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The Growth of the Very Large CD8+ T Cell Clones in Older Mice Is Controlled by Cytokines

Chia-Chi Ku, John Kappler, and Philippa Marrack

Olde human beings and mice often develop large clones of CD8+ T cells. In mice these cells are phenotypically very similar to memory CD8+ T cells. Like memory CD8+ T cells, most members of the clones are in continuous slow division, apparently independently of Ag stimulation. Proliferation of the CD8+ clonal T cells is inhibited in mice treated with Ab to the IL-2R β-chain that blocks signaling by either IL-2 or IL-15. However, inhibition of IL-2 increases the numbers of dividing clonal cells. Therefore, like normal memory CD8+ T cells, expansion of the clones is driven by IL-15 and inhibited by IL-2 and is probably limited by the amounts of IL-15 and IL-2 present in the host. Control by these two cytokines may account for the fact that, although the clones can be very large, they do not overwhelm or kill their hosts. Nevertheless the clonal cells compete successfully with normal memory CD8+ T cells for growth. Perhaps the clonal cells use IL-15 more effectively or are more resistant to the inhibitory effects of IL-2. Thus they might affect the immune response of their hosts by competing for factors that stimulate and inhibit normal CD8+ memory T cells. The Journal of Immunology, 2001, 166: 2186–2193.

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Materials and Methods

Mice

Six- to 8-wk-old C57BL/6J and B6D2F1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Fifteen-month-old C57BL/6 and B6D2F1 mice were purchased from the National Institute on Aging colony maintained at Charles River Laboratories (Wilmington, MA). In previous experiments mice of various strains including C57BL/6J were obtained from The Jackson Laboratory and aged at National Jewish Medical and Research Center (Denver, CO) (1, 10, 11). We observed no difference in the properties of aged C57BL/6J mice obtained from The Jackson Laboratory or Charles River Laboratories.
All of the animals were kept in a specific pathogen-free animal facility at the National Jewish Medical and Research Center. Mice over the age of 15 mo were screened for CD8+ clones by anti-VP and anti-CD8 staining of the peripheral blood (11, 12). Mice of the same strain aged younger than 4 mo were used as the comparison group. Six- to 8-wk-old C57BL/6 β2-microglobulin-deficient (β2MKO)4 mice were purchased from Taconic Farms (Germantown, NY).

T cell purification

Spleen cells were treated briefly with ammonium chloride solution to lyse RBC and then washed in balanced salt solution (BSS) once. T cells were purified from lymph node (LN) and spleen cells as previously described (27, 28). Briefly, the cells were resuspended in 1–1.5 ml of BSS plus 5% FCS and loaded on sterile nylon wool columns, which had been washed and soaked in BSS plus 5% FCS for at least 30 min at 37°C before loading the cells. The cells were incubated on columns for 30–40 min at 37°C and eluted in an appropriate volume of BSS plus 5% FCS.

In experiments in which the total numbers of T cells in mice were counted, counts included cells in the spleen and the axillary, brachial, inguinal, mesenteric, peri-aortic, and superficial cervical LNs.

Abs and cell staining

Some anti-mouse Vβs, anti-CD4 (GK1.5), anti-CD8 (53-6.7), and anti-Clβ (H597) mAbs were prepared and conjugated with fluorescein or biotin (bio) in our laboratory. All other PE-, CyChrome-, and allophycocyanin-labeled mAbs were purchased from PharMingen (San Diego, CA). These included a panel of PE-labeled anti-mouse surface proteins, which were as follows: anti-CD28, 37.51; anti-CD44, IM7; anti-CD45RB, 16A; anti-CD62 ligand, MEL-14; anti-CD69, H1.2F3; anti-IL-2Ra (anti-CD25), 3C7; anti-IL-2Rβ (anti-CD122), TM-β1; anti-IL-2γ (anti-CD122), TSU Gl2; anti-Fas (anti-CD95), Jo2; and biotinylated anti-IL-7Rα (anti-CD127), B12-1. Anti-mouse Bel-2 and streptavidin-CyChrome (SAv-CyC) were also obtained from PharMingen. Cells were stained and analyzed as previously described (27–29).

CFSE (Molecular Probes, Eugene, OR) was dissolved in DMSO and diluted in sterile BSS. Nylon wool-purified T cells from old mice were isolated and prepared as described above. The cells were labeled with 1 μM CFSE at 1 × 10^5 cell/ml for 15 min at 37°C and then washed twice in BSS (30). CFSE-labeled cells (0.5–1 × 10^7) were i.v. injected into nonirradiated, syngeneic young mice. The CFSE signal from these cells was detected on the FL-1 channel of FACSscan flow cytometer (Becton Dickinson, San Jose, CA).

To analyze incorporation of 5-bromo-2'-deoxyuridine (BrdU) by CD8+ T cells, T cells were stained and sorted for CD8+ expression. They were then stained with anti-β2M and the Vβ expressed on the clone and SAv-CyC, treated with acid and stained with fluorescein anti-BrDU (Becton Dickinson) as previously described (31).

Anti cytokine and anti-cytokine receptor Abs given to animals were as follows: 5C7, anti-IL-2Ra (32); A7R34, anti-IL-7Rα (33); TM-β1, anti-IL-2β (34); S4B6, anti-IL-2 (35); and M25, anti-IL-7 (36). All the Abs used were rat in origin. Abs were purified from culture supernatants by passage over protein G columns. F(ab′)2 preparations of the anti-receptor Abs were prepared by pepsin digestion at pH 4.0 and judged to be <1% contaminated with intact Ig by SDS PAGE. Mice were given 1 mg of each Ab or F(ab′)2 preparation daily i.p.

T cell transfer from old mice to lethally irradiated young mice

Normal or β2MKO young mice were treated with 0.04 ml rabbit anti-CD8 antibody (The Jackson Laboratory) to deplete these populations of T cells (37). Two days later, bone marrow cells were harvested from these animals. Young C57BL/6 or β2MKO mice were lethally irradiated with 950 rad and immediately reconstituted with 1 × 10^7 syngeneic bone marrow cells. Four days later, spleen and LN T cells were isolated from individual old mice with CD8+ expansions bearing known Vβs. Equal numbers of these T cells from old mice were transferred i.v. into the lethally irradiated C57BL/6 and β2MKO recipients such that each recipient was given 0.5–1 × 10^7 T cells. The animals were bled at intervals thereafter to monitor survival or growth of the transferred cells.

Abbreviations used in this paper: β2MKO, β2-microglobulin-deficient; BSS, balanced salt solution; LN, lymph node; bio, biotin; SAv-CyC, streptavidin-CyChrome; BrdU, 5-bromo-2'-deoxyuridine.

Results

Identification of CD8+ T cell clones in old mice

We and others have shown previously that healthy older mice or humans contain very large clones of CD8+ T cells (1–7, 11, 12). These clones have been identified in many ways: by cDNA sequencing of the junctional regions of their TCR α- and β-chains, by complementarity-determining region-3 length analysis and by the presence of an unexpectedly high percentage of CD8+ T cells bearing a particular Vα and/or Vβ. For convenience, in this paper, clones of CD8+ T cells will be defined by the presence in a mouse of a percentage of CD8+ T cells bearing a particular Vβ that is more than two SDs above the percentage of CD8+ T cells bearing Vβ in young animals. Because the spectrum of percentages of CD8+ T cells bearing particular Vβs is very predictable in young animals this identification of CD8+ clones by Vβ analysis has proven to be extremely reliable. In every case, TCR sequencing or Vβ analysis has shown that CD8+ expansions defined by Vβ staining are indeed clones.

However, it must be borne in mind that some percentage of the cells, defined as clones by Vβ staining, will not be members of the clones. For example, in one mouse we studied, about 25% of the CD8+ T cells bore Vβ9. In young mice of the same strain, the percent of CD8+ T cells bearing Vβ9 was only about 5%. Therefore, we could estimate that about 5/25 × 100 (20%) of the CD8+ Vβ9+ T cells in the old mouse were not members of the clone, but rather the normal CD8+ Vβ9+ T cells of the animal. Thus there will always be a small contamination by normal cells of the cells identified as part of the clone. This is unavoidable, because molecular methods of identifying clonal cells destroy the cells and live cells were needed for the experiments of this paper. However, in some cases we have confirmed that the behavior observed was due to members of the clone, because the TCR α- and β-chain junctional sequences of the population studied were shown to be the same before and after the experiment.

Members of the large CD8+ clones have the surface characteristics of memory T cells

T cells from nine old mice containing CD8+ clones and from young mice were stained with various Abs to surface molecules. Results from a representative experiment are shown in Fig. 1. The phenotypes of CD8+ T cells from the old mice were similar, whether or not they were members of a CD8+ clone. Their surface markers were, on the whole, characteristic of memory or activated cells because they were CD44high, CD45RBmedium-low and they bore high levels of IL-2Rβ (but see below; Refs. 25, 38, and 39). Unlike the large CD8+ clones found in humans (40, 41) the mouse CD8+ clones were, like the other CD8+ cells in young and old mice, CD28+. We previously reported that such cells were CD28+. However, using an improved anti-CD28 Ab, we found that all of the cells in the nine clones we have recently analyzed were CD28. The staining with anti-CD28 was not nonspecific, because the Ab did not stain T cells from CD28 KO mice (data not shown).

Many of the clonal CD8+ T cells are dividing in mice

Most CD8+ memory and memory phenotype cells divide, albeit slowly, in mice (23–25). We used two methods to find out whether this is also true for members of the clones. First, several old mice that contained large CD8+ clones were given BrdU continuously in their drinking water. Twenty-one days later, the animals were sacrificed and the percentage of clonal CD8+ T cells that had incorporated BrdU was measured. As shown in Table I, both
clonal and nonclonal cells divided during this time. However, the percentage incorporating BrdU was higher for the CD8\(^+\) clonal than for the CD8\(^+\) nonclonal cells.

In a second type of experiment, cells from old mice that contained large CD8\(^+\) clones and cells from young mice used as a control, were labeled with CFSE and transferred to young, normal, syngeneic recipients. The recipients were sacrificed at intervals after the transfer and division of the donor cells analyzed by examination of their CFSE profiles. The results of a typical experiment are shown in Fig. 2.

Very few of the CD8\(^+\) T cells from young donors divided after transfer. However, many of the nonclonal CD8\(^+\) T cells from old mice did divide. These results were consistent with the division of cells of the memory, but not naive, phenotype (24, 25, 42). The environment of normal young mice also supported division of the CD8\(^+\) clonal cells from the old mice. Almost all of the clonal cells had gone through at least one round of division within 36 days of transfer.

This result was somewhat different from that observed when BrdU was used to measure the division of the clones directly in their original (old) hosts, which showed that only about 50% of the clonal cells divided within 21 days (Table I). This difference suggested that the conditions in young mice may be more conducive to the division of memory CD8\(^+\) cells. Such an interpretation was supported by experiments in which CSFE labeled CD8\(^+\) T cells from old mice were transferred to either normal young and old mice. In this case, both the clonal and nonclonal CD8\(^+\) T cells divided rather more frequently in young than old mice (Table II). Perhaps this difference between old and young recipients is due to the fact that older mice contain more dividing CD8\(^+\) T cells than young animals do (25) and these may compete for stimulatory factors with the transferred cells.

Thus, like nonclonal CD8\(^+\) memory T cells, most members of the CD8\(^+\) clones are capable of and do divide in both young and old animals, suggesting that their behavior is an intrinsic property of the clones.

**Clonal CD8\(^+\) cells divide more quickly and accumulate faster than nonclonal cells**

The CFSE profiles and cell recoveries between days 6 and 36 after transfer in the experiments illustrated in Fig. 2 allowed an estimate of the rate of cell division and numbers of recovered CD8\(^+\) clonal and nonclonal cells in the young recipients. In an average of four experiments, the recovered clonal CD8\(^+\) cells had divided approximately every 15 days, whereas the recovered nonclonal CD8\(^+\) T cells had divided approximately every 22 days.

To find out whether the clonal or nonclonal cells had actually increased in number over the course of these experiments, we counted the total numbers of clonal or nonclonal CFSE labeled CD8\(^+\) T cells recovered from mice 6 and 36 days after transfer. The results of such experiments are shown in Fig. 3. For three of four CD8\(^+\) clones, there was a dramatic increase in cell number between days 6 and 36. This was not true for the nonclonal cells, which were found in approximately equal numbers per mouse 6 and 36 days after transfer.

For both the clonal and nonclonal CD8\(^+\) T cells, the increase in numbers was less from 6 to 36 days than would have been predicted from the average division rates. Therefore, progeny from both groups must also be dying during the expansion. However, overall, the interplay between division and death leads to a net expansion of the clonal CD8\(^+\) T cells, but not of the nonclonal memory CD8\(^+\) T cells.
Survival and division of clonal CD8+ T cells is not class I MHC dependent

The long-term maintenance of normal CD8+ memory T cells is well documented. In the normal immune response, following a sometimes extremely large Ag-driven expansion, most progeny CD8+ T cells disappear leading to a steady state in which a residual population of memory T cells is maintained, often for the life of the animal (16–22, 43, 44). Several experiments have shown that, unlike naive or actively responding CD8+ T cells, this persistent memory population is not dependent on Ag or even MHC class I for its survival (24, 45–47). However, as discussed above, these memory T cells do maintain their number by an equilibrium between slow cell division and cell death. Recently, we have shown that their division and survival is dependent on IL-15 and inhibited by IL-2 (25, 26).

Our experiments led us to hypothesize that the clonal CD8+ T cells were in fact derived from memory T cells through some alteration that allowed them to successfully compete with normal memory T cells during this maintenance phase. If this were the case, we predicted that the survival and expansion of the clonal cells should also be independent of MHC class I, dependent on IL-15, and inhibited by IL-2. Alternatively, these cells to be

Table I. A large percentage of the CD8+ clonal cells in old mice are dividing

<table>
<thead>
<tr>
<th>Donor</th>
<th>Cell Type</th>
<th>Expt.</th>
<th>Days on BrdU Water</th>
<th>Percent of CD8+ Cells Labeled With BrdU</th>
<th>Clonal</th>
<th>Nonclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD17</td>
<td>Vβ8.3</td>
<td></td>
<td>23</td>
<td></td>
<td>54.7</td>
<td>55.5</td>
</tr>
<tr>
<td></td>
<td>Clone</td>
<td>2</td>
<td>21</td>
<td></td>
<td>40.4</td>
<td>28.4</td>
</tr>
<tr>
<td>BD18</td>
<td>Vβ2 Clone</td>
<td>2</td>
<td>21</td>
<td></td>
<td>54.7</td>
<td>33.9</td>
</tr>
</tbody>
</table>

* Two 26-mo-old B6D2F1 mice were given BrdU in their drinking water for the indicated time. In one of the mice, 54% of the CD8+ T cells bore Vβ8.2 (Expt. 1) and, in the other, 50% of the CD8+ T cells bore Vβ8.3 (Expt. 2). LN and spleen T cells were isolated and stained with PE-anti-C8D, and the CD8+ cells were purified by sorting. The CD8+ T cells were then stained with biotinylated Ab to the Vβ expressed on the clone and SA-V-CyC, acid-treated, and stained with FL-anti-BrdU. BrdU-labeled cells were identified by comparing the histogram profiles of T cells from mice given BrdU with those of mice that had not been given BrdU.

Table II. CD8+ clonal and nonclonal cells from old mice divide more frequently in young recipients than in old recipients

<table>
<thead>
<tr>
<th>Donor</th>
<th>Cell Type</th>
<th>Percent ± SEM of Cells That Had Not Divided By 21 Days After Transfer Into Recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD17</td>
<td>Vβ8.3</td>
<td>9.3 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Clone</td>
<td>34.4 ± 13.3</td>
</tr>
<tr>
<td>BD18</td>
<td>Vβ2 Clone</td>
<td>11.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>23.8 ± 0.9</td>
</tr>
<tr>
<td>BD17</td>
<td>Nonclonal</td>
<td>29.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>56.5 ± 17.7</td>
</tr>
<tr>
<td>BD18</td>
<td>Nonclonal</td>
<td>54.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Young</td>
<td>63.3 ± 1.0</td>
</tr>
</tbody>
</table>

* T cells were purified from two 26-mo-old B6D2F1 mice that contained CD8+ clones bearing identifiable Vβs. In BD17, 59% of the CD8+ T cells bore Vβ8.3; in BD18, 29% of the CD8+ T cells bore Vβ2. The T cells were labeled with CFSE and transferred i.v. into two sets of three nonirradiated syngeneic recipients which were either 2-mo-old (young) or 17-mo-old (old). T cells were isolated and analyzed 21 days after transfer as described in Fig. 2. Results shown are the means and SE of the mean of three identical recipients.

FIGURE 2. Clonal and nonclonal CD8+ T cells from old mice divide in nonirradiated syngeneic young mice. T cells from a 19-mo-old C57BL/6 mouse and from another 19-mo-old C57BL/6 mouse that did not contain a detectable CD8+ clone were labeled with CFSE. Each set of T cells was transferred i.v. into four nonirradiated C57BL/6 young mice such that each recipient was given 10^7 T cells. The recipients were sacrificed at various times after transfer. For recipients of cells from the old mouse containing the CD8+ clone, T cells from spleens and LNs were stained with bio-anti-Vβ8.2 plus SA-V-CyC and PE-anti-C8D. For recipients of T cells from the other old mouse or the young mouse, cells were stained similarly except for the substitution of anti-Cβ for anti-Vβ8.2. For all samples, CFSE fluorescence was detected in the FL-1 channel on the FACScan. Data shown are the CFSE fluorescence profiles of the cells which were as follows: A, Vβ8.2+ CD8+ clonal cells from an old mouse; B, Vβ8.2- CD8- nonclonal cells from the old mouse, which also contained the Vβ8.2+ CD8+ clone; C, Cβ+ CD8- nonclonal cells from an old mouse, which did not contain a large CD8+ clone; D, Cβ- CD8+ cells from a young mouse. Data shown are typical of three independent experiments with four different CD8+ clones (one donor contained two CD8+ clones).
maintained by some chronically presented Ag, they should be dependent on MHC class I and perhaps IL-2 as well.

We performed several experiments to test these ideas. First, we transferred T cells from old mice into β2MKO animals. APCs in these recipients express class I MHC very poorly and hence are unlikely to present Ag to CD8\(^+\) T cells. Unfortunately, CD8\(^+\) T cells from normal animals were rejected very rapidly from normal β2MKO mice (data not shown) because of recognition of class I MHC protein on their surfaces by host T cells (48, 49). Hence, as an alternate approach, the experiments were performed in β2MKO animals that had been T cell depleted, lethally irradiated, and reconstituted with T cell-depleted β2MKO bone marrow 4 days before transfer of the T cells to be tested. Control C57BL/6 recipients were treated similarly. Because transfer into irradiated mice causes the immediate, short term proliferation of donor T cells (50, 51), the fate of the transferred cells was not evaluated until months later, after the effects of irradiation had waned.

An example of such an experiment is shown in Fig. 4. T cells were purified from an old C57BL/6 mouse that contained a CD8\(^+\) clone bearing V\(\beta\)6. These cells were transferred into the reconstituted normal and β2MKO C57BL/6 mice and growth of the clone was evaluated by sampling peripheral blood at intervals thereafter. After a short recovery period in the uninjected reconstituted C57BL/6 mice, normal host CD8\(^+\) V\(\beta\)6 T cells rose to a steady level of about 4% of PBL. In the uninjected reconstituted β2MKO mice, of course, there were virtually no CD8\(^+\) V\(\beta\)6 T cells (Fig. 4B, ○).

It was clear that the donor CD8\(^+\) V\(\beta\)6 clonal cells grew in both of these types of recipients. Because in both types of recipients, the clone took a long time to reach a percentage that was detectable by anti-V\(\beta\) staining, there was no evidence that its proliferation was unduly affected by the irradiation. In the C57BL/6 recipients, the clonal cells reached a level in PBL up to 3-fold higher than that of the host V\(\beta\)6 CD8\(^+\) cells in the uninjected host (Fig. 4A, compare ○ and ○). The clonal cells increased in percentage in the PBL of the β2MKO recipients as well (Fig. 4B, ●). After subtraction of the 4% background due to host V\(\beta\)6 CD8\(^+\) T cells in the wild-type recipients, comparison of the rates of appearance of the V\(\beta\)6 CD8\(^+\) clonal cells revealed that they accumulated a little more slowly in β2MKO hosts than they did in normal mice. Perhaps this reflects some survival advantage conferred on the T cells by expression of class I in the wild-type recipients.

To measure the actual number of the clonal cells rather than just their percentages in PBL, recipient mice were sacrificed about 200 days after transfer and their LN and spleen cells were pooled. Three types of cells were analyzed. From the β2MKO mice both CD8\(^+\) V\(\beta\)6 and CD8\(^+\) V\(\beta\)6 donor T cells were analyzed, because the presence of class I on the donor cells allowed us to distinguish them accurately from the host cells. In the case of the C57BL/6 recipients, only the greatly expanded CD8\(^+\) V\(\beta\)6 cells were analyzed, because we could not accurately distinguish the donor CD8\(^+\) V\(\beta\)6 cells from the cells already present in the host. The results of transfer of four different clones in four independent experiments (I-IV) are given in Fig. 5.

All four CD8\(^+\) clones expanded in the C57BL/6 recipients (Fig. 5A) with sometimes greater than 10-fold more cells recovered than injected. Given that only 5–10% of the cells survive the initial injection, this is a very dramatic expansion. Three of the four CD8\(^+\) clones also expanded similarly in the β2MKO recipients (Fig. 5B). In the case of the other clone (III), although fewer cells were recovered than injected, again this probably represents a net expansion, given the low initial survival of the injected cells. The nonclonal CD8\(^+\) T cell also survived in the β2MKO recipients (Fig. 5C), but their expansion was on average less dramatic than seen with the clonal cells. Overall these results indicate that the clones, like nonclonal memory CD8\(^+\) T cells, do not need host-presented class I MHC (or, by inference, Ag) to survive and divide.

Division of clonal CD8\(^+\) T cells is IL-15 dependent and inhibited by IL-2

In mice, the continuous division of CD8\(^+\) T cells of memory phenotype is driven by IL-15 and inhibited by IL-2 (25, 26). To find out whether this is also true for the members of the CD8\(^+\) clones, T cells were isolated from three old mice, each with an identifiable CD8\(^+\) clone. The cells were labeled with CFSE and cells from...
to the animals was confirmed by the fact that the cells isolated from the animals stained poorly or not at all with Abs against the appropriate receptors (data not shown). Division of the transfected cells in the animals was assessed from their CFSE profiles after harvesting. The results for cells transferred from one of the old donors are shown by Fig. 6.

As we have previously reported, division of the nonclonal CD8^+ T cells was inhibited by treatment with anti-IL-2Rβ and stimulated by anti-IL-2 plus anti-IL-2Rα (25). Similar results were observed for the members of the CD8^+ clone. During this relatively short experiment, about one third of the surviving transfected clonal cells had divided in the recipient given normal rat Ig. This result was unaffected by anti-IL-7 plus anti-IL-7Rα F(ab)'2. The presence of anti-IL-2Rβ (Fab)'2 inhibited division of the clone. This was due to blockage of signals delivered by IL-15 rather than IL-2, because specific inhibition of IL-2 with anti-IL-2 plus anti-IL-2Rα (Fab)'2 actually stimulated division by the clone. As summarized in Table III, similar results were obtained with cells from two other old mice. In all cases, blockage of IL-2 increased the rate of appearance of dividing clonal cells and anti-

### Table III. Accumulation of proliferating CD8^+ clonal cells is stimulated by IL-15 and inhibited by IL-2^2

<table>
<thead>
<tr>
<th>Mouse ID</th>
<th>Cell Type</th>
<th>Rat IgG</th>
<th>Anti-IL-2 + Anti-IL-2Rβ F(ab)'2</th>
<th>Anti-IL-7 + Anti-IL-7Rα F(ab)'2</th>
<th>Anti-IL-2Rβ F(ab)'2</th>
</tr>
</thead>
<tbody>
<tr>
<td>B654</td>
<td>Vβ5^+ clone</td>
<td>14.6</td>
<td>70.2</td>
<td>12.4</td>
<td>4.9</td>
</tr>
<tr>
<td>B654</td>
<td>Vβ8.2^+ clone</td>
<td>22.8</td>
<td>75.5</td>
<td>18.3</td>
<td>7.0</td>
</tr>
<tr>
<td>B655</td>
<td>Vβ8.3^+ clone</td>
<td>28.6</td>
<td>67.0</td>
<td>19.6</td>
<td>20.6</td>
</tr>
<tr>
<td>NIA07</td>
<td>Vβ3^+ clone</td>
<td>31.9</td>
<td>84.2</td>
<td>36.0</td>
<td>10.9</td>
</tr>
<tr>
<td>B654</td>
<td>Nonclonal</td>
<td>14.3</td>
<td>100.0</td>
<td>19.8</td>
<td>10.0</td>
</tr>
<tr>
<td>B655</td>
<td>Nonclonal</td>
<td>20.5</td>
<td>66.7</td>
<td>21.5</td>
<td>14.2</td>
</tr>
<tr>
<td>NIA07</td>
<td>Nonclonal</td>
<td>37.7</td>
<td>76.1</td>
<td>39.8</td>
<td>13.1</td>
</tr>
</tbody>
</table>

^Mice were treated and their cells were analyzed as described in Fig. 6. Shown are the percentages of transferred, CFSE-labeled, nonclonal CD8^+ T cells that had divided after transfer.
IL-2Rβ (Fab′)2 inhibited division of the clones. Inhibition was not complete. This may either have been due to incomplete blockage of the IL-15R by the anti-IL-2Rβ or to stimulation of growth of the clones by factors in addition to IL-15. However, overall, these results showed that division of the clonal cells, like that of the nonclonal memory CD8+ T cells, is stimulated, at least in part, by IL-15 and inhibited by IL-2.

**Discussion**

The results described here show that the clonal CD8+ T cells have properties that are very similar to those of nonclonal memory CD8+ T cells. The profiles of surface proteins on the two types of cells are similar, with both bearing high levels of CD44 and amounts of IL-2Rβ and CD28, which are increased by comparison with naive cells. Previous experiments and the data reported here indicate that CD8+ memory T cells divide in the absence of Ag. It appears that the clonal CD8+ T cells can do this too, because they expand in β2-MKO mice, animals that should not be able to present Ag to these cells. As for their nonclonal counterparts, division of the clonal cells is inhibited by anti-IL-2Rβ and stimulated by anti-IL-2 plus anti-IL-2Rα. Anti-IL-2Rβ inhibits the action of IL-2 and IL-15 equally well (data not shown). However, the inhibitory effects of anti-IL-2Rβ must be due to inhibition of IL-15 action, because inhibition of IL-2 action increased the appearance of dividing clonal cells. Thus, like their nonclonal counterparts, division of the CD8+ clones is stimulated by IL-15. This conclusion is supported by the fact that both types of cells contain high levels of Bcl-2, a protein that is induced in T cells by IL-2 and IL-15.

The inhibition experiments described here indicate the presence of both IL-15 and IL-2 in our old and young pathogen-free mice. IL-15 is made constitutively in animals (60), but IL-2 is present only in infections; IL-15 and IL-2 are present in both old and young mice. IL-15 is made constitutively in animals (60), but IL-2 is present only in infections; IL-15 and IL-2 are present in both old and young mice. Anti-IL-2Rβ plus anti-IL-2Rα inhibits the action of IL-2 and IL-15 equally well (data not shown). Preliminary results suggest that IL-2 may operate in vivo via the IL-2 dependent CD25+ regulatory cells that have been described by others (data not shown, and Refs. 56–59).

The inhibition experiments described here indicate the presence of both IL-15 and IL-2 in our old and young pathogen-free mice. IL-15 is made constitutively in animals (60), but IL-2 is thought to be produced only by activated T cells, so its source in these animals is not clear. Because the clonal cells are thought to be relatively nonproductive, the clonal cells probably do not make the IL-2 themselves. A recent paper showed that IL-2 is bound to extracellular matrix, even in animals that are not overtly confronting with Ag (61), so that IL-2 made at earlier times may be stored and active at this site.

What is it that allows some CD8+ T cells to grow into the very large clones at the expense of the other CD8+ T cells in older animals? The data presented in this paper show that after transfer into normal recipients, the clones increase in numbers whereas the nonclonal cells do not, even though some of these latter cells are in fact dividing. Thus either the clonal T cells divide more rapidly or are less likely to die than the normal memory phenotype T cells. Our data do not allow us to distinguish these two possibilities. In our CFSE-labeling experiments, the clonal cells do appear on average to have been through more rounds of division at any time point than the nonclonal T cells. However, we cannot tell whether this is due to an intrinsically faster division rate or to the higher probability of survival of proliferating clonal vs nonclonal T cells.

What molecular changes might account for these differences between clonal and nonclonal CD8+ T cells? One possibility is that the clonal T cells are more sensitive to IL-15. In preliminary experiments in vitro, where we do not see the inhibitory effects of IL-2, the clonal cells outgrow their nonclonal counterparts in cultures containing IL-15 (data not shown). It seems unlikely that this is due to differences in surface expression of IL-2Rβ because the clonal cells have only slightly more of this protein (186 ± 16 in arbitrary fluorescence units) than the nonclonal cells (179 ± 9).

However, perhaps this small difference or differences in other components of the receptor or its downstream signaling molecules account for the behavior of the two types of cells.

The successful competition of the clonal CD8+ T cells for apparently the same niche as that occupied by normal memory CD8+ T cells raises the possibility that they may inhibit the normal maintenance of memory T cells. We have no direct evidence for this possibility at the moment, although the observation that the clonal and nonclonal cells grow more slowly in old than in young recipients suggests, very indirectly, that some sort of competition for growth factors may occur in vivo. Alternatively, the susceptibility of the clonal cells to the inhibitory effects of IL-2 raises another intriguing possibility. These cells may act as a decoy for this inhibitory mechanism and in fact protect normal memory CD8+ T cells.

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