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Normal Thymic Selection of TCR Transgenic CD4 T Cells, but Impaired Survival in the Periphery Despite the Presence of Selecting MHC Molecules

Thomas Barthlott, Rebecca J. Wright, and Brigitta Stockinger

In this paper, we investigate selection in the thymus and survival in the periphery of CD4 T cells, which carry a major histocompatibility class II-restricted transgenic TCR (A18 TCRtg) specific for a natural self Ag, the fifth component of complement (C5). A18 TCRtg thymocytes develop normal numbers of CD4 single-positive (SP) thymocytes, but do not show pronounced overselection as do some other TCR transgenic strains. CD4 SP cells are mature as judged by termination of CD8 synthesis, resistance to cortisone, and functional competence. The kinetics of positive selection, determined by BrdU labeling, are very fast. CD4 SP thymocytes are demonstrable within 2 days of labeling, and within 8 days after labeling a large proportion (20%) of lymph node T cells are recent thymic emigrants. The high number of recent thymic emigrants suggests rapid turnover of CD4 T cells in the periphery, which was confirmed by thymectomy and determination of CD4 T cell life spans. A18 TCRtg T cells have a t1/2 of ~6 wk, despite the presence of selecting MHC molecules. This explains the failure to accumulate high numbers of peripheral T cells and suggests that the MHC-bound ligand(s) responsible for initiating survival signals is limiting for the selection and maintenance of A18 transgenic CD4 T cells. The Journal of Immunology, 1998, 161: 3992–3999.

P ositive selection of T cells in the thymus is crucial for the generation of a T cell repertoire capable of recognizing foreign Ags in conjunction with self MHC molecules. A delicate integration of continuous signals at the CD4/CD8 double-positive (DP)2 TCR intermediate stage, mediated by the TCR, the coreceptors CD4 and CD8, and MHC ligands with bound peptides, ensures that only thymocytes bearing TCRs with the appropriate avidity are given a survival signal. DP thymocytes bearing TCRs unable to interact with self MHC undergo programmed cell death. Positively selected DP thymocytes up-regulate TCR expression and differentiate to either CD4 or CD8 single-positive (SP) thymocytes, depending on the MHC restriction of the expressed TCR; i.e., MHC class I-restricted thymocytes develop into CD8 SP cells, whereas MHC class II-restricted thymocytes develop into CD4 SP cells (1–3). There are exceptions to this rule, since some TCRs can be selected into both lineages (4–6). Mature SP thymocytes are exported into peripheral lymphoid organs and are considered long-lived cells (7). Recent data provided evidence that peripheral, naive T cells need signals mediated by the restricting MHC ligand for long term survival (8–12). Thus, following positive selection in the thymus, a continuous selection process is operative in the periphery. The precise nature and origin of these survival signals, however, are at present unknown.

We have developed a transgenic TCR (A18 TCRtg) model to study thymic selection of cells expressing a TCR specific for a peptide derived from a natural self Ag in the mouse; this is the serum protein complement C5, recognized in the context of MHC class II H2-Ek (13). Thymocytes in these A18 TCRtg mice are selected into the CD4 lineage in C57BL/6 H-2b mice and are deleted at the late DP stage in C57BL/6 H-2b mice. In the periphery, however, only very few CD4 T cells can be detected. Generation of CD4 SP thymocytes in A18 TCRtg mice is comparable with normal mice, but does not show the substantial skewing into the CD4 compartment reported for other CD4 TCR transgenic mice (14, 15).

While overselection into the CD4 lineage is not necessarily a feature of transgenic mice carrying MHC class II-restricted TCRs, CD4 T cells appear to accumulate in the periphery of these mice, even on a Rag2−/− background (Refs. 4 and 16; and P. M. Allen, personal communication). In the A18 mice, however, the number of peripheral CD4 T cells falls short of what is found in other transgenic strains.

In this paper, we address the question of whether the paucity of peripheral T cells is the consequence of abnormal selection processes in the thymus. Two possible scenarios could apply. One is that the A18 TCR avidity for H2-Ek and the positively selecting ligand(s) may be too low and the A18 TCR is therefore not efficiently selected. This could cause defective final maturation at the CD4 SP stage and compromise the export of CD4 T cells into the periphery. Alternatively, the A18 TCR could have too high an avidity for the selecting MHC/ligand complexes and therefore be on the brink of negative selection, from which only few CD4 T cells escape.

Previous data obtained from the analysis of F1 mice generated by breeding A18 Rag2−/− TCR mice with BM3 Rag2−/− TCR mice are compatible with both scenarios. In these F1 mice, constitutively expressing both the MHC class II-restricted A18 TCR and an MHC class I-restricted, H-2Kd-specific TCR, we observed a substantial increase in the number of CD4 cells in thymus and periphery (17). Thus, in a low avidity scenario for the A18 TCR, the BM3 TCR could have mediated positive selection. In a high avidity scenario a reduction in expression levels for the A18 TCR due to expression of a second receptor could have avoided negative selection. The results presented in this paper indicate that the A18
TCR is appropriately selected, but not overselected like some MHC class II-restricted transgenic receptors. The CD4 SP thymocytes that are generated are mature and efficiently exported into the periphery. Instead, the lack of CD4 T cell accumulation in lymphoid organs is due to the short life span of these cells. We discuss these findings in conjunction with the recent observations concerning peripheral T cell selection and survival.

Materials and Methods

Animals

Mice of strain A/J and A18 TCRtg RagI-/- CSII-/- (on an A/J H-2b background) were kept in conventional animal facilities at the National Institute for Medical Research, Mill Hill. A18 TCRtg Rag1-/- CSII-/- mice were bred to Rag1-/- CSII-/- SWR (H-2q) mice to generate H-2k/q coexpressing mice. As controls, A18 TCRtg Rag1-/- CSII-/- mice heterozygote for the TCR was generated by crossing them to Rag1-/- CSII-/-/A/J mice.

Flow cytometry and monoclonal Abs

Analytical flow cytometry was conducted using a FACScan (Becton Dickinson, Mountain View, CA), and the data were processed using Cellquest software (Becton Dickinson). Three-color stainings were performed with FITC-, phycoerythrin, and biotin-conjugated mAbs followed by streptavidin-R-PE (Red670 Life Technologies, Paisley, U.K.). Anti-CD4 phycoerythrin (H129.19) was purchased from PharMingen (San Diego, CA) and anti-CD8 (YTS 169.4), anti-TCR Vβ8.3 (7G8.2), and anti-HSA (YBM5.10) were conjugated with biotin or FITC using standard procedures. F3 mAb was used as supernant and detected with biotinylated anti-rat IgM (Becton Dickinson). Three-color stainings were performed with FITC-, phycoerythrin, and biotin-conjugated mAbs followed by streptavidin-R-PE (Becton Dickinson). Analytical flow cytometry was conducted using a FACScan (Becton Dickinson). Three-color stainings were performed with FITC-, phycoerythrin, and biotin-conjugated mAbs followed by streptavidin-R-PE (Becton Dickinson).

BrdU labeling and detection

Mice were injected i.p., twice within 4 h, with 1 mg BrdU (Sigma) in PBS for analysis of the selection kinetics in the thymus. For continuous BrdU labeling, mice received one i.p. injection with 1 mg BrdU in PBS and then were given 0.8 mg/ml BrdU in the drinking water, which was changed every three days. Single-cell suspensions were stained with anti-CD4 and anti-CD8 mAb, re-suspended in 25 ml of ice cold 0.15 M NaCl, and fixed by the dropwise addition of ice cold 95% ethanol for 30 min on ice. After washing with PBS, the samples were fixed in 100 ml PBS/1% paraformaldehyde/0.01% Tween for 30 min at room temperature, and the samples were analyzed immediately after using linear settings for fluorescence 3 to assess 7-AAD staining.

BrdU labeling and detection

Mice were injected twice i.p. with 1 mg demethylase (Sigma) in 250 ml PBS within 24 h and analyzed 24 h after the second injection.

Results

CD4 T cell numbers in A18 TCRtg Rag1-/- mice

The percentage of CD4 SP thymocytes in A18 TCRtg mice on the positively selecting H-2b, CSII-/- background is ~9%, and thymic cellularity is not drastically different from nontransgenic A/J mice. In the periphery, however, very few CD4 T cells can be detected (Table I). Spleens from A18 TCRtg mice have a low cellularity, ~4 x 106, and the percentage of CD4 T cells is ~3-fold lower than in A/J mice. In lymph nodes, the percentage of CD4 T cells is only slightly lower than in A/J mice, but the cellularity is reduced 50-fold.

Table 1. Total cell numbers (x 106) and the percentage of CD4 T cells in thymus, spleen, and lymph nodes from A/J and A18 TCRtg Rag1-/- mice

<table>
<thead>
<tr>
<th></th>
<th>A/J</th>
<th>A18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>93.9 ± 0.4 (n = 32)</td>
<td>79.4 ± 33.3 (n = 45)</td>
</tr>
<tr>
<td>% CD4 SP</td>
<td>13.5 ± 2.5 (n = 30)</td>
<td>8.7 ± 2.3 (n = 35)</td>
</tr>
<tr>
<td>Spleen</td>
<td>28.5 ± 13.1 (n = 22)</td>
<td>4.0 ± 1.8 (n = 43)</td>
</tr>
<tr>
<td>% CD4</td>
<td>20.4 ± 4.9 (n = 18)</td>
<td>6.0 ± 2.7 (n = 19)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>86.0 ± 33.0 (n = 24)</td>
<td>1.7 ± 1.3 (n = 27)</td>
</tr>
<tr>
<td>% CD4</td>
<td>59.0 ± 5.1 (n = 20)</td>
<td>46.6 ± 10.9 (n = 22)</td>
</tr>
</tbody>
</table>

a Pool of mesenteric and inguinal lymph nodes, x 106.

Phenotypic and functional characterization of A18 thymocytes

During positive selection into the CD4 lineage, thymocytes down-regulate the differentiation marker F3Ag. This down-regulation occurs in the DP stage and is directly correlated to the efficacy of positive selection; i.e., the more pronounced the skewing into the CD4 compartment, the more pronounced the down-regulation of F3Ag at the DP stage (18). We stained thymocytes from wild-type A/J and A18 TCRtg mice with anti-CD4, anti-CD8, and F3 mAb (Fig. 1). Expression of the tgTCR mediates selection into the CD4 compartment, comprising 10% in A/J and A18 TCRtg mice. Gating on mature F3Aghigh thymocytes shows the enrichment in SP and positively selected CD4lowCD8low DP cells (19). No significant differences in wild-type and A18 TCRtg mice were observed, indicating that there is no overselection into the CD4 lineage for this TCR.

CD4 SP thymocytes from A18 TCRtg retain a low level of CD8 expression on their cell surface as determined by flow cytometry. Mature cells selected into the CD4 lineage, however, normally cease to express CD8. Residual CD8 expression could reflect inefficient positive selection; i.e., the signals generated in the DP stage might not be sufficient to induce complete silencing of the CD8 gene. Alternatively, it could be due to a rapid transition from the DP to the CD4 SP stage, so that there is not enough time for the intracellular biochemical machinery to completely abrogate CD8 expression on the cell surface. To test these possibilities, we performed a coexpression assay, which allowed us to distinguish between active protein synthesis and “leftover” protein expression after the corresponding gene has been switched off due to the long half life of the protein (20). Thymocytes from A18 TCRtg mice were treated with trypsin, which cleaves off the co-receptors CD4 and CD8. Following overnight incubation in suspension cultures, the cells were analyzed to assess CD8 reexpression on CD4 SP cells (Fig. 2). Trypsinized thymocytes now showed a distinct CD4 SP population, with a mean fluorescence intensity (MFI) value for CD8 identical to the DN population. This shows that thymocytes with a CD8+ phenotype have been selectively eliminated due to the presence of a constitutively rearranged transgenic TCR.

Another criterion for maturity of thymocytes is their resistance to corticoid-induced cell death. A18 TCRtg mice were injected with dexamethasone, and the cellular composition of the thymi was analyzed 24 h later (Fig. 3). As expected, thymi from treated mice contained only immature DN (TCRlow and predominantly HSAhigh) and mature CD4 SP cells (TCRhigh and HSAlow), while the bulk of DP thymocytes was eliminated due to dexamethasone-induced apoptosis.

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Taken together, these data show that, although positive selection is not highly efficient, the CD4 SP thymocytes generated have received all of the signals required at the DP stage to develop a mature phenotype. As shown previously, A18 CD4 SP thymocytes are also functionally competent; they respond to Ag stimulation in vitro with production of IL-2 and IFN-γ (21).

Reduction of the selecting MHC ligand leads to decreased positive selection of A18 TCRtg CD4 SP thymocytes

The following experiment was designed to address the question of whether a proportion of A18 thymocytes is lost due to high avidity interactions during positive selection, resulting instead in negative selection. A18 TCRtg mice were crossed with Rag1-/- H-2q mice, which are also C5 negative. The H-2q background is neutral and nonselecting for A18 CD4 T cells, and mature A18 T cells are not activated by class II-bearing APC from the thymus or periphery of H-2q mice (data not shown). In the resulting F1 generation, H-2k expression is reduced due to codominant expression of the MHC H-2q allele. In case the A18 thymocytes are on the brink of negative selection, reduction of one of the ligands participating in positive selection would be expected to lead to an increase in the number of thymic CD4 SP cells. In contrast, if the A18 TCR is normally weakly positively selected, the reduction in selection ligands should lead to a further reduction in CD4 SP cells. Figure 4 shows that the latter is the case. In an H-2k/q F1, the amount of CD4...
SP cells is reduced nearly threefold compared with an A18 TCRtg heterozygous H-2\(^{k/k}\) mouse (2.5 to 6.4%, respectively). However, no significant changes in the peripheral CD4 compartments are observed, and CD4 spleen cells from both mice respond identically to stimulation with the antigenic peptide (data not shown). Interestingly, a significant population of CD8 T cells are generated, in agreement with previous findings reporting that the lack of CD4 selection can result in an increased selection into the CD8 compartment (5). These results suggest that the paucity of peripheral CD4 T cells in A18 TCRtg mice is not due to negative selection of thymocytes.

**Kinetics of CD4 SP development in the thymus**

To investigate the kinetics of CD4 T cell production in the thymus, mice were injected with the thymidine analogue bromodeoxyuracil (BrdU), and BrdU incorporation in the different thymic populations was chased during a 4-day period. BrdU is mainly incorporated during the proliferative phase at the DN/DP transition so that newly generated and selected thymocytes can be traced with an anti-BrdU Ab. Table II shows the results of two experiments. Within 2 days, an average of 21% of CD4 thymocytes in A18 TCRtg mice were BrdU\(^{+}\), compared with only 6% in nontransgenic mice. On day 4, the CD4 SP compartment of nontransgenic mice contained 17% of recently selected cells, compared with 38% in the A18 thymus. These data demonstrate that A18 CD4 SP thymocytes are generated very fast, first appearing within 2 days, whereas A/J thymocytes need at least 4 days to generate an equivalent percentage of newly selected CD4 SP thymocytes. Constitutive expression of a rearranged TCR chains on most A18 thymocytes probably accelerates the positive selection process at the DP

| Table II. Percentage of BrdU\(^{+}\) cells in thymic subpopulations after a single BrU pulse* |
|-----------------|----------|--------|----------|--------|----------|--------|
| Day        | DN       |         | DP       |         | CD4 SP   |         |
| 0\(^b\)     |          |         |          |         |          |         |
| Exp. 1     | 0.2      | 0.8     | 0.9      | 0.6     | 0.2      | 0.2     |
| Exp. 2     | 0.2      | 1.5     | 0.2      | 0.4     | 0.1      | 0.4     |
| 1           |          |         |          |         |          |         |
| Exp. 1     | 32.0     | 54.4    | 55.7     | 73.1    | 7.6      | 7.4     |
| Exp. 2     | 31.2     | 38.0    | 65.0     | 67.2    | 7.8      | 2.9     |
| 2           |          |         |          |         |          |         |
| Exp. 1     | 10.8     | 8.3     | 33.9     | 12.5    | 3.7      | 19.2    |
| Exp. 2     | 20.8     | 18.7    | 59.2     | 20.1    | 8.2      | 23.8    |
| 3           |          |         |          |         |          |         |
| Exp. 1     | 7.1      | 7.7     | 17.8     | 9.9     | 8.1      | 45.4    |
| Exp. 2     | 12.1     | 3.7     | 15.1     | 5.2     | 12.8     | 30.4    |
| 4           |          |         |          |         |          |         |
| Exp. 1     | 8.3      | 1.8     | 3.7      | 3.8     | 18.7     | 37.9    |
| Exp. 2     | 4.9      | ND      | 3.9      | ND      | 16.4     | ND      |

\(^a\) A18 TCRtg Rag\(^{null}\) and A/J mice were injected with BrdU, and the BrdU incorporation in thymic subpopulations was determined at different time points after the injection. The percentages of BrdU\(^{+}\) CD4 SP thymocytes on days 2, 3, and 4 are underlined. ND, not done.

\(^b\) Uninjected control.
stage. We conclude that the DP/SP transition is not a limiting step in the generation of A18 T cells.

Export of CD4 SP cells into the periphery

To compare the export of CD4 cells from thymus to periphery in A18 TCRtg and nontransgenic A/J mice, we gave them BrdU in the drinking water for 8.5 days to ensure continuous labeling of cells. After the labeling period, 95% of the A18 and 70% of the A/J CD4 SP thymocytes were BrdUpos (data not shown). This suggests that the mature nondividing and therefore BrdUneg CD4 SP thymocytes, present before the addition of BrdU, must either have left the thymus or have died during the 8.5-day labeling period. To discriminate between these two possibilities, BrdU-labeled CD4 T cells were traced in the lymph nodes. Figure 5 shows that 18% of the CD4 T cells in A18 TCRtg mice have incorporated BrdU, compared with 4% in A/J lymph nodes. BrdU incorporation in CD4 T cells is not due to proliferation in this population, since analysis with the DNA-binding dye 7-AAD (Fig. 6) shows that in A18 TCRtg mice neither CD4 SP thymocytes nor CD4 T cells in lymph nodes contain a significant number of cells in cycle. Nontransgenic A/J mice, on the other hand, have some cycling CD4 cells in their lymph nodes, as would be expected in mice kept in a conventional animal house facility. Taken together, these results imply that the paucity of peripheral A18 CD4 T cells is not due to excessive cell death at the CD4 SP stage nor to inefficient export from the thymus. Instead, the high proportion of recent thymic emigrants found 8 days after BrdU labeling suggests that there is a high turnover of A18 CD4 T cells in the periphery.

Life span of peripheral A18 CD4 T cells

Since the low numbers of peripheral A18 CD4 T cells are not due to inefficient thymic positive selection and export, other mechanisms must be responsible for this phenotype. A18 CD4 T cells are phenotypically naive; i.e., they are CD44low, CD25low, CD69neg, CD62Lhi, and CD45RBhi (data not shown), which makes it unlikely that these cells are undergoing deletion after interaction with an unknown ligand in the periphery. To address the question of their life span, A/J and A18 TCRtg mice were thymectomized, and the number of CD4 T cells in the blood was determined over time (Fig. 7). During the 7 wk of the experiment, no reduction of A/J CD4 T cells could be observed, although there was some degree of experimental variation on the different time points. On the other hand, more than half of the thymectomized A18 mice showed a progressive loss of peripheral CD4 cells, suggesting a 1/2 for these T cells between 4 and 6 wk. Although there is variation between mice, the trend in A18 TCRtg mice is for a decrease in CD4 T cells over time. The number of A18 CD4 T cells observed in the blood
correlated with the numbers found in spleen and lymph nodes analyzed at the end of the 7-wk period (data not shown). We conclude that the most likely reason for the low numbers of CD4 T cells found in the periphery of A18 TCRtg mice is their reduced life span following export from the thymus.

Discussion

The generation of TCRtg mice has made it possible to dissect selection events in the thymus. Numerous examples exist to show that the amount of SP thymocytes selected is directly correlated to the overall avidity of interactions taking place between the tg TCR, the coreceptors, and the MHC ligands (22–26). These observations led to the avidity threshold model for positive and negative selection, suggesting that low to intermediate avidity interactions lead to positive selection and high avidity interactions lead to negative selection. The number of peripheral CD4 T cells, however, is not directly correlated to the efficiency of positive selection in the thymus. Mice selecting with low efficiency into the CD4 lineage in the thymus nevertheless have numbers of peripheral CD4 T cells comparable with TCRtg mice selecting very efficiently into the CD4 lineage (4, 14–16, 27).

The A18 TCRtg mice are an exception to the so-far-described MHC class II-restricted TCRtg mice. They are selected reasonably well into the CD4 lineage, comprising ~10% of the thymus, and the thymus cellularity is comparable to nontransgenic controls, but the amount of peripheral tg CD4 T cells is very low. On average, spleen and lymph nodes of the A18 mice contain ~5 × 10^5 CD4 T cells. In contrast, DO-11-10 mice on a scid background have ~1.5 × 10^7 CD4 T cells in the spleen and lymph nodes (M. Jenkins, personal communication). Our data rule out gross abnormalities in the thymic selection processes as a reason for the low numbers of peripheral T cells. One concern was the possibility that the relatively low numbers of CD4 SP thymocytes generated in comparison with other CD4 TCR transgenic strains reflected a degree of negative selection due to a too high avidity of the transgenic TCR for positively selecting ligands. While there is no direct way to distinguish between cell loss due to failed positive selection or negative selection, the data presented in this paper, as well as previously published observations, make it very unlikely that this is the case. 1) TUNEL staining of thymus from C5-negative A18 TCRtg mice revealed very few apoptotic cells in contrast to thymus from C5-positive A18 TCRtg mice (28). 2) Negative selection in the presence of the self Ag C5 does not take place until the very late DP stage, although all DP thymocytes and a proportion of DN thymocytes express TCR, suggesting that the A18 TCR interaction with its ligand is of relatively low avidity (13), (iii) in this paper, we show that lowering the avidity of interaction further by reducing expression of the MHC ligand results in a further decrease in CD4 SP thymocytes. The opposite effect would have been expected, had the relatively low number of CD4 SP thymocytes been the consequence of negative selection due to avidity interactions that were too high.

The data are compatible with the assumption that the avidity of TCR/MHC ligand interactions for this transgenic TCR are on the low side and therefore might not favor extensive production of CD4 SP. However, this conclusion seems somewhat at odds with the high sensitivity of functional activation seen in mature T cells (or CD4 SP thymocytes) with nanogram amounts of Ag (13, 21). An alternative explanation could lie in the kinetics of CD4 SP production. A18 SP cells are generated very rapidly, so that within 2 to 3 days, 30 to 45% of the CD4 SP population is derived from cycling DP precursors as defined by BrdU labeling, whereas in nontransgenic controls only a small number of CD4 SP is detected 2 days after BrdU labeling. While in nontransgenic mice, 20 to 30% of the DP pool are still labeled on day 2 after BrdU injection, A18 DP thymocytes have lost the BrdU label nearly completely at that time point. This suggests that the DP stage is very short lived, so that the low cell output from positive selection might be due to a restricted “[time window]” at the DP stage. In theory, the presence of a single TCR specificity on a positively selecting background should allow 100% positive selection. This is not the case, however, even in TCRtg mice, which generate SP thymocytes with higher efficiency than the A18 (29, 30). One reason for this could be the availability of selecting stromal microenvironments, which were shown to be rate limiting for positive selection, because during thymocyte selection each thymocyte appears to engage with only one rather than multiple stromal cells (31). Whereas
in transgenic mice on a Rag<sup>−/−</sup> background there should not be any competition with other T cells, one commodity that might be limiting is the selecting ligand(s), so that the kinetic feasibility of engaging stromal cells displaying the relevant ligand may be low for A18 thymocytes.

A related problem may face peripheral CD4 T cells. We have excluded the possibility that the low number of peripheral T cells is due to a defect in export from the thymus. Instead, the relatively high percentage (18%) of BrdU-labeled CD4 T cells in A18 TCR<sub>tg</sub> mice, compared with 4% in A/J controls, suggests that a high turnover of CD4 T cells must take place in A18 lymph nodes. Analysis of peripheral A18 T cells in thymectomized mice supported this assumption, because in half of the thymectomized mice a clear reduction of peripheral CD4 T cells could be observed during a 7-wk time course. A number of recent reports provided evidence that an indefinite life span for naive T cells is not guaranteed once the T cells have left the thymus. Although the life spans vary from one report to another, the general consensus is that for long term survival of naive T cells, continuous engagement of the restricting MHC ligand is required (8–12). In the A18 TCR<sub>tg</sub> model, however, peripheral T cell survival is compromised even in the presence of the restricting MHC molecule.

One could envisage that the rapid positive selection of A18 thymocytes fails to provide some crucial signals in the thymus necessary to make them responsive to survival signals in the periphery. The requirement for a thymus in generation of long-lived CD4 T cells even after completion of positive selection has been shown previously (32). However, the fact that A18 SP CD4 T cells are corticoid resistant and functionally mature argues against this hypothesis. Akkaraju et al. observed that spleen and lymph nodes of transgenic mice with hen egg lysozyme-specific TCRs also had markedly reduced cellularity on a Rag<sup>−/−</sup> background, compared with a Rag<sup>+/+</sup> background, and suggest that these mice may not have a normal splenic architecture, including B cells, which might compromise their survival (33). Similarly, the A18 mice have more T cells on a Rag<sup>−/−</sup> background, but it is difficult to interpret these higher numbers of CD4 T cells without a clonotypic Ab for their identification because of the presence of cells with endogenously rearranged TCR or two TCR. We are currently back-crossing our mice to TCR α-chain knock-out mice to investigate the involvement of B cells in T cell survival. However, abnormally low T cell numbers do not seem to be a general problem in TCR<sub>tg</sub> mice on Rag<sup>−/−</sup> or scid backgrounds, as was mentioned earlier.

Given that it is unlikely that the MHC interaction necessary for survival of peripheral T cells depends on recognition of “empty” MHC molecules, this could indicate that, in the periphery as well, the availability of an MHC-bound ligand might be limiting for the A18 TCR. It is possible that the A18 TCR is very restricted in its Ag recognition pattern, i.e., might not be as promiscuous as other tg TCR described. We have never observed any cross-reactivity with potential Ags present in a variety of other MHC haplotypes such as H-2<sup>b</sup>, H-2<sup>d</sup>, H-2<sup>c</sup>, whereas other TCR expressed in transgenic mice have known cross-reactivities (34, 35). The nature of MHC ligands mediating survival signals are not known, but it is logical to assume that self peptides must be of importance. Whether the self peptides that are responsible for peripheral selection are the same ones mediating thymic selection is another unknown variable at present.

It is worth mentioning that C5 in a C5-deficient mouse has no autologous counterpart, due to the complete absence of this protein from the repertoire. In contrast, other transgenic TCR with specificity for molecules like hen egg lysozyme, OVA, or cytochrome c, may in some way be influenced by the presence of the autologous mouse proteins. While presentation of these is not likely to lead to activation or negative selection, it is conceivable that peptides derived from such autologous proteins could yield alternative ligands, which are known to give partial activation signals that can have both beneficial and harmful effects on peripheral T cells (36). In addition, certain cytokines have been reported to promote Ag-independent proliferation of naive T cells; this apparently does not result in up-regulation of standard activation markers (37, 38). Although mature naive CD4 T cells in the A18 TCR<sub>tg</sub> mice do not appear to cycle, there is considerable variability in the numbers of CD4 T cells in individual mice. Given our present lack of knowledge concerning the identity of ligands responsible for providing survival signals via the TCR, it will be important to determine whether cytokines, perhaps secreted by cells of the innate immune system in response to environmental stimuli, could play a role in promoting T cell survival.

References