Effects of different antigenic microenvironments on the course of CD8$^+$ T cell responses in vivo

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Abstract

The influence of microenvironment on the course of CD8$^+$ T cell responses in vivo was investigated by injecting H-2K$^b$-specific T cells from donor TCR transgenic (TCR-Tg) mice into H-2K$^b$-Tg mice. H-2K$^b$ expression in recipients was either ubiquitous (CBK mice) or restricted to myeloid and erythroid cells (K$p^+$ mice). Donor T cells proliferated as extensively and acquired similar surface phenotypes in spleen of both recipient types. Thus, neither the restricted pattern of H-2K$^b$ expression nor the significantly reduced level of H-2K$^b$ expression by myeloid cells in K$p^+$ recipients affects the ability of the splenic microenvironment to prime T cell proliferation in vivo. However, an unsustained burst of cytolytic activity was generated rapidly in spleen of CBK recipients, whereas relatively little cytolytic activity was generated in K$p^+$ spleen. This indicates that effector T cells were not generated efficiently in spleen of K$p^+$ recipients even though extensive T cell proliferation was taking place in this microenvironment. Furthermore, activated donor T cells dispersed rapidly throughout primary and secondary lymphoid organs of K$p^+$ recipients, whereas few T cells migrated from spleen in CBK recipients. Consequently, the course of CD8$^+$ T cell responses and the anatomical distribution of activated T cells are profoundly influenced by the nature of the antigenic microenvironment encountered in vivo. We conclude that T cells rapidly proliferate and acquire new tissue-homing characteristics but do not differentiate into cytolytic effector cells at the site of priming when they encounter myeloid cells expressing low levels of antigen in vivo.

Introduction

Naive T cells respond to contact with cells displaying antigen by undergoing a complex series of morphological, phenotypic and functional changes that result in the acquisition of altered growth, differentiation and tissue-homing characteristics (reviewed in 1,2). Initial contacts between naive T cells and antigen-presenting cells (APC) take place within secondary lymphoid organs that drain the site of primary pathological challenge. The adaptive immune system has evolved to maximize the probability that very low numbers of APC displaying foreign antigen will induce a rapid, effective and highly specific response against invading pathogens to prevent spread of infection. For most antigens, T cells with appropriate receptor (TCR) specificities are present at very low frequencies in the naive T cell repertoire. Thus, initial contacts that result in specific immune responses in vivo must involve vanishingly small numbers of T cells and APC. Consequently, mimicking physiological microenvironments in which priming events take place is difficult to accomplish in vitro. To circumvent this, cognate antigens, in the form of peptides or virus, have been introduced directly into TCR transgenic (TCR-Tg) mice or into non-transgenic mice harbouring a cohort of antigen-specific T cells from TCR-Tg mice.
CD8 T cell responses in vivo

(3-10) so that subsequent T cell responses can be monitored relatively easily in vivo. Not surprisingly, route of antigen administration and antigen dose are critical factors that influence the vigour and course of T cell responses in vivo, and their ultimate outcome in terms of virus clearance, tolerance induction and development of T cell memory (3). In most studies, a wide variety of different cell types were potentially able to present antigen to naive T cells because cognate antigen was either (i) expressed by many types of cell (5,7-10) or (ii) administered as a soluble peptide able to associate with all cells expressing appropriate surface MHC molecules (3,4). In this study we have introduced naive H-2K\(^b\)-specific, CD8\(^+\) T cells from TCR-Tg donor mice (11) into recipient (H-2K\(^b\)-Tg) mice that differ only in the tissue-specific pattern and level of H-2K\(^b\) expression (11,12). The rationale for this experimental approach is to provide the means to investigate how T cell responses proceed in microenvironments that are similar to those in which immune responses to foreign antigens are initiated in vivo. In particular, we concentrated on events that occur in microenvironments where few cells other than myeloid cells express low levels of antigen. Our results indicate that the balance between T cell proliferation, differentiation and acquisition of altered tissue-homing characteristics is profoundly influenced by the pattern and level of antigen expression encountered in vivo.

Methods

Mice

CBA/Ca mice were bred at the National Institute for Medical Research. All transgenic mice were generated and bred at the NIMR under barriered conditions and according to regulations laid down by the UK Home Office. BM3 TCR-Tg mice used as a source of donor T cells have been described (11). KB (12) and CBK (11) are H-2K\(^b\)-Tg mice. All transgenic mice were used as a source of donor T cells in vivo. Not surprisingly, route of antigen administration and antigen dose are critical factors that influence the vigour and course of T cell responses in vivo, and their ultimate outcome in terms of virus clearance, tolerance induction and development of T cell memory (3). In most studies, a wide variety of different cell types were potentially able to present antigen to naive T cells because cognate antigen was either (i) expressed by many types of cell (5,7-10) or (ii) administered as a soluble peptide able to associate with all cells expressing appropriate surface MHC molecules (3,4). In this study we have introduced naive H-2K\(^b\)-specific, CD8\(^+\) T cells from TCR-Tg donor mice (11) into recipient (H-2K\(^b\)-Tg) mice that differ only in the tissue-specific pattern and level of H-2K\(^b\) expression (11,12). The rationale for this experimental approach is to provide the means to investigate how T cell responses proceed in microenvironments that are similar to those in which immune responses to foreign antigens are initiated in vivo. In particular, we concentrated on events that occur in microenvironments where few cells other than myeloid cells express low levels of antigen. Our results indicate that the balance between T cell proliferation, differentiation and acquisition of altered tissue-homing characteristics is profoundly influenced by the pattern and level of antigen expression encountered in vivo.

H-2K\(^b\) expression

H-2K\(^b\) expression by different cell lineages in H-2K\(^b\)-Tg mice (Table 1) was assessed directly by staining with H-2K\(^b\)-specific antibodies or indirectly in a more sensitive bioassay in which cells were used to stimulate IFN-\(\gamma\) secretion by BM3 splenocytes (11). Expression by myeloid cells from bone marrow (11) or thymus (unpublished results) was tested after prolonged culture with granulocyte macrophage colony stimulating factor. Levels of H-2K\(^b\) expression are much higher on myeloid cells from CBK mice than KB mice (11 and unpublished results).

Adoptive transfer of TCR-Tg\(^+\) T cells

Pooled spleen and lymph node cells (4x10\(^6\)) from BM3 TCR-Tg donor mice were injected into the tail vein of CBA mice or H-2K\(^b\)-Tg mice. Mice were killed on subsequent days after transfer. At least three mice were injected per time point. Control studies revealed that BM3 T cells continued to circulate in secondary lymphoid organs of CBA recipients for >100 days. Throughout this prolonged period TCR-Tg\(^+\) T cells remained small, neither increased nor decreased significantly in numbers and did not acquire characteristic markers of activation (e.g. CD44, data not shown).

Cytotoxicity assays

Ex vivo cytotoxic activity of splenocytes was assessed in a standard \(^{51}\)Cr-release assay (11). Tests were performed in triplicate in round-bottomed microtitre plates at responder:target (A:T) ratios of 100:1, 30:1, 10:1 and 3:1. Target cells were incubated for 90 min at 37°C with 100 \(\mu\)Ci \(^{51}\)Cr (Amersham, UK) before being mixed with responder cells. After 5 h at 37°C, 25 \(\mu\)l of supernatant was collected from each well and counted (Betaplate 1205; Pharmacia, UK). Regression analysis was performed as described by Simpson and Chandler (13). EL-4 (H-2\(^b\)) and P1.HTR (H-2\(^d\)) were used as targets. To normalize between different time points mean specific lysis values from experimental samples were divided by the mean specific lysis (at A:T = 10:1) from simultaneous control samples (BM3 TCR-Tg spleen cells stimulated with CBK cells in vitro for 3 days) and the result expressed as a percentage. H-2K\(^b\)-specific lysis in injected CBA mice is due entirely to donor BM3 TCR-Tg cells since no cytotoxic activity is detected in CBA mice after a 3 day mixed lymphocyte culture with CBK stimulator cells (unpublished results).

Cytofluorimetric analysis

Thymus, spleen, axillary and mesenteric lymph nodes were removed and cells teased out into medium. Cells (1x10\(^6\)) in PBS were stained at 4°C in the presence of 0.02% Na azide, 1% BSA. Anti-CD8-FITC (53-6.7) and anti-CD4-phycocerythrin (PE) (GK1.5) conjugates were obtained from Becton Dickinson (Mountain View, CA). Anti-TCR antibody [Ti-98 (14)] was biotinylated according to standard procedures and developed with streptavidin-Tricolor (CalTag, San Francisco, CA). Cells from spleen and mesenteric lymph nodes were stained for the following T cell activation markers: MEL-14-FITC (anti-L-selectin), PS/2-biotin (anti-VLA-4), FD44.1.8.2-FITC (anti-LFA-1), 7D4-FITC (anti-CD25) and IM7-1-FITC (anti-CD44, Pharmingen, Cambridge Bioscience, Cambridge, UK). Cells were also stained with anti-CD8-PE (YTS, 169.4; CalTag) or anti-CD8-FITC and Ti-98 conjugated to biotin or FITC. Cells from spleen and mesenteric lymph nodes were stained for the following T cell activation markers: MEL-14-FITC (anti-L-selectin), PS/2-biotin (anti-VLA-4), FD44.1.8.2-FITC (anti-LFA-1), 7D4-FITC (anti-CD25) and IM7-1-FITC (anti-CD44, Pharmingen, Cambridge Bioscience, Cambridge, UK). Cells were also stained with anti-CD8-PE (YTS, 169.4; CalTag) or anti-CD8-FITC and Ti-98 conjugated to biotin or FITC. Cells were analysed on a Becton Dickinson FACScan with four logarithmic scales. Viable lymphocytes for acquisition were selected using forward and side scatter characteristics (set by eye). Resulting profiles were analysed using Cell Quest software. Markers were set on TCR-Tg\(^{high}\) and TCR-Tg\(^{+}\) in

<p>| Table 1. Pattern of H-2K(^b) expression in H-2K(^b)-Tg mice |</p>
<table>
<thead>
<tr>
<th>Cell lineage</th>
<th>Cell type</th>
<th>H-2K(^b)-Tg mouse (promoter)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CBK (H-2)</td>
</tr>
<tr>
<td>Lymphoid</td>
<td>T cells</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>B cells</td>
<td>+</td>
</tr>
<tr>
<td>Erythroid</td>
<td>red blood cells</td>
<td>+</td>
</tr>
<tr>
<td>Myeloid</td>
<td>dendritic cells</td>
<td>+</td>
</tr>
<tr>
<td>Stromal</td>
<td>(keratinocyte, epithelial)</td>
<td>-</td>
</tr>
</tbody>
</table>
CD8 T cell responses in vivo

Fig. 1. FACS analyses of splenocytes from H-2K<sup>b</sup>-Tg recipient mice 3 days after transfer of BM3 cells. Two-dimensional dot plots show staining profiles with anti-CD4-PE (vertical axis) and anti-CD8-FITC (horizontal axis); 10,000 events were collected. Numbers on two-dimensional dot plots indicate the percentage of total events falling into each quadrant. Histograms show staining profile of biotinylated anti-TCR-Tg (Ti-98) on the gated CD8<sup>+</sup>CD4<sup>-</sup> population. Markers shown on the TCR-Tg histograms define the percentage of donor TCR-Tg<sup>+</sup>CD8<sup>+</sup> T cells expressing high or low levels of surface TCR-Tg, markers were set using stained CD8<sup>+</sup> T cells from control BM3 (TCR-Tg<sup>low</sup>) or CBA (TCR-Tg<sup>+</sup>) mice.

Assessment of proliferation in vivo

For three-colour staining of CD8, TCR and 7-amino-actinomycin D (7AAD) (15) cells were stained as above in a mixture of FITC-conjugated anti-TCR-Tg [Ti-98 (14)] and anti-CD8-
PE. After two washes in FACS medium cells were stained with 7AAD (1 μg/ml) in 0.3% saponin for 30 min at room temperature.

Results

Proliferation of CD8+ T cells in H-2Kb-Tg mice

T cell responses in recipient CBK and Kβ mice (Table 1) were analysed following adoptive transfer (i.v.) of pooled splenocytes and lymph node cells from BM3 (TCR-Tg) donor mice. Donor T cells in all secondary lymphoid organs and, in some cases, in primary lymphoid organs were characterized by cytfluorimetry using an anti-clonotypic [anti-TCR-Tg (14)] mAb and a number of other T cell markers. In addition, splenocytes were stained with a DNA intercalating dye, 7AAD (15), to assess T cell proliferation and were tested for the ability to lyse labelled EL-4 target cells directly (ex vivo assay). The progress of T cell responses was monitored for a period of 14 days in each case but here we concentrate on early events that occur from 3 to 6 days after adoptive transfer.

Three days after transfer, the total number of CD8+ T cells present in spleen of CBK and Kβ recipients had increased significantly (Fig. 1) and most (~90%) T cells were of donor origin (i.e. TCR-Tg+). In contrast, the splenic microenvironment of control CBA mice did not foster expansion of donor CD8+ T cells. Numbers of donor CD8+ T cells present in spleen were at least 10 times higher (2–3x10^7) in CBK and Kβ than in control CBA (~2x10^6) recipients injected at the same time (Table 2). These numbers were three to four times higher than the total number of CD8+ T cells injected (~7x10^6) showing that donor T cells had proliferated, rather than preferentially accumulated, in recipient spleen. Confirmation that donor T cells were proliferating in CBK and Kβ spleen was obtained by staining cells with 7AAD. On average, around a quarter of gated TCR-Tg+, CD8+ cells stained brightly (i.e. contained more than a diploid quota of DNA) 3 days after transfer. This reveals that the majority of donor T cells in both recipients were proliferating. Furthermore, few cells, if any, stained dull with 7AAD (i.e. contained less than a diploid quota of DNA) in either recipient, indicating that the majority of donor CD8+ T cells were viable and not undergoing apoptosis in spleen. The number of donor CD8+ T cells detected in spleen did not continue to increase at later times after transfer (Table 2) and decreased rapidly after 4 days in both CBK and Kβ recipients.

Table 2. Proliferation of CD8+ TCR-Tg+ T cells after adoptive transfer

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Day</th>
<th>CD8:CD4</th>
<th>Gated TCR-Tg+ CD8+ splenocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total^b Low^b High^b</td>
</tr>
<tr>
<td>CBK</td>
<td>3</td>
<td>2.5</td>
<td>22 22 0.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.0</td>
<td>30 26 4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.7</td>
<td>12 10 2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.5</td>
<td>11 11 1</td>
</tr>
<tr>
<td>Kβ</td>
<td>3</td>
<td>3.9</td>
<td>31 4 27</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8.0</td>
<td>23 5 18</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.5</td>
<td>8 3 5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.8</td>
<td>10 7 3</td>
</tr>
<tr>
<td>CBA(i)</td>
<td>—</td>
<td>0.4</td>
<td>2 0 2</td>
</tr>
</tbody>
</table>

^aCell numbers (in millions) were averaged from three mice in each group.

^bNumbers of T cells expressing low or high levels of TCR-Tg (see Methods).

Fig. 2. Staining and analysis of splenocytes using 7AAD. Histograms, measured as linear 7AAD fluorescence, show staining profiles of gated CD8+ splenocytes from BM3 controls and CBK and Kβ mice 3 days after adoptive transfer of BM3 cells. Percentages shown indicate the proportion of CD8+ donor cells undergoing apoptosis (left marker), or in G_1 (centre marker), or S, M, G_2 (right marker) phases of the cell cycle. CD8+ TCR-Tg+ cells are cycling in both H-2Kb Tg mice.
These results demonstrate that APC in splenic microenvironments of CBK and Kβ mice induce rapid expansion of donor CD8^+ T cells. As the kinetics of T cell expansion was similar in CBK and Kβ recipients, we conclude that the ability of splenic microenvironments to induce rapid proliferation of H-2K^b-specific CD8^+ T cells in vivo is not affected by (i) limiting expression of H-2K^b to myeloid and erythroid cells only in Kβ mice (Table 1) nor by (ii) the marked reduction in level of surface H-2K^b expressed by myeloid cells in Kβ mice [relative to CBK (11 and unpublished results)]. Myeloid cells, such as dendritic cells, are efficient inducers of T cell proliferation because they express ligands (such as CD80) that provide co-stimulatory signals necessary for activating naive T cell responses. It is highly unlikely that erythroid cells, which do not express co-stimulatory ligands, can induce T cell expansion and, hence, myeloid cells must mediate the T cell responses observed in Kβ recipients. Indeed, red blood cells from Kβ mice did not induce T cells from BM3 mice to respond in vitro (data not shown).

**Differentiation of CD8^+ T cells in H-2K^b-Tg mice**

Although the kinetics of CD8^+ T cell expansion and subsequent contraction were comparable in CBK and Kβ spleen, qualitative aspects of CD8^+ T cell responses differed profoundly (Table 2). In CBK recipients 3 days after adoptive transfer splenic CD8^+ T cells of donor origin displayed a marked reduction in the level of surface TCR-Tg expression (TCR-Tg<sub>low</sub>) relative to naive CD8^+ T cells from control BM3 mice (TCR-Tg<sub>high</sub>). In contrast, TCR-Tg levels were unchanged, relative to naive BM3 T cells, in Kβ recipients (Fig. 1 and Table 2). Reduction in the level of surface TCR expression following T cell activation has been described by others and appears to correlate with differentiation of T cells (16). By this criterion T cell responses followed a different course in the two types of recipient mice. Further evidence confirming that T cells responded differently in CBK and Kβ mice was obtained from analyses of cytolytic activity (ex vivo assay) and of cell surface phenotypes of donor T cells. Three days after transfer donor T cells in CBK spleen had differentiated into blast cells that lysed EL-4 (H-2K^b) target cells in an ex vivo killing assay (Fig. 3A). Functional analyses carried out at later times revealed that the burst of cytolytic activity detected in CBK spleen 3 days after transfer was short lived and declined rapidly on subsequent days until no cytolytic activity was detected on day 6 (Fig. 3B). This pattern of functional activity was not observed in Kβ recipients. Three days after transfer splenocytes did not lyse EL-4 target cells ex vivo (Fig. 3A) even though >90% were of donor origin at this time. Furthermore a burst of cytolytic activity was not detected on subsequent days and activity remained low (<25% of controls) until 6 days after transfer. These data reveal that the splenic microenvironment of Kβ recipients is not conducive to rapid and efficient generation of cytolytic effectors in situ, and suggests that T cell responses follow a fundamentally different path in CBK and Kβ spleen in terms of the relationship between proliferation and differentiation of CD8^+ T cells.

Analyses of various cell surface markers were carried out to determine whether CD8^+ T cells displayed phenotypic characteristics typical of naive or activated T cells. In CBK spleen 3 days after transfer CD8^+ T cells displayed phenotypes characteristic of activated, differentiated T cells (Fig. 4). Levels of IL-2R (CD25), VLA-4 (CD49d), pgp-1 (CD44), LFA-1 (CD11a) were elevated and levels of L-selectin (MEL-
CD8 T cell responses in vivo

Fig. 4. Analyses of cell surface markers on splenocytes 3 days after transfer of BM3 cells. Cells were stained with anti-CD8-PE, anti-TCR (Ti-98) biotinylated or FITC, and the following cell surface activation markers MEL-14-FITC, VLA-4-biotin, LFA-1-FITC, CD44-FITC and IL-2R-FITC. Flow cytometric analyses were performed on splenocytes from CBK (thin line) or Kβ (thick line) recipients. Splenocytes from BM3 mice were used as controls (shaded panel). Histograms show staining profiles of each surface marker on gated CD8$^+$ TCR-Tg$^+$ T cells.

Table 3. Analysis of CD8$^+$ TCR-Tg$^+$ T cells in mesenteric lymph nodes

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Day</th>
<th>Gated TCR-Tg$^+$ CD8$^+$ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total$^a$ (%)$^c$</td>
</tr>
<tr>
<td>CBK</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>Kβ</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td>CBA(i)</td>
<td>—</td>
<td>0.6</td>
</tr>
</tbody>
</table>

$^a$Cell numbers (in millions) were averaged from three mice in each group.
$^b$Numbers of T cells expressing low or high levels of TCR-Tg (see Methods).
$^c$Percentage of CD8$^+$ T cells that are TCR-Tg$^+$

issues we assessed the tissue distribution of donor T cells in each recipient.

Of all tissues analysed, expansion of CD8$^+$ T cells of donor origin was most evident in spleen of CBK and Kβ recipients, particularly at early times following adoptive transfer. This may be a consequence of introducing donor cells via the tail vein as spleen would be the first secondary lymphoid organ infiltrated by most naive T cells administered via this route (17). However, cytofluorimetric analyses of primary (bone marrow and thymus) and secondary lymphoid organs (mesenteric and axillary lymph nodes) revealed significant differences in the anatomical distribution of activated CD8$^+$ T cells following adoptive transfer into CBK or Kβ recipients.

At the peak of donor T cell expansion in mesenteric lymph nodes (Table 3, day 4) both the number and the proportion of donor CD8$^+$ T cells found were much lower in CBK (~3x10$^6$ and ~20% respectively) compared to Kβ (~1.2x10$^7$ and ~95%) recipients. Thus, throughout the period when activated donor T cells were undergoing extensive proliferation and differentiation in CBK spleen very few donor T cells were found in the lymph nodes. This shows that few naive T cells circulated to the lymph nodes and/or most donor T cells did not leave the spleen after activation in CBK mice. The majority of donor T cells in lymph nodes expressed low levels of TCR-Tg in CBK recipients (~95% at day 4) whereas most expressed high levels in Kβ recipients (~75% at day 4). This correlates with observations made in spleen and indicates that the distinctive pathways of differentiation observed in spleen also occur in other tissues. Presumably, this is a consequence of the different antigenic microenvironments encountered by donor T cells in all tissues. Large cohorts of donor T cells were found in thymus and bone-marrow of Kβ recipients 3 days after transfer (data not shown). In contrast, naive T cells did not circulate to primary lymphoid tissues in control CBA mice (data not shown). This is consistent with changes in the expression of surface markers associated with tissue homing (e.g. pgp-1, VLA-4, LFA-1 and L-selectin) and confirms that donor CD8$^+$ T cells acquire altered tissue-homing characteristics as a result of activation in Kβ mice. Donor T cells were

14, CD62L) were decreased when compared with naive CD8$^+$ T cells from control BM3 mice. Similar changes in the levels of all surface markers analysed were detected on CD8$^+$ T cells in Kβ spleen indicating that these T cells were also activated. However, changes in surface levels of IL-2R, pgp-1 and L-selectin were not as marked as in CBK spleen.

**Tissue distribution of activated CD8$^+$ T cells**
Failure to detect cytolytic activity in spleen of Kβ recipients might arise because activated T cells migrate from spleen in large numbers after priming and enter circulation relatively rapidly. Additionally, activated T cells may be retained more effectively in CBK than in Kβ spleen, perhaps because of the higher density of cells expressing antigen. To address these
not found in thymus of CBK recipients and only 3% of bone marrow cells were TCR-Tg+ 3 days after transfer (data not shown). This may reflect a difference in homing characteristics of activated T cells in CBK mice but could also arise because few T cells migrate from spleen after activation in these recipients.

These data show that donor T cell expansion was largely confined to one organ, the spleen, in CBK recipients with comparatively few activated T cells migrating from spleen or being generated in situ in other primary or secondary lymphoid tissues. This prominent sequestration of the T cell response suggests that the entire programme of T cell activation, proliferation and differentiation to produce cytolytic effector cells is induced by APC and the microenvironment prevalent in spleen. In comparison, the T cell response observed in Kβ recipients is not confined to spleen since activated T cells rapidly permeate all primary and secondary lymphoid organs. Nevertheless, as in CBK recipients, spleen is the initial epicentre of proliferation in Kβ recipients since peak expansion of donor CD8+ T cells is delayed by 1 day in peripheral lymph nodes.

Discussion

Our observations illustrate how the type of microenvironment encountered by naive CD8+ T cells in vivo exerts a profound influence on the subsequent programme of T cell responses. The two lineages of H-2Kb-Tg mice, CBK and Kβ, used in these experiments express H-2Kb on different types of cells and at different levels under the control of H-2 class I or human β-globin gene promoter elements respectively. With the exception of erythroid cells, myeloid cells are the only cells to express H-2Kb in Kβ tissues and the level of H-2Kb expressed is relatively low since expression was detected only by recourse to a sensitive bioassay (11). In contrast, all cells express H-2Kb in CBK mice at elevated levels (11,12, unpublished results) Presumably, encounters in situ between APC displaying cognate (foreign) antigen and naive, antigen-specific T cells occur rarely and involve relatively low amounts of antigen, especially during initial priming stages. Furthermore, antigen is presented by myeloid cells that migrate to secondary lymphoid organs draining sites of infection or inflammation (18,19). In effect, our system allows us to increase enormously the number of encounters between APC displaying antigen and antigen-specific CD8+ T cells. To minimize potential artifacts arising from increases in the number of these encounters and for T–T collaboration, we titrated the number of splenocytes so that the minimum dose was introduced (4×10⁷) that still allowed us to assess the functional state of T cells in vivo. At this dose ~7×10⁶–1×10⁷ CD8+ T cells are injected into recipients and these represent ~2–3% of total CD8+ T cells in control CBA recipients. Furthermore, CBK recipients are effective controls for artifacts arising from increased T–T collaboration during T cell responses. With these factors in mind, we consider that T cell responses in Kβ recipients take place under circumstances that are more realistic than those which prevail in CBK mice.

The kinetics of donor T cell proliferation in spleen is almost identical in Kβ and CBK recipients. We assume that rapid expansion is induced by splenic APC of myeloid origin since myeloid cells are potent stimulators of naive CD4+ and CD8+ T cells (1,16,18–23). However, the balance between proliferation and differentiation, as measured by phenotypic and functional criteria, differs profoundly. In Kβ spleen, relatively little cytolytic effector activity was detected, even though the organ contained a large number of activated donor CD8+ T cells. This cannot be explained by a slower rate of T cell proliferation since the burst of cytolytic activity detected in CBK spleen at day 3 occurred when similar numbers of activated donor T cells were present. Thus, T cell responses in CBK and Kβ spleen are distinguished by a distinct difference in acquisition of effector function but not by the kinetics of T cell proliferation. Our observations suggest that terminally differentiated effector T cells are not generated in large numbers in secondary lymphoid tissues where priming takes place in vivo. Generation of cytolytic effector T cells in situ may compromise continuous stimulation of T cell responses since APC displaying antigen (which are therefore targets for cytolytic effectors) will be few in number during the early stages of an immune response.

One explanation for the absence of a burst of cytolytic activity in Kβ spleen is that T cells did not differentiate along identical pathways in each recipient. However, donor T cells in CBK and Kβ recipients displayed surface phenotypes typical of activated T cells, although levels of some surface markers analysed did differ. Alternative differentiation of T cells may occur if distinct signals were received by naive T cells as they entered the splenic microenvironments and/or as they underwent subsequent proliferation. At both stages there are tangible reasons why distinct signals may be provided in the two different microenvironments. First, myeloid cells express different levels of H-2Kb and this may contribute directly to the strength of the signal received by naive T cells when they initially encounter APC. In turn, this could influence the course of T cell differentiation such that weaker signals (equated with lower antigen levels in Kβ mice), whilst effectively inducing T cell proliferation, fail to induce efficient differentiation of CD8+ T cells in vivo. Evidence that the level of antigen expressed by APC correlates with the degree of subsequent differentiation of CD4+ T cell clones in vitro has been reported recently (16). Another potential source of differential signalling arises from the presence of non-myeloid cells expressing antigen in CBK spleen. These cells may deliver additional signals to T cells, reinforcing or modulating signals delivered by APC of myeloid origin. Such 'secondary' signals could induce rapid differentiation of effector T cells because of continuous stimulation by all cells in this microenvironment. Furthermore, frequent contact with cells expressing antigen may explain why activated T cells do not emerge from spleen in large numbers in CBK mice. This scenario would not occur in Kβ spleen because very few cells express H-2Kb. Indeed, the large number of donor T cells present in Kβ spleen may saturate all available contact sites with myeloid APC early in the response such that T cells generated de novo via proliferation do not encounter any cells expressing H-2Kb in this microenvironment. Lack of contact with cells expressing H-2Kb may explain why so many activated donor T cells migrated out of Kβ spleen and found their way into primary and other secondary lymphoid tissues. Limited access to APC displaying antigen may also be a
feature of T cell responses to foreign antigens in vivo since APC will induce rapid clonal expansion in their vicinity once they encounter appropriate naive T cells. Thus, differences in the nature of signals delivered at priming and/or subsequent stages in the T cell response provide potential explanations for the distinct pathways of differentiation observed in vivo.

An alternative explanation for our observations in Kβ spleen is that activated donor T cells migrated rapidly from spleen before they had fully differentiated into cytolytic effector T cells. In this event, T cell differentiation could occur at similar rates in Kβ and CBK mice except that rapid dispersal of activated T cells in Kβ but not CBK mice resulted in a burst of cytolytic activity only when CBK splenocytes were tested. We cannot distinguish between this and an explanation based on alternative pathways of differentiation. However, it is striking that most donor T cells found in peripheral lymph nodes of Kβ mice express high levels of TCR-Tg rather than the lower levels characteristic of fully differentiated cytolytic T cells observed in CBK spleen. This suggests that generation of cytolytic cells is at least partially compromised in Kβ mice.

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Abbreviations

7-AAD 7-amino-actinomycin D
APC antigen-presenting cells
PE phycoerythrin
Tg transgenic

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