conditions in humans and mice. Specifically, Jak3 −/− mice are characterized by a block in B, NK, and γδ T cell development (6–8). Although there is a reduction in the cellularity of the thymus, αβ thymocyte development appears to progress normally. Despite this, Jak3 −/− mice have plentiful numbers of peripheral T cells, but they are predominantly CD4 +, with a virtual absence of mature CD8 + T cells. Furthermore, these cells resemble activated and/or memory cells in that they are large and express surface markers (CD44 high, CD25, and CD69) characteristic of prior activation (9). These T cells expand in the periphery, leading to an increase in overall T cell numbers. Interestingly, when TCR-transgenic mice are crossed with Jak3 −/− mice, the peripheral T cell pool is dramatically reduced in numbers and the cells remain phenotypically naïve (CD44low, CD25low, and CD69low) (10), suggesting that the activation and expansion of Jak3 −/− T cells bearing heterogeneous TCRs may be oligoclonal and dependent on Ag receptor-specific stimulation.

CTLA-4 is a CD28 homologue that acts as a negative regulator of T cell activation. Interaction of CTLA-4 with its ligands, B7-1 and B7-2 (CD80 and CD86), inhibits T cell proliferation and reduces TCR and CD28 signaling during T cell activation (11). Unlike the Jak3 −/− mice, CTLA-4 −/− mice have normal thymocyte numbers and development (12); yet the peripheral T cell phenotype of the two mouse strains is remarkably similar. Peripheral

3 Abbreviations used in this paper: Jak, Janus kinase; LCMV, lymphocytic choriomeningitis virus; CDR, complementarity-determining region; CD62L, L-selectin.

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CTLA-4−/− T cells are predominantly CD4+, and virtually all of them express activation markers (CD44high, CD25, and CD69).

Furthermore, the mice die at about 3 wk of age due to a lymphoproliferative disorder (13, 14). In contrast to the findings with Jak3−/− TCR-transgenic mice, this disease process can be delayed, but not prevented, by introducing an MHC class II-restricted TCR transgene into the CTLA-4−/− mice (15, 16). These data suggested that, unlike the Jak3-deficient T cells, the activation of CD4+ T cells in the CTLA-4−/− mice may not be dependent on specific Ag stimulation.

To better understand the nature of the T cell activation/expansion and loss of homeostasis in both Jak3−/− and CTLA-4−/− mice, we analyzed the diversity of the peripheral TCR repertoires in each mouse line. We reasoned that, if specific Ag is involved in the activation and expansion of the peripheral T cells, this would be reflected in the TCR repertoire as a skewed distribution of the activation and expansion of peripheral T cells in unmanipulated Jak3−/− mice is Ag driven. In contrast, the TCR repertoire of activated peripheral T cells from CTLA-4−/− mice ex vivo remains diverse and unbiased, comparable to that seen in wild-type animals. These results demonstrate that the nature of the T cell expansion in Jak3−/− and CTLA-4−/− mice is distinct and suggest a unique role for each of these molecules in the regulation of T cell homeostasis.

Materials and Methods

Mice

Jak3−/− and Jak3+/− mice (6, 8) and CTLA-4−/− and CTLA-4+/− mice (13) have been described previously. Mice have been backcrossed to Piscataway, NJ). The Jak3−/− and littermate controls were 2 wk of age. All experiments using Jak3−/− and CTLA-4−/− mice were conducted using their respective littersmates on the same day. Male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were used for the lymphomycotic choriomeningitis virus (LCMV) infections.

Cell staining and sorting

For Ab staining experiments, splenocytes were isolated and depleted of RBC. Cells were then stained with anti-CD44-FITC (clone IM7), anti-CD69-FITC (clone MEL 14) (Bioscience, San Diego, CA). Samples were analyzed on a Becton Dickinson FACStar to 94% purity.

Virus stocks and immunization

PFUs (4 × 103) of the LCMV Armstrong strain were used to infect mice i.p. Splenocytes were isolated 8 days after infection.

RNA extraction and cDNA synthesis

Total RNA was isolated from whole spleens and thymi using TRizol as described by the manufacturer (Life Technologies, Grand Island, NY). Briefly, 2 ml of TRizol was used for the thymi of Jak3+/− mice, spleens of Jak3−/− and Jak3+/− mice, and spleens of CTLA-4−/− and CTLA-4+/− mice, while 1 ml of TRizol was used for the thymi of Jak3−/− mice, and for 1–10 × 106 sorted thymocytes. RNA was resuspended in 10–15 μl of diethyl pyrocarbonate water. Total cDNA was synthesized from an average of 4 μl of RNA using the Pharmacia kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Figure 1. Activated/memory cell phenotype of Jak 3−/− and CTLA-4−/− T cells. Splenocytes were stained with Abs to CD4 plus a panel of activation markers. Histograms show expression of CD44 and CD69 on gated CD4+ populations. Thin lines represent heterogeneous controls and thick lines represent Jak3−/− (left panels) or CTLA-4−/− (right panels).
primers. The PCR products are then used as templates in elongation reactions using several fluorescently labeled Jβ-specific primers. The resulting run-off reactions are displayed as a spectrum of size peaks for each CDR3 region. In naive T cells, CDR3 lengths are distributed as a Gaussian curve. An increase in a given peak within the spectrum indicates preferential expansion of a particular T cell clone. As previously established, CDR3 spectratyping provides an exquisitely sensitive means of assessing the heterogeneity of the TCR repertoire in a given population of T cells (17).

To examine the heterogeneity of TCR repertoires in the Jak3–/– and CTLA-4–/– mice, CDR3 spectratyping was performed on splenocytes using combinations of primers for eight different Vβs and six Jβs (see Materials and Methods). Representative data are shown in Fig. 2A for three Vβs in combination with three Jβs. Splenocytes from Jak3–/– and CTLA-4–/– littermate control mice display a Gaussian distribution, typical of a diverse and unbiased TCR repertoire (3, 17). This diversity was observed with all combinations of Vβs and Jβs examined (Fig. 2A and data not shown). Interestingly, the same diverse repertoire is observed in splenocytes from CTLA-4–/– mice and was even observed in T cells from CTLA-4–/– mice with extremely advanced lymphoproliferation (Fig. 2A and data not shown). Conversely, CDR3 spectratype analysis of splenocytes from Jak3–/– mice shows a skewed phenotype. The magnitude of skewing appears to be biologically relevant, as it is comparable in magnitude to the skewing observed at the peak of the CTL response to an LCMV infection (day 8) using a primer specific for Vβ8.1 (Fig. 2B and Ref. 3).

To confirm this initial observation, these experiments were repeated with a total of five Jak3–/– mice, seven Jak3–/– mice, four CTLA-4–/– mice, and four CTLA-4–/– mice, each analyzed with four Vβ primers coupled with six Jβ primers. Of these, three of the Jak3–/– and five of the Jak3–/– mice were analyzed with primers specific for an additional four Vβs in combination with six Jβs. In all cases, the data supported the initial observation that peripheral T cells from Jak3–/– mice have a profoundly skewed TCR repertoire, whereas peripheral T cells from the other three groups tested, including the CTLA-4–/– mice, exhibited a normal, diverse, and unbiased repertoire (data not shown). These data strongly support the idea of Ag-dependent activation and expansion of Jak3–/– T cells, as only a limited number of T cell clones appears to be expanded in these mice. In contrast, the activation and expansion of CTLA-4–/– T cells appears to occur by an Ag-independent mechanism, as an unlimited number of T cell clones is expanding in the absence of CTLA-4. Interestingly, skewing of the CDR3 region was detected in the Jak3–/– T cells despite the

### FIGURE 2

**Jak3–/–**, but not CTLA-4–/– peripheral T cells show a skewed TCR repertoire. **A**, Total RNA was extracted from Jak3+/+ and Jak3–/– (8 wk of age), CTLA-4+/+ and CTLA-4–/– (2 wk of age) splenocytes and subjected to spectratype analysis as described in Materials and Methods. A representative example of the data from two mice of each genotype is displayed for three Vβs, each analyzed with three Jβs. **Row 1** (Jak3+/+ and CTLA-4+/+) and **row 4** (Jak3–/– and CTLA-4–/–) are littermate controls, while **row 2** (Jak3+/+ and CTLA-4–/–) and **row 3** (Jak3–/– and CTLA-4+/+) are littermate controls. **B**, Total RNA was extracted from naive and day 8 LCMV-infected C57BL/6 splenocytes and subjected to spectratype analysis using a Vβ8.1-specific primer.

### FIGURE 3

TCR repertoire skewing in Jak3–/– mice occurs in the periphery, not in the thymus. **A**, Total RNA was isolated from Jak3+/+ and Jak3–/– (8–9 wk of age) thymi and spleens and subjected to spectratype analysis. A representative example of the data for two mice of each genotype is displayed for three Vβs, each analyzed with three Jβs. The first Jak3+/+ and the second Jak3–/– are littermate controls while the second Jak3+/+ and the first Jak3–/– are littermate controls. **B**, Total RNA was extracted from sorted CD4+ thymocytes from Jak3+/+ and Jak3–/– thymi and subjected to spectratype analysis. A representative example of the data for two Vβs, each analyzed with three Jβs, is shown.
fact that there was no detectable skewing of the TCR repertoire as assessed by Vβ8 and Vα usage determined by flow cytometry (21).

To address the possibility that skewing of the TCR repertoire in Jak3−/− mice is occurring during thymic selection, rather than as a result of peripheral T cell activation and expansion, we examined the TCR repertoire of Jak3−/− thymocytes. As can be seen in Fig. 3A, thymocytes from Jak3−/− mice show the typical Gaussian distribution of a diverse TCR repertoire, whereas splenocytes show a highly skewed repertoire. To confirm this and eliminate the potential contribution of unselected thymocytes, we examined the TCR repertoire of purified CD4+ single-positive thymocytes from Jak3−/− and control (Jak3+/+) mice. Both the Jak3+/+ and the Jak3−/− CD4+ single-positive thymocytes exhibited a diverse and unbiased TCR repertoire (Fig. 3), demonstrating that the skewing observed in the TCR repertoire of peripheral Jak3−/− T cells does not occur as a result of altered positive or negative selection in the thymus.

These data, along with our previous studies of TCR-transgenic Jak3−/−/− mice, support the following model. Jak3−/− αβTCR+ thymocytes undergo normal positive and negative selection in the thymus and migrate to the periphery as resting naive T cells. Once in the periphery, naïve Jak3−/− T cells are susceptible to apoptosis, due to the lack of IL-7 signaling in the absence of Jak3. The dependence of naive T cells on IL-7 for survival may be mediated by Bcl-2 induction, as has been suggested by both in vitro and in vivo studies (22-24). However, a fraction of the Jak3−/− T cells encounter specific Ags in the periphery, and these cells get activated and expand. Once activated, the Jak3−/− T cells will become independent of IL-7 for survival and will accumulate in the periphery due to the absence of IL-2R-mediated up-regulation of Fas ligand necessary for activation-induced cell death. Consistent with this hypothesis, Nakajima et al. have previously shown that common γ-chain signaling is essential for the deletion of activated peripheral CD4+ T cells, most likely by inducing Fas ligand expression (19, 25).

Similar to the Jak3-deficient mice, CTLA-4−/− animals do not appear to have defects in αβTCR+ thymocyte selection, and single-positive thymocytes emigrate to the periphery as mature naive T cells (12). However, almost immediately upon entering the periphery, CTLA-4−/− T cells become activated (12). The subsequent accumulation of these T cells in the periphery does not appear to be due to a defect in apoptosis (14, 26). Instead, we have proposed that CTLA-4−/− T cells can become activated as a result of some of the TCR-MHC interactions necessary for peripheral T cell survival/homeostasis (18, 27, 28). Furthermore, as CTLA-4-mediated inhibition is more profound in previously activated T cells, compared with naive T cells (15, 29), the absence of CTLA-4 would be magnified upon restimulation of the CTLA-4−/− T cells in vivo. This model predicts that exogenous Ags would not be necessary for activation of CTLA-4−/− T cells and that there should be no skewing of the TCR repertoire. The results presented here support this model. Also consistent with this model is the observation that CD4+ T cells become activated in H-2M3×M3 mice in the absence of CTLA-4 (C. A. Chambers, unpublished observation).

In summary, these CDR3 spectratype results demonstrate that the expansion of T cells observed in Jak3−/− mice does not occur during thymic development, but instead, takes place in the periphery of the mice and involves a restricted number of T cell clones. In contrast, the expansion of T cells in the periphery of CTLA-4−/− mice does not appear to be restricted to a limited number of T cell clones, as the diversity of the TCR repertoire in CTLA-4−/− mice is comparable to immunized wild-type mice. Therefore, two very similar phenotypes of peripheral T cell activation and expansion are clearly derived by distinct mechanisms.