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The Jak Family Tyrosine Kinase Jak3 Is Required for IL-2 Synthesis by Naive/Resting CD4⁺ T Cells

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The Jak family tyrosine kinase, Jak3, is involved in signaling through cytokine receptors using the common γ-chain. Mice deficient in Jak3 have mature T cells, all of which have an activated/memory cell phenotype but are unresponsive to in vitro stimulation. Due to this activated phenotype, it has been impossible to determine whether Jak3 plays a role in the responsiveness of naive/resting T cells. To circumvent this difficulty, we generated naive/resting Jak3-negative T cells by two genetic approaches. After stimulation, these cells failed to produce significant amounts of IL-2. Although no signaling defect could be detected, we did find that naive/resting Jak3-negative T cells have substantially reduced levels of the transcription factor NF-AT1 and moderately reduced levels of c-Jun and c-Fos. On the basis of these data, we propose that Jak3-dependent cytokine signals may be required to maintain the normal levels of basal transcription factors required for immediate responsiveness to Ag activation. The Journal of Immunology, 1999, 163: 5411–5417.

The Jak family tyrosine kinase, Jak3,²* plays a crucial role in signaling through several cytokine receptors. It fulfills this function by associating with the cytokine receptor common γ-chain, which is a component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (1–3). Mice deficient for Jak3, recently reported by several groups (4–7), were found to have several severe defects. Among the more unusual findings in Jak3 knockout (KO) (4) mice is the phenotype of their peripheral T cells. Jak3⁻/⁻ T cells are predominantly CD44high, CD62Llow, and CD69high and therefore resemble T cells that have previously experienced Ag activation signals (4, 8–10). Interestingly, peripheral T cells from mice that are deficient in other components of the IL-2R signaling system, including the IL-2R α-chain, the IL-2R β-chain, and the common γ-chain (γc), display the same phenotype as Jak3⁻/⁻ T cells (11–14). Thus, the inability to transmit IL-2 signals appears to result in the accumulation of activated T cells. In contrast to these data, experiments in which Jak3 or γc KO mice were crossed to TCR transgenic lines have shown that T cells that express the transgenic TCR are mainly in the naive/resting state (9, 15, 16). These data indicate that the activation of T cells that occurs in the absence of Jak3 or γc still requires specific signals through the TCR.

In addition to their activated phenotype, Jak3⁻/⁻ CD4⁺ T cells were found to secrete greatly decreased amounts of IL-2 when stimulated in vitro by cross-linking of the TCR and the costimulatory receptor, CD28 (4, 8). We have recently provided evidence that this defect is probably the result of two different factors (15). First, Jak3⁻/⁻ CD4⁺ T cells were found to have greatly decreased survival in culture (15), as has also been reported for T cells deficient in γc (16). This feature of Jak3⁻/⁻ T cells results in the death of the majority of T cells long before the 24 or 48 h points when IL-2 secretion was assessed. Second, cross-linking of the TCR and the costimulatory receptor was found to be a suboptimal stimulus for activated T cells from wild-type mice, provoking a greatly reduced level of IL-2 production from activated compared with that from naive/resting CD4⁺ T cells (15). Thus, the reduced IL-2 response of Jak3⁻/⁻ CD4⁺ T cells can be explained in part by their activation state plus the stimulus used and in part by their reduced survival in culture. This hypothesis was substantiated by our previous findings that after stimulation for only 5 h, IL-2 production by Jak3⁻/⁻ CD4⁺ T cells was comparable to that by normal activated T cells (15). Thus, activated T cells from Jak3 KO mice appear to have no inherent defect in the signaling pathway(s) that leads to IL-2 gene transcription.

Here we provide evidence showing that, in contrast to activated Jak3⁻/⁻ CD4⁺ T cells, naive/resting Jak3⁻/⁻ CD4⁺ T cells are severely defective in IL-2 synthesis after stimulation. These results suggest that Jak3 is important in maintaining the capability of naive/resting CD4⁺ T cells to produce IL-2 in response to TCR and costimulatory receptor signals.

Materials and Methods

Mice

Jak3-deficient mice, Jak3⁻/⁻ tg⁰ mice expressing a wild-type Jak3 transgene under control of the Lck proximal promoter, and 2B4 TCR transgenic Jak3⁻/⁻ mice have been described previously (4, 8, 15). Jak3⁻/⁻ and Jak3⁻/⁻ tg⁰ mice were of a mixed C57BL/6 (H-2b) and 129 (H-2b) background, and 2B4 TCR transgenic Jak3⁻/⁻ mice were generated by crossing these Jak3⁻/⁻ mice to 2B4 TCR transgenics on a B10.BR (H-2b) background. The absence of Vβ3-deleting superantigens in 2B4 TCR transgenic mice was assessed by staining peripheral blood CD4⁺ T cells with Abs against both chains of the transgenic TCR.

Flow cytometry

Splenocytes (1 × 10⁶) were stained with combinations of the following Abs: anti-CD4-PE, anti-CD8-Red (Life Technologies, Grand Island, NY), anti-CD62L-biotin, and anti-CD44-biotin (PharMingen, La Jolla, CA). The α- and β-chains of 2B4 TCR were detected with A2B4-FITC (17) and...
anti-Vβ3-biotin (KJ25; PharMingen) Abs, respectively. Detection of biotinylated Abs was with streptavidin-CyChrome (PharMingen). For analysis of intracellular IL-2, splenocytes were stimulated with plate-bound anti-CD3 (PharMingen) plus anti-CD28 as previously described (8, 15) or with PMA (10 ng/ml) plus ionomycin (1 μM) as indicated. Alternatively, stimulations were conducted in the presence of 10 μM moth cytochrome c peptide 92–103 in the absence or the presence of an equal number of irradiated, T cell-depleted B10.BR splenocytes. Following stimulation for 5 h in the presence of the protein secretion inhibitors monensin and brefeldin A, cells were stained with Abs to CD4 and CD44, fixed with paraformaldehyde, permeabilized with 0.5% saponin, and stained with anti-IL-2-FITC (PharMingen) as previously described (8, 15). For intracellular staining of transcription factors, freshly isolated splenocytes were stained with Abs to CD4 and CD62L, fixed with paraformaldehyde, permeabilized with 0.5% saponin, and stained with rabbit polyclonal antisera against NF-AT1 (67.1) (18, 19), c-Fos, c-Jun, or NF-κB p65 (Santa Cruz Biotechnol- ogy, Santa Cruz, CA), followed by detection with goat anti-rabbit FITC (Molecular Probes, Eugene, OR). Cells were analyzed on a Becton Dick- inson FACScan flow cytometer (Mountain View, CA), and data were an- alyzed with CellQuest software (Becton Dickinson).

**Immunofluorescence**

Splenocytes (5 × 10^6) were allowed to attach to poly-γ-lysine (Sigma)- coated coverslips for 2 h in a humidified 37°C, 5% CO₂ incubator. Ad- herent cells were stimulated with 10 ng/ml PMA and 1 μM ionomycin for 5 min, followed by fixation with 3% paraformaldehyde (19). Washed cells were stained with anti-CD4-Red (Life Technologies), permeabilized with 0.5% Triton X-100, blocked with 10% FBS (HyClone, Logan, UT), and stained with polyclonal rabbit serum against NF-AT1 (67.1). Detection of anti-NF-AT1 Ab was achieved using goat anti-rabbit Oregon Green (Mo- lecular Probes), followed by confocal microscopy.

**Results and Discussion**

**II-2 accumulation after stimulation is greatly reduced in naive/resting Jak3^-/- CD4^+ T cells**

To assess IL-2 synthesis after stimulation of Jak3^-/- T cells we used the intracellular IL-2 staining assay (15). This assay offers two important advantages over the traditional assay that measures secreted IL-2 in the supernatants of stimulated cells. First, due to the short time period of the T cell stimulation, cell death during the in vitro culture period is minimized, and in addition, live cells can be selectively gated during the flow cytometric analysis. Second, IL-2 synthesis in naive/resting vs activated T cells can be distin- guished by simultaneously staining cells with Abs to cell surface molecules such as CD44 or CD62L. Using this assay, we have previously shown that Jak3^-/- CD4^+ T cells, all of which are activated, are equally competent to produce IL-2 in response to stimulation as wild-type activated CD4^+ T cells (15). These results led us to conclude that the signaling pathways that lead to IL-2 gene transcription are intact in CD4^+ T cells from Jak3 KO mice.

To expand on these findings, we set out to examine IL-2 syn- thesis in naive/resting Jak3^-/- CD4^+ T cells. Because significant numbers of naive/resting CD4^+ T cells cannot be detected in Jak3 KO mice at any age (15), we used Jak3 KO mice crossed to two different lines of transgenic mice. The first line, tgthy, contains the wild-type Jak3 gene transcription are intact in CD4^+ T cells from Jak3 KO mice.

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**FIGURE 1.** Decreased IL-2 synthesis by naive/resting CD4^+ T cells from Jak3^-/- tg^{bry} mice. Splenocytes from Jak3^-/- and Jak3^-/- tg^{bry} mice were stimulated in vitro for 5 h. Cells were then stained with anti-CD4 and anti-CD44 Abs, and IL-2 synthesis was detected by intracellular staining with anti-IL-2 Ab. A, Splenocytes cultured in medium alone (Med.), in the presence of plate-bound anti-CD3 plus anti-CD28 Abs (CD3 + CD28), or with phorbol ester, calcium ionophore, and anti-CD28 Ab (PMA+Iono.+CD28). B, Splenocytes from Jak3^-/-, Jak3^-/-, and Jak3^-/- tg^{bry} mice of two different ages were left unstimulated (Med.) or were stimulated with PMA, iono- mycin, and anti-CD28 Ab (PMA+Iono.+CD28). All dot plots show data gated on live CD4^+ T cells. Histograms illustrate the CD44 profiles of gated CD4^+ T cells, and the dot plots show IL-2 vs CD44 staining of gated CD4^+ T cells. The numbers in the quadrants represent the percentages of IL-2-positive cells in each T cell subset: naive/resting (CD44^{lo}) and ac- tivated/memory (CD44^{hi}).
old, the Jak3 protein becomes completely undetectable in the periphery (8). Despite the absence of detectable Jak3 protein, these mice contain a substantial, albeit variable, number of naive/resting T cells. Jak3 KO mice were also crossed to a second line of transgenic mice, Jak3−/− mice used for these experiments, expressing the transgenic TCR are in a naive/resting state, as previously described (15).

Using these two lines of mice we were able to obtain sufficient numbers of naive/resting Jak3−/− CD4+ T cells for stimulation assays. The presence of naive/resting (CD44low) CD4+ T cells in Jak3−/−tg thy mice is shown in Fig. 1. As mentioned above, the fraction of CD44low cells varied from animal to animal, but in all mice used for these experiments, >30% of CD4+ T cells were CD44low. Splenocytes were then stimulated with various mitogens, and intracellular accumulation of IL-2 was measured after 5 h. When cultured in medium alone for 5 h, very little intracellular IL-2 was detectable in any of the T cell populations (Fig. 1).

Cross-linking of the TCR plus costimulatory receptor (CD3 plus CD28) induced a strong response from control Jak3+/−/ CD4+ T cells; as shown in Fig. 1A, a larger percentage of CD44low (naive/resting) cells respond to this stimulus than CD44high (activated/memory) cells. Interestingly, cross-linking of CD3 plus CD28 failed to produce a strong response from Jak3−/−tg thy CD4+ T cells. This was particularly apparent in the CD44low (naive/resting) compartment, where the percentage of IL-2-producing cells was significantly decreased compared with the corresponding subset of CD4+ T cells from control mice (Table I).

A similar outcome was observed when T cells were stimulated with a much stronger combination of mitogens, consisting of phorbol ester (PMA), a calcium ionophore (ionomycin), and the anti-CD28 Ab. The increased efficacy of this stimulus is apparent with the control T cells; substantially more IL-2 was produced in response to PMA, ionomycin, and anti-CD28 Ab than was elicited by cross-linking of CD3 and CD28 (Fig. 1A). Interestingly, this stronger stimulus had a differential effect on CD44high vs CD44low CD4+ T cells from Jak3−/−tg thy mice. Specifically, CD44high CD4+ T cells from these mice responded similarly to control T cells, producing much more IL-2 in response to this stimulus than in response to CD3 plus CD28 cross-linking (Fig. 1A). In contrast, the CD44low CD4+ T cells from these mice had a markedly reduced response to PMA, ionomycin, and anti-CD28 Ab compared with control CD44low T cells (9% responding vs 40%; see Fig. 1A and Table I). Thus, in response to both stimuli examined, naive/resting, but not activated, Jak3-deficient CD4+ T cells are severely defective in producing IL-2 (Table I).

To examine this phenomenon further, we examined IL-2 synthesis in CD4+ T cells from Jak3−/−tg thy mice of varying ages. The rationale for this experiment was as follows. We have previously observed that in very young Jak3−/−tg thy mice (2–3 wk old) low levels of Jak3 protein can be detected in peripheral T cells; however, Jak3 protein is completely undetectable in peripheral T cells from older Jak3−/−tg thy mice (>3–4 wk) (8). Thus, we examined whether the loss of Jak3 protein from peripheral T cells correlated with the loss of IL-2 synthesis capability. Indeed, when splenocytes from Jak3−/−tg thy mice of different ages were examined, we found that CD44low (naive/resting) CD4+ T cells lose their ability to produce IL-2 with increasing age (Fig. 1B). In contrast, IL-2 synthesis by CD44high CD4+ T cells was not affected by the age-dependent loss of the Jak3 protein (Fig. 1B). These results further support the observation that naive/resting, but not activated, CD4+ T cells are highly dependent on Jak3 for the maintenance of their ability to produce IL-2 following stimulation.

As a second approach to assess IL-2 synthesis in naive/resting Jak3−/− CD4+ T cells, we crossed Jak3 KO mice to 2B4 TCR transgenic mice. We and others have previously shown that in TCR transgenic Jak3 or γc KO mice the majority of T cells that express the transgenic TCR are in a naive/resting state (9, 15, 16). Unfortunately, TCR transgenic Jak3 KO mice have very low numbers of peripheral T cells (15), making the analysis of these cells extremely difficult. Therefore, we further crossed the 2B4 TCR transgenic Jak3−/− mice to the tg thy transgenic mice. In these mice, thymic maturation is restored, leading to dramatically increased numbers of peripheral T cells; as described above, the Jak3 protein is rapidly lost from the 2B4 TCR transgenic peripheral T cells. As shown in Fig. 2A, the spleens of 2B4 TCR transgenic Jak3−/−tg thy mice contain a significant fraction of naive/resting CD4+ T cells. Interestingly, the majority of 2B4+ CD4+ T cells expressed low levels of CD44, whereas most 2B4+ CD4+ T cells were CD44high (Fig. 2A). When splenocytes from H-2b 2B4 TCR transgenic Jak3−/−tg thy or control animals were cultured with moth cytochrome c peptide 82–103, the cognate Ag of the 2B4 TCR, a substantial response was obtained from the control 2B4 TCR transgenic T cells, whereas a very weak response was observed with T cells from the 2B4+ Jak3−/−tg thy mice (Fig. 2A). The addition of irradiated, T cell-depleted B10.BR (H-2b) splenocytes did not increase the IL-2 response in either case, excluding the possibility of a defect due to impaired Ag presentation by Jak3-deficient APC. Furthermore, when cells were stimulated with the combination of PMA, ionomycin, and anti-CD28 Ab, the control T cells produced a strong IL-2 response, whereas the 2B4+ Jak3−/−tg thy CD4+ T cells synthesized very little IL-2 (Fig. 2A).

Finally, we measured IL-2 synthesis by naive/resting 2B4 TCR transgenic Jak3−/− T cells in the absence of the Jak3 (tg thy) transgene. Stimulation of the cells by TCR plus costimulatory receptor cross-linking (CD3 and CD28) elicited a strong response from both naive/resting (CD44low CD62Lhigh) and activated (CD44high CD62Llow) CD4+ T cells from control mice (Fig. 2B). In contrast, naive/resting CD4+ T cells from 2B4 TCR transgenic Jak3−/− mice were nearly completely unresponsive (Fig. 2B and Table I).

Table I. Summary of intracellular IL-2 staining data for naive/resting CD4+ T cells

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<tr>
<td>Unstimulated</td>
<td>0.51 ± 0.31 (n = 10)</td>
<td>0.49 ± 0.34 (n = 13)</td>
<td>NA</td>
<td>0.23 ± 0.14 (n = 6)</td>
<td>0.24 ± 0.1 (n = 4)</td>
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<td>oCD3 + oCD28</td>
<td>19.6 ± 5.9 (n = 9)</td>
<td>3.0 ± 1.8 (n = 11)</td>
<td>0.0001</td>
<td>11.2 ± 7.2 (n = 3)</td>
<td>0.9 ± 0.7 (n = 2)</td>
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<tr>
<td>PMA + lono. + oCD28</td>
<td>25.5 ± 13.2 (n = 5)</td>
<td>5.4 ± 2.7 (n = 6)</td>
<td>0.01</td>
<td>5.9 (n = 1)</td>
<td>1.6 (n = 1)</td>
<td>NA</td>
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<td>MCC pep + APCs</td>
<td>NA</td>
<td>NA</td>
<td>0.45 ± 0.5 (n = 3)</td>
<td>0.8 ± 0.4 (n = 2)</td>
<td>0.006</td>
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*p Values derived from the Student’s t test assuming unequal variance.

**Table I. Summary of intracellular IL-2 staining data for naive/resting CD4+ T cells**
Thus, using two different strategies to generate mice containing naive/resting Jak3-deficient CD4+ T cells, we have shown that these cells are severely defective in synthesizing IL-2 in response to antigenic or pharmacological stimulation. In contrast, activated Jak3-deficient CD4+ T cells produce IL-2 to a level comparable to normal activated CD4+ T cells (15) (Fig. 1, A and B). These findings suggest that naive/resting and activated CD4+ T cells differ in their requirement for Jak3 to maintain function. The biochemical basis of this difference could be explained by one of the following models. Jak3 might play a previously unsuspected role in a signaling pathway that leads to IL-2 synthesis. For instance, Jak3 activation could synergize with other activation signals, thus decreasing the stimulation threshold required for induction of the IL-2 gene. According to this hypothesis, naive/resting CD4+ T cells would be highly dependent on a Jak3 signal, whereas activated CD4+ T cells would not be. This model proposes a direct role for Jak3 in IL-2 gene induction. An alternative model that is more in accordance with the known role of Jak3 in cytokine receptor signaling is that Jak3 plays an indirect role in IL-2 production. Specifically, Jak3-mediated cytokine signals might be required to maintain the capacity of naive/resting CD4+ T cells to produce IL-2, whereas activated CD4+ T cells might have differentiated into a Jak3-independent state.

Normal NF-AT1 translocation in both activated and naive/resting Jak3-deficient CD4+ T cells

To identify the role of Jak3 in naive/resting CD4+ T cells, we attempted to localize the biochemical basis of the IL-2 synthesis defect in naive/resting Jak3-deficient CD4+ T cells. For this purpose, we chose to analyze CD4+ T cells from 2B4 TCR transgenic Jak3−/− mice, rather than Jak3−/−tgthy mice, to ensure the complete absence of the Jak3 protein. However, the extremely small numbers of T cells in these mice (∼1–2% of splenocytes) confounded any attempts at analyzing signal transduction by conventional biochemical methods. For this reason we resorted to assays that could be performed at the single-cell level.

We have previously reported that activated Jak3-deficient T cells undergo relatively normal tyrosine phosphorylation of proteins following TCR cross-linking (15). We have recently analyzed mitogen-activated protein kinase (MAPK) signaling in Jak3-deficient T cells, and again found no gross defect in the activation of the MAPKs Erk-1 and Erk-2 following cross-linking of the TCR (data not shown). Furthermore, our previous studies demonstrated that calcium mobilization is not significantly defective in Jak3−/− T cells (15). We confirmed this latter finding with naive/resting Jak3−/− 2B4+ T cells (data not shown). These data together with our observation that stimulation with PMA plus ionomycin does not elicit a normal IL-2 response from naive/resting Jak3−/− CD4+ T cells suggested that any signaling defect is likely to be relatively distal from the TCR.

One measure of relatively late events after T cell stimulation is the translocation of transcription factors to the nucleus (20, 21). After translocation, these cooperate to induce cytokine gene transcription. One class of transcription factors important for cytokine gene transcription is the nuclear factor of activated T cells (NF-AT) family (22–24), which has been shown to play a crucial role in the activation of IL-2 synthesis (25). Therefore, we examined whether there is any defect in the nuclear translocation of NF-AT1 in activated Jak3-deficient CD4+ T cells. This analysis was performed by indirect immunofluorescence. As shown in Fig. 3, when cells were left unstimulated, the majority of NF-AT1 was present in the cytoplasm of CD4+ T cells, as shown by the rings of green fluorescence (19, 26). When the cells were stimulated with PMA plus ionomycin, NF-AT1 efficiently translocated into the nucleus.

A. Jak3+/− 2B4+ Jak3−/− tgthy2B4+

CD4+ CD4+2B4+ CD4+2B4+ CD4+ CD4+2B4+ CD4+2B4+ CD44 Med. Med. Med. Med. 0.8 0.8 0.8 0.8 6.4 6.4 6.4 6.4 2.1 2.1 2.1 2.1 5.9 5.9 5.9 5.9 7.7 7.7 7.7 7.7 PMA + Iono. + CD28 PMA + Iono. + CD28 PMA + Iono. + CD28 PMA + Iono. + CD28

B. Jak3+/− 2B4+ Jak3−/− 2B4+

CD25 CD25 CD25 CD25 CD44 Med. Med. Med. Med. 90.4 90.4 90.4 90.4 12.2 12.2 12.2 12.2 14.2 14.2 14.2 14.2 1.2 1.2 1.2 1.2 5.1 5.1 5.1 5.1

FIGURE 2. Reduced IL-2 response from 2B4 TCR transgenic Jak3−/− CD4+ T cells. A. Splenocytes from 2B4 TCR transgenic Jak3+/− and Jak3−/− tgthy mice were cultured for 5 h in medium alone (Med.) or were stimulated with plate-bound anti-CD3 and plate-bound anti-CD28 Abs (PMA + Iono. + CD28). Cells were stained with combinations of Abs against CD4, CD44, KJ25, and IL-2. All plots show data gated on live T cells. Histograms show CD44 profiles of gated CD4+ T cells. The numbers in the quadrants represent the percentages of IL-2-positive cells in each T cell subset: naive/resting (CD44low) and activated (CD44high).
of control (Jak3+/−, 2B4+) CD4+ T cells (Fig. 3). This was also true for both the Jak3+/− CD4+ T cells (all CD44high before stimulation) as well as the CD4+ T cells from the Jak3−/− 2B4+ animal (∼35% CD44low/−65% CD44high before stimulation). Thus, there is no defect in the nuclear translocation of NF-AT1 in either previously activated or naive/resting Jak3−/− CD4+ T cells.

Despite the fact that NF-AT1 translocation into the nucleus appeared normal, we always observed reduced staining of NF-AT1 in some CD4+ T cells from the Jak3−/− 2B4+ mice (large arrows in Fig. 3) compared with that in CD4+ T cells from the control mice. Furthermore, the fraction of Jak3−/− 2B4+ CD4+ T cells with reduced NF-AT1 staining was always similar to the fraction of naive/resting CD4+ T cells found in the same splenic sample (as determined by flow cytometry; data not shown). Thus, it seemed likely that the cells showing weak NF-AT1 staining are, in fact, naive/resting Jak3-negative CD4+ T cells. To verify this hypothesis we quantitated the levels of NF-AT1 protein in resting/naive vs activated control and Jak3-deficient CD4+ T cells.

**Reduced NF-AT1 protein levels in naive/resting Jak3−/− T cells.**

As mentioned above, the extremely small numbers of T cells found in 2B4 TCR transgenic Jak3−/− mice do not allow standard biochemical analyses, such as immunoblotting. For this reason, we used intracellular staining followed by flow cytometry to measure the levels of NF-AT1 and several other transcription factors in naive/resting vs activated CD4+ T cells. Splenocytes from control, Jak3−/−, and 2B4 TCR transgenic Jak3−/− mice were stained with Abs to CD4 and CD62L, and then permeabilized for intracellular staining. Following permeabilization, cells were stained with Abs to NF-AT1, c-Jun, c-Fos, or NF-kB p65. Selective gating was used to specifically analyze the expression levels of these transcription factors in CD62Llow (naive/resting) compared with CD62Lhigh (activated) CD4+ T cells. As shown in Fig. 4, Jak3−/− and Jak3−/− mice contained mostly naive/resting and activated CD4+ T cells, respectively, whereas Jak3−/− 2B4+ mice contained a mixture of activated and naive/resting T cells. The expression levels of NF-AT1, c-Jun, c-Fos, and NF-κB p65 were similar in both subsets of wild-type CD4+ T cells and correlated well with those observed in CD62Llow CD4+ T cells from Jak3−/− mice (Fig. 4 and data not shown). It should be noted that Jak3−/− mice almost completely lack CD62Lhigh CD4+ T cells, and thus, results obtained from these cells should be interpreted with caution. Interestingly, when CD4+ T cells from 2B4 TCR transgenic Jak3−/− mice were examined, the level of NF-AT1 was significantly reduced in CD62Lhigh cells compared with that in the analogous subset of wild-type CD4+ T cells. In contrast, CD62Llow CD4+ T cells from Jak3−/− mice contained levels of NF-AT1 that were similar to those in wild-type CD4+ T cells.
cells from 2B4 TCR transgenic Jak3−/− animals expressed nearly comparable levels of NF-AT1 as the activated/memory T cells from wild-type and Jak3−/− mice. As shown in Fig. 4, the expression levels of c-Jun and c-Fos were only slightly reduced in naive/resting T cells from Jak3−/− mice; in contrast, NF-κB p65 levels were indistinguishable from the normal levels found in wild-type controls.

Our data clearly show that in the absence of the tyrosine kinase Jak3, naive/resting CD4+ T cells are severely defective in producing IL-2 after stimulation. This defect is distinct from that of activated/memory CD4+ T cells from Jak3-deficient mice. In the former case, the cells most likely fail to produce normal amounts of IL-2 because of their limited life span in culture. However, when intracellular IL-2 accumulation in activated Jak3-deficient CD4+ T cells is measured after only 5 h of stimulation, these cells were found to have a similar capacity to produce IL-2 as normal activated CD4+ T cells (15). In contrast, naive/resting Jak3-deficient CD4+ T cells produce little or no intracellular IL-2 after 5 h of stimulation. Thus, there is a defect in the sustained synthesis of IL-2 in activated Jak3-deficient CD4+ T cells due to premature cell death, while in naive/resting Jak3-deficient CD4+ T cells there is a defect much earlier in the IL-2 response.

Our previous analyses of early T cell signaling events in activated Jak3-deficient CD4+ T cells, including tyrosine phosphorylation, calcium mobilization, and MAPK activation, failed to uncover any defects that would account for the impaired function of these cells (15). In agreement with these results, we found no significant defect in TCR-induced calcium mobilization in naive/resting Jak3-deficient CD4+ T cells. Furthermore, a relatively late event in the T cell signaling cascade(s) that leads to IL-2 gene induction, the nuclear translocation of NF-AT1 following stimulation, was intact in both activated and naive/resting Jak3-deficient CD4+ T cells. In contrast, naive/resting Jak3-deficient CD4+ T cells have substantially reduced levels of the transcription factor NF-AT1. Interestingly, this deficiency distinguishes naive/resting Jak3-deficient CD4+ T cells from wild-type T cells as well as from activated Jak3-deficient CD4+ T cells and thus correlates perfectly with the functional capabilities of these cell populations.

Recent studies of NF-AT1-deficient mice have indicated that T cells from these mice have no defect in IL-2 gene induction after stimulation (27, 28). Therefore, this difference alone cannot be responsible for the functional defect we observed in naive/resting Jak3-negative T cells. Nonetheless, simultaneous inhibition of all NF-AT family members has been achieved in mice expressing a dominant negative NF-AT protein in T cells. Interestingly, T cells from these mice show dramatically reduced IL-2 gene induction after TCR stimulation (25). Therefore, reduced levels of multiple NF-AT family members could explain the reduced IL-2 expression observed in Jak3-deficient naive T cells. In addition, we propose that the substantial deficit in NF-AT1 protein levels and the more modest decreases in c-Jun and c-Fos protein levels may reflect a more global decrease in the levels of basal transcription factors. It has been clearly demonstrated that signaling through γc-containing cytokine receptors is necessary for the survival of naive/resting T cells (16, 29, 30). These cytokine signals may also be required to maintain the expression levels of transcription factors that are

**FIGURE 4.** Levels of transcription factors in naive/resting vs activated Jak3−/− CD4+ T cells. Splenocytes from Jak3+/+, Jak3−/−, and 2B4 TCR transgenic Jak3−/− mice were stained with Abs to CD4 and CD62L, fixed, permeabilized, and stained with Abs to NF-AT1, c-Jun, c-Fos, or NF-κB p65. Expression of CD62L on gated CD4+ T cells is shown in the top left panels. The gates used for examination of transcription factor expression in naive/resting (CD62Lhi) vs activated (CD62Llo) CD4+ T cells are indicated. The filled histograms show the specific staining of the transcription factors; the open histograms show background staining with normal rabbit serum.
poised for immediate response to Ag activation signals. Our demonstration that naive/resting CD4+ T cells are highly dependent on Jak3 to maintain a state that enables them to synthesize IL-2 following stimulation represents a novel role for Jak3; further elucidation of the biochemical basis of this phenomenon will certainly provide important information about the mechanisms regulating the resting T cell pool.

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References