Crucial Role of Jak3 in Negative Selection of Self-reactive T Cells

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Summary

Jak3 mediates growth signals through cytokine receptors such as interleukin-2 (IL-2), IL-4, and IL-7, and its deficiency results in autosomal recessive SCID in mice and humans. In spite of the severely reduced number of lymphocytes in Jak3-deficient mice, the differentiation profile of thymocytes was normal and mature T cells accumulated in the periphery with age. However, we found that self-reactive T cells were not deleted in the thymus and the peripheral tissues in Jak3-deficient mice. All peripheral T cells were in the activation state and thus were unable to be activated further, as demonstrated by the failure of eliciting Ca\(^{2+}\) response upon T cell receptor (TCR) stimulation. From the analysis of TCR-transgenic Jak3-deficient mice, only self-reactive T cells appeared to be in the activated state and anergic. These findings demonstrate a crucial function of Jak3 in the negative selection of autoreactive T cells and the maintenance of functional peripheral T cells.

Materials and Methods

Mice. C57BL/6 mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). DO11.10 TCR-transgenic mice (DO-Tg) were provided by Dr. D. Loh (Nippon Roche Research Center, Kamakura, Japan) (17). Jak3-deficient mice were previously described (15).

Analysis of Cell Proliferation and IL-2 Secretion. For proliferation assay, splenocytes (2\times10^6) from 8-wk-old mice were stimulated with Con A (2.5 \mu g/ml), PMA (5 ng/ml) plus A23187 (100 ng/ml), and anti-CD3\e mAb (145-2C11) cross-linking for 48 h, pulsed with 37 kBq \[^{3}H\]thymidine (Amersham Corp., Arlington Heights, IL) for the last 8 h of culture, and harvested. \[^{3}H\]thymidine uptake was measured with a MicroBeta\textsuperscript{TM} liquid scintillation counter (Pharmacia, Uppsala, Sweden). For IL-2 production, proliferation of the IL-2-dependent cell line CTLL-2 was measured. CTLL-2 cells (6 \times 10^4) were cultured with supernatants from the proliferation assay after stimulation for 48 h. Cells were pulsed with \[^{3}H\]thymidine and harvested as described above.

Measurement of Intracellular Ca\(^{2+}\) Response. Intracellular Ca\(^{2+}\) mobilization was measured using Epics Elite ER-3 (Coulter Corp., Hialeah, FL) with a Cell Quest analyzing program. 2 \times 10^7 cells were incubated with 4 \mu M Indo-1 (Molecular Probes, Inc., Eugene, OR) for 30 min at 37°C for loading. For TCR stimulation, cells were incubated with anti-CD3\e mAb (145-
**Results**

**Failure of Deletion of Self-reactive T Cells in the Thymus and Periphery of Jak3-deficient Mice.** When the Vβ repertoire of splenic T cells from Jak3-deficient mice with C57BL/6 background was analyzed, there was no difference in the frequency of Vβ usage between Jak3+/− mice and wild-type littermates (data not shown). To analyze the repertoire of T cells reactive to endogenous MMTV products presented on I−E molecule, we backcrossed with BALB/c and analyzed the Vβ usage. As shown in Fig. 1 A, the frequency of mature T cells expressing Vβ5 and Vβ11, but not others, was significantly increased in Jak3+/− mice as compared with heterozygous mice. These Vβ-expressing T cells are known to be deleted in BALB/c mice, whereas C57BL/6 mice fail to delete them due to the lack of MHC class II I-E molecules. Although extensive analysis could not be performed on thymocytes due to their limited number in Jak3−/− mice, similar increases of CD4 single-positive thymocytes expressing Vβ5 and Vβ11 were observed (Fig. 1 B). It is noteworthy that, while the percentage of Vβ11+ T cells in Jak3−/− mice with H−2d background was almost restored to the level of C57BL/6 mice, the frequency of Vβ5 was only partly recovered. These data demonstrate that Jak3-deficient mice failed to delete self-reactive T cells. Furthermore, the observation that the percentage of Vβ10+ T cells was reduced in Jak3−/− mice suggests additional defects.

**Peripheral T Cells in Jak3-deficient Mice Are Preactivated.** In addition to the existence of autoreactive T cells in thymus, spleen, and lymph node, all peripheral T cells from Jak3−/− mice were activated as determined by surface expression of several markers. These T cells expressed high levels of CD44 and CD69 (18–20) and a low level of Mel-14, representing the phenotype of activated T cells (Fig. 2 A). To analyze further the origin of the preactivated T cells in the periphery of Jak3−/− mice, we crossed Jak3−/− mice with OVA-specific DO-Tg mice whose TCR (DO-TCR) was detected by staining with anti-clonotypic mAb KJ1-26 for the TCRαβ dimer and anti-Vβ8 mAb F23.1 for the TCRβ chain. ~20% of the T cells from DO-Tg mice expressed endogenous TCR, but the rest of the cells were
stained with KJ1-26 and F23.1 (17). Whereas KJ1-26 positive and negative populations from DO-Tg mice did not show a significant difference in CD44 expression, the DO-TCR-expressing (KJ1-26 high) T cells from DO-Tg Jak3−/− mice were CD44 low (Fig. 2B). In contrast, most of the T cells expressing endogenous TCR (KJ1-26 low) were CD44 high, which is the same phenotype as Jak3−/− splenic T cells (Fig. 2B). This was also shown by Mel-14 expression. Whereas DO-TCR expressing T cells (Vß8 high) were composed of both Mel-14 high and Mel-14 low populations, T cells expressing endogenous TCR (Vß8 low) were all Mel-14 low (Fig. 2B). These data demonstrate that T cells with endogenous TCR but not DO-TCR-expressing T cells were re-activated in DO-Tg Jak3−/− mice, suggesting that splenic T cells in Jak3−/− mice may be autoreactive and have been activated with self-antigens.

Defects of TCR Signaling in Peripheral T Cells from Jak3-deficient Mice. Since splenic T cells in Jak3−/− mice are in the activated state, we asked whether these T cells might be functionally unresponsive to further stimulation. Indeed, splenic T cells from Jak3−/− mice failed to proliferate upon stimulation with either Con A or anti-CD3e mAb cross-linking, regardless of the presence of exogenous IL-2. Furthermore, these T cells did not proliferate even after stimulation with PMA and Ca²⁺ ionophore (Fig. 3A). In contrast with the splenic T cells, thymocytes from Jak3−/−
mice responded to both anti-CD3ε cross-linking and stimulation with PMA plus Ca²⁺ ionophore (15). As shown in Fig. 3B, thymocytes from Jak3⁻/⁻ mice secreted a considerable amount of IL-2 compared with normal thymocytes, while splenic T cells from Jak3⁻/⁻ mice produced very little. Cell surface staining revealed no difference in TCR expression between Jak3⁻/⁻ and wild-type mice (15). These data demonstrated that splenic T cells in Jak3⁻/⁻ mice possess defects in the signal transduction pathway leading to IL-2 production upon TCR stimulation, in addition to growth signal defects.

To investigate the defects in TCR activation, we analyzed intracellular Ca²⁺ mobilization as an indicator of the early signal transduction pathway upon TCR stimulation. As shown in Fig. 4A, thymocytes from Jak3⁻/⁻ mice elicited almost comparable Ca²⁺ response to that of thymocytes from wild-type mice upon stimulation with both anti-CD3ε mAb cross-linking and Ca²⁺ ionophore. In Figure 3. Functional analysis of thymocytes and splenic T cells from Jak3-deficient mice. (A) Proliferation of splenic T cells upon mitogenic stimulation. Splenocytes (2 × 10⁶) from Jak3 homozygous (−/−), heterozygous (+/−) mutant mice, and wild-type littermates (+/+), were stimulated with anti-CD3ε mAb (145-2C11, 10 μg/ml), Con A (2.5 μg/ml), IL-2 (40 U/ml), Staphylococcal enterotoxin B (10 μg/ml), and the combination of PMA (5 ng/ml) and A23187 (100 ng/ml). Cells were cultured for 48 h and pulsed with [³H]thymidine for 8 h. (B) IL-2 production of thymocytes and splenic T cells upon mitogenic stimulation. Thymocytes and splenic T cells from Jak3-homozygous (−/−), and heterozygous (+/−) mutant mice, and wild-type littermates (+/+), were stimulated with 145-2C11, Con A, and PMA plus A23187 as described in (A). All results were presented as mean ± SD from triplicate cultures.
contrast, splenic T cells from \( \text{Jak}^{3/-/} \) mice failed to elicit Ca\(^{2+}\) response upon TCR cross-linking in spite of the fact that these cells showed Ca\(^{2+}\) flux upon stimulation with Ca\(^{2+}\) ionophore (Fig. 4 B). These data clearly demonstrate that splenic T cells from \( \text{Jak}^{3/-/} \) mice have defects in early Ca\(^{2+}\) signaling upon activation through the TCR complex.

**Discussion**

Mutations in \( \gamma_c \) and Jak3 in the patients of XSCID (11) and AR-SCID (12–13), respectively, caused growth defects in T cells because \( \gamma_c \) and Jak3 are associated (2, 8) and are both required for growth signal in T cells (9). The failure of cell growth has been thought to be due to defective cyto-

kine receptor signaling. In the present study, we have demonstrated that, in addition to the growth defects, AR-SCID model mice have defects in negative selection of self-reactive T cells. Thus, Jak3-deficient mice possess forbidden autoreactive T cells in the thymus and periphery. The reason these mice do not develop autoimmune diseases may be because these autoreactive T cells are anergic to further stimulation. Although Jak3 deficiency resulted in a dramatic decrease in the number of precursor cells in the thymus, once they were seeded in the thymus, thymocyte differentiation appeared to take place normally (15). However, we found that Jak3 deficiency resulted in a failure to eliminate self-reactive thymocytes, consequently leading to the accumulation of autoreactive but anergic peripheral T cells.

How Jak3 is involved in negative selection is unknown at present. One possibility is that Jak3-mediated growth signal is crucial for the subsequent deletion of self-reactive thymocytes in addition to signals through TCR. The other intriguing possibility is that Jak3 is directly involved in T cell activation. Our observation that PMA plus Ca\(^{2+}\) ionophore did not stimulate IL-2 production in splenic T cells from \( \text{Jak}^{3/-/} \) mice, as well as the previous finding that Jak3 is crucial for preventing the induction of anergy in T cells (21), are consistent with this idea.

Signaling defects in splenic T cells from \( \text{Jak}^{3/-/} \) mice were observed in association with reactivated status, namely the high expression of activation markers such as CD44 and CD69 as well as the downregulation of Mel-14. Splenic T cells were reactivated in Jak3-deficient mice and were all refractory to further activation. From the analysis of DO-Tg\( \text{Jak}^{3/-/} \) mice, we showed that the appearance of reactivated and refractory T cells depended on the specificity of TCR. Because thymocytes from Jak3-deficient mice do not exhibit the activated phenotype and proliferate, secrete IL-2 and exhibit Ca\(^{2+}\) flux upon TCR stimulation, preactivation of splenic T cells probably takes place during immigration after leaving the thymus or within the periphery. Considering that only T cells with endogenous TCRs exhibited the activated phenotype, it is likely that these T cells were activated with self-peptides in the periphery, while OVA-specific T cells could not be activated in the absence of OVA peptide. The fact that defects in negative selection were influenced by TCR specificity is consistent with our observation that some VB\(^+\) T cells were completely restored from deletion, while some others were only partly recovered by Jak3 deficiency (Fig. 1). Such autoreactive T cells are in an anergic state after activation. Alternatively, provided that all T cells had been activated during immigration from thymus to the periphery and then returned to the resting state, T cells may fail to return in the absence of Jak3, although some of them can still return to the resting state depending on their TCR specificity. In either case, Jak3 plays a pivotal role in maintaining the normal phenotype and function of peripheral T cells.

Further analysis will be required to elucidate the molecular basis of defects of thymic negative selection and signaling in peripheral lymphocytes from Jak3-deficient mice.
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