Antigen-specific T-T interactions regulate CD4 T-cell expansion

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The regulation of CD4 T-cell numbers during an immune response should take account of the amount of antigen (Ag), the initial frequency of Ag-specific T cells, the mix of naive versus experienced cells, and (ideally) the diversity of the repertoire. Here we describe a novel mechanism of T-cell regulation that potentially deals with all of these parameters. We found that CD4 T cells establish a negative feedback loop by capturing their cognate major histocompatibility class (MHC)/peptide complexes from Ag-presenting cells and presenting them to Ag-experienced CD4 T cells, thereby inhibiting their recruitment into the response while allowing recruitment of naive T cells. The inhibition is Ag specific, begins at day 2 (long before Ag disappearance), and cannot be overcome by providing new Ag-loaded dendritic cells. In this way, CD4 T-cell proliferation is regulated in a functional relationship to the amount of Ag, while allowing naive T cells to generate repertoire variety. (Blood. 2008;112:1249-1258)

Introduction

Because the frequency of T cells specific for any antigen (Ag) is low, T-cell proliferation is an important part of primary immune responses. However, proliferation must stop at some point to allow Ag-specific T cells to become effectors (and to accommodate a limited body size). In a typical primary immune response, CD4 T cells proliferate extensively and generate effector cells before a contraction phase sets in. The extent of proliferation depends upon the initial frequency of the Ag-specific T cells and is therefore much greater for naive T cells than for the more numerous memory T cells.1-3 Because memory and naive cells reach the same plateau, even though memory cells respond faster, there must be mechanisms that regulate T-cell proliferation early during an immune response.4,5 It has been suggested that T-cell proliferation is related to the disappearance of Ag or antigen-presenting cells (APCs).6-8 to exhaustion of the APCs,9 to suppression by regulatory T cells,10 or to competition among responding T cells.11-14 However, disappearance or exhaustion of APCs or Ag should generate higher final plateaus for the faster-responding memory T cells; and pure competition for waning antigen-presenting cells and presenting them to Ag-experienced CD4 T cells, thereby inhibiting their recruitment into the response while allowing recruitment of naive T cells. The inhibition is Ag specific, begins at day 2 (long before Ag disappearance), and cannot be overcome by providing new Ag-loaded dendritic cells. In this way, CD4 T-cell proliferation is regulated in a functional relationship to the amount of Ag, while allowing naive T cells to generate repertoire variety. (Blood. 2008;112:1249-1258)

Methods

Mice

Marilyn TCR-transgenetic Rag2−/− mice are specific for the Dby-H-Y male antigen presented by A8.16 For Marilyn T cells expressing diphtheria toxin receptor (DTR), we crossed Marilyn Rag2−/− CD45.1 mice to Lat-DTR knockin mice (harboring, in the 3′ untranslated region of the Lat gene, a human diphtheria toxin receptor (DTR) cassette, driven by an internal ribosomal entry site [A.K. and B.M., manuscript in preparation]) to finally obtain Marilyn-Lat-DTR.Rag2−/− CD45.1/2 mice. B6-OT-II19 and B6 mice were from CRL (Charles River Laboratories, L’Arbesle, France). Live animal experiments were done according to French Veterinary Department guidelines.

Cell preparation

Effector and memory T cells were generated in vivo as described,19 by transferring 10⁵ Marilyn or OT-II LN cells into CD3ε−/− hosts together with 3 × 10⁹ mitomycin-treated male spleenocytes or LPS-matured bone marrow dendritic cells (DCs) loaded with OVA323-339 peptide (Neosystem, Strasbourg, France), respectively. Effector or memory T cells were purified from the spleen and lymph nodes 5 days later or at least 6 weeks later, respectively, using anti-CD45.1-PE antibody and anti-PE microbeads. Such positive purification does not alter T-cell behavior (Figure S1, available on the Blood website; see the Supplemental Materials link at the top of the online article).

Naive, effector, or memory T cells were labeled with 5 μM CFSE (Invitrogen, Carlsbad, CA) in PBS containing 0.1% BSA, for 8 minutes at 37°C.

Depletion experiment and flow cytometry

To deplete Marilyn-Lat-DTR-Rag2−/− T cells in vivo, we injected 500 ng diphtheria toxin (DT; List Biological Laboratories, Campbell, CA) intravenously twice, 8 hours apart, the day before the second transfer.

Four- to 8-color cytometry was performed with directly conjugated antibodies: anti-CD45.1-phycocerythrin (PE), anti-Vß6-PE, anti-Vα2-PE,
anti-Brdu-PE, anti-CD62-L-PE, anti-CD44-PE, anti-IA-ß-PE, anti-CD45RB-PE, anti-ßTCR-chrome, anti-CD45.2-peridinin chlorophyll protein (PerCP)–cyanin 5.5 (Cy5.5), anti-CD69-biotin, anti-CD44-biotin, anti-VA2-biotin, and anti-Vß6-biotin; biotinylated antibodies were revealed with streptavidin-allophycocyanin APC/APC-Cy7 (APC or APC-Cy7; Pharmingen, San Diego, CA), and analyzed using FacsCalibur, Aria, or LSRII flow cytometers (Becton Dickinson, San Jose, CA).

**Generation of dendritic cells and immunizations**

Bone marrow–derived dendritic cells (BMDCs) were generated by 10- to 15-day culture in granulocyte-macrophage colony-stimulating factor (GM-CSF)–containing conditioned medium as previously described, matured by 20-hour treatment with 10 µg/ml lipopolysaccharide (LPS; Sigma-Aldrich, St Louis, MO), pulsed with 40 nM Dby (NAGFN-SNRAASSRSS) peptide for 2 hours, and washed 3 times before injection. This peptide dose is just at plateau for in vitro proliferation and at 30% of maximum for IFN-γ production (Figure S2G and H, respectively).

In some experiments (Figure 4A), BM was obtained from mice expressing a fusion protein of Ab-GFP.21 BMCs were injected into the footpads of both hind feet of female B6 mice that had been injected intravenously the day before with 10^6 LN cells from CD45 congenic Marilyn mice.

**In vitro experiments**

CFSE-labeled Dby-loaded BMDCs (2 × 10^6) and CD45.2 Marilyn T cells (2 × 10^6) were cocultured overnight in RPMI-1640 plus 10% fetal calf serum in 6-well plates, negatively sorted (CD11c–/CFSE–) by fluorescence-activated cell sorting (FACS), and fixed with PBS plus 0.01% glutaraldehyde on ice for 1 minute (stopped with 0.2 M glycine-PBS). Fixed Marilyn T cells (5 × 10^5) were then incubated for 1 or 3 days with 2 × 10^5 CD45.1 CFSE-labeled naive or memory Marilyn cells that had been purified by FACs-based negative selection (CD11c–) for the naive cells from which DCs had been removed by FACs-based negative selection; CD11c–/CD45.2– for the naive cells; and CD11c+/CD45.2+ for the memory cells. DC contamination was less than 0.02%.

**Results**

**Naive and memory CD4 T cells are differentially recruited into ongoing immune responses in vivo**

To unravel the mechanisms controlling CD4 T-cell expansion during a localized immune response, where new and returning T cells continuously enter the draining lymph node (LN), we measured the responses of a monoclonal population of H-Y-specific CD4 T cells (from the TCR transgenic [Tg] Rag2–/– Marilyn mouse)16 that were transferred into normal female B6 mice 1 day before immunizing the recipients in the footpad with LPS-activated female bone marrow–derived dendritic cells (DCs) loaded with the H-Y peptide. To track cell divisions, we labeled the transferred Marilyn T cells with CFSE, and to discriminate them from host cells, we used CD45.1 Marilyn cells and CD45.2 hosts (Figure 1A). For naive cells, we used LN cells from Marilyn mice. For Ag-experienced T cells, we transferred naive Marilyn T cells together with H-Y–expressing male cells into CD3ε–/– hosts and recovered them after 5 days (effector cells) or more than 6 weeks (memory cells) later.16

When transferred 1 day before priming, both naive and memory T cells proliferated extensively, then decreased in number (Figure 1B). This proliferation was Ag specific, as there was no response to DCs not bearing H-Y peptide (Figure S2B and Joncker et al22). As expected, the memory cells peaked 1 to 2 days earlier than naive cells, and returned to their original numbers, whereas naive cells returned to levels somewhat higher than the input number.

During a typical response, early arriving cells are stimulated, divide, and then migrate to B-cell follicles or leave the node to pursue their effector function. Both new and recirculating cells can also arrive at later times. To track the fate of later arriving T cells, which might arrive when the Ag-specific cell numbers are contracting, we injected a second cohort of naive or memory CFSE-labeled T cells 6 days after priming and studied their phenotype at day 12 (Figure 1C). In the absence of a first cohort of responding T cells (where the low frequency of host H-Y–specific CD4 T cells are likely to be expanding), naive, effector, and memory T cells all responded (Figure 1C top panels). However, the presence of a first cohort (Figure 1C bottom panels) radically changed the situation. The late-arriving naive T cells proliferated less (56% of the cells did not divide at all, compared with 21% in the absence of a first cohort), and memory and effector T cells were almost completely inhibited. Thus, the presence of a first cohort of Tg CD4 T cells had a major effect on the proliferation of CD4 T cells that arrived later, especially on effector and memory cells (even if the naive and memory cells were transferred together and stimulated in the same LN; Figure S3). Why were the late-arriving cells responding less well?

The simplest possibility was that they had run out of Ag. LPS-activated DCs are thought to last only a few days.23 Thus there might have been a rapid decay of Ag to a level too low to sustain T-cell proliferation. To test this, we examined the kinetics of available MHC-II/H-Y peptide complexes by transferring new naive CD45.1 Marilyn cells at different time points after the injection of DCs, and measuring their proliferation. Figure 1D shows that the Ag persisted at least 18 days, even when the frequency of responding T cells was increased at day 0 by injecting 10^6 Marilyn cells.

Another possibility was that our memory populations were somehow inexplicably less responsive to Ag than the naive cells. Figure 1E shows that this was not the case, as naive and memory cells responded similarly in vivo to DCs loaded with a titration of peptide doses.

**Physiologic numbers of endogenous T cells inhibit memory T-cell proliferation**

It has been suggested that low proliferative responses can be artifacts of nonphysiologically large numbers of Ag-specific cells resulting from adoptive transfer of TCR-Tg T cells.11,13 To check whether the lower proliferation of late-arriving cells was due to the large number (10^6) of first-cohort Marilyn cells injected at day 0, we compared the responses of naive and memory Marilyn cells injected at different times into normal B6 female hosts, which have very low frequencies of endogenous CD4 T cells specific for H-Y. The patterns were the same. Marilyn cells injected at time 0 proliferated extensively, most of them dividing more than 6 to 7 times, and completely diluting the CFSE dye (Figure 2Ai,ii), but cells injected at day 6 proliferated much less, and once again the memory cells divided fewer times than the naive cells (Figure 2Ai,iv). To lower the endogenous frequency of H-Y–specific cells even further, we used OT-II hosts on a normal B6 background, which have sufficiently large numbers of T cells in the periphery to prevent space-induced expansion but, because 95% of their CD4 T cells are specific for ovalbumin, have a 20-fold lower frequency of anti-H-Y T cells compared with normal B6 females. In these hosts, both naive and memory Marilyn T cells injected at day 6 proliferated better than in normal B6 hosts, although not as well as...
Physiologic numbers of Tg T cells preferentially inhibit memory T-cell proliferation

Having checked for the effect of physiologic numbers in the first cohort, we turned to the second. We decreased the number of responding T cells by 200-fold, transferring only 5000 cells in the first and second cohorts to generate a specific T-cell frequency of approximately 0.001%. We injected the second cohort of T cells 6 days after priming and examined their proliferation pattern 8, instead of 6, days later, to account for the smaller inoculum size. In the absence of the first cohort, both naive and memory cells proliferated extensively, only 1% to 6% of cells remained undivided (Figure 2B left panels), but once again the presence of a first cohort preferentially inhibited the memory cells (Figure 2B right panels). Thus, the preferential inhibition of memory T-cell proliferation is observed with cell frequencies within the physiologic range.

The preferential inhibition of Ag-experienced T-cell proliferation occurs with endogenous APCs

Another potentially nonphysiologic aspect of our studies concerned the use of bone marrow–derived DCs, which may not represent any normal in vivo DC population. We therefore designed an experiment where the transferred T cells would encounter Ag on normal endogenous APCs. We used an MCA101 tumor cell line, transfected with DBY-H-Y,25 which cannot directly present to Marilyn Tg T cells, as indicated. Antigen persists in vivo at least 18 days. H-Y peptide–loaded LPS-matured DCs were injected into the footpad of female B6 mice, previously injected (right panels) or not (left panels) with naive CD45.1 CFSE-labeled Marilyn LN cells. At the indicated time points, naive CD45.1 CFSE-labeled Marilyn LN cells were injected intravenously. Dot plots of gated TCR CD45.1+ cells from the DLN were representative of at least 3 experiments with 2 mice each per group. The percentage of undivided cells, among total Tg Marilyn T cells, is indicated. (D) Antigen persists in vivo at least 18 days. H-Y peptide–loaded LPS-matured DCs were injected into the footpad of female B6 mice, previously injected (right panels) or not (left panels) with naive CD45.2 CFSE-labeled Marilyn LN cells. At the indicated time points, naive CD45.1 CFSE-labeled Marilyn LN cells were transferred intravenously. Dot plots of gated TCR CD45.1+ cells from the DLN injected at the indicated time after DC priming and analyzed 6 days later. Representative of 2 independent experiments with 2 mice per group for each one. (E) Functional avidity measurement in vivo. Naive (106) or memory (2 × 106) CD45.1 CFSE-labeled Marilyn cells were injected intravenously into B6 mice, which were then primed by injection into the footpad of 106 LPS-matured DCs loaded with the indicated amount of H-Y peptide. Five days later, the number of naive and memory T cells recovered in the DLN was measured.
memory cells (88% undivided memory vs 39% undivided naive cells). Overall, therefore, the inhibition of proliferation due to an ongoing immune response seems to be a physiologic effect produced by normal endogenous T cells and normal endogenous APCs.

Inhibition requires the continued presence of the first cohort of T cells

To determine the mechanism by which memory T cells are preferentially inhibited, we began by asking if the early-arriving T cells must be continuously present. As a first cohort, we injected Marilyn T cells expressing a DTR (A.K. and B.M., manuscript in preparation) into OT-II recipients (to minimize H-Y–specific host T cells, which would be resistant to DT), primed them 1 day later with H-Y-DCs, and killed them 5 days after that with an injection of DT, which induced approximately 90% depletion of the first cohort. On day 6, we injected a second cohort of CFSE-labeled naive or memory Marilyn cells. As usual, both naive and memory T cells proliferated well in the absence of a first cohort (Figure 3Ai,ii), whereas the presence of a first cohort moderately inhibited the naive cells (Figure 3Ai iii) and severely inhibited the memory cells (Figure 3Ai v). Removing the first cohort a day before transfer of the second cohort almost completely restored the proliferation of both the naive and memory inocula (Figure 3Av,vi). Thus, although some Ag is certainly stripped from the APCs by the early-responding T cells,26 the release of inhibition when the early-responding T cells are removed suggests that decreases in APC-associated Ag cannot alone account for the inhibition of proliferation of later-arriving memory T cells.

The inhibition is Ag specific

It has been shown in several systems that CD4 T cells can influence other T cells by educating,27 licensing,28,29 suppressing,10,30 or otherwise modifying Ag the APCs they bind to. In these cases, the Ag specificity of the “educator” T cell needs not be the same as that of the T cell that is ultimately influenced. To determine whether the early-responding T cells inhibit late-arriving memory T cells by modifying the APCs, we asked whether the effect was Ag specific. We added a second TCR-Tg T cell with a different Ag specificity but the same restriction element: OT-II (specific for OVA/Ab). We transferred either Marilyn or OT-II into CD45.1 B6 hosts, then immunized with DCs loaded with both OVA and H-Y peptides. After 6 days, we transferred a mixture of CFSE-labeled Marilyn and OT-II cells (both naive or both memory) and analyzed them 6 days later, gating for CD45.2-V60hi (Marilyn) or CD45.2-V60lo (OT-II).

If the inhibitory effects were mediated by DC modifications, or by soluble factors, then ongoing responses to either OVA or H-Y should inhibit both the OT-II and Marilyn T cells in the second cohort. However, this was not the case; a first cohort of Marilyn T cells had no effect on OT-II cells (Figure 3Bvi,vi compared with 3Bi,ii), although it inhibited the proliferation of a second cohort of Marilyn cells (Figure 3Bvi-viii and 3Bi,iv). Similarly, a first cohort of OT-II T cells (Figure 3Bix-xii) inhibited proliferation of OT-II but not of Marilyn cells. As before, memory T cells were inhibited more strongly (Figure 3Bviii,x). These results show that the inhibitory effect of early-responding T cells is (1) generalizable to more than one T-cell clone; (2) not due to global modification of the DCs or the LN environment, and (3) not due to interclonal competition. Finally, in the absence of a first cohort, memory T cells can respond to the APCs, showing that the preferential inhibition of memory T cells is not due to an inability to functionally encounter the APCs.
Activated CD4 T cells capture and present MHC-II in vivo during an ongoing immune response

Although known for decades,\textsuperscript{31,32} it was recently rediscovered that T cells can specifically acquire MHC/peptide complexes from the cells with which they interact.\textsuperscript{26,33-35} In vitro, Ag presentation by T cells can induce activation of naive T cells versus anergy of activated T cells.\textsuperscript{35} In vivo, both inhibition\textsuperscript{36} and activation\textsuperscript{37} have been seen. If the presentation of captured complexes by T cells resulted in an inhibition of proliferation, one would see exactly the sort of inhibition that we had seen.

We therefore asked whether early-responding Marilyn T cells might acquire MHC-II molecules. We injected naive CD45.1 Marilyn T cells into CD45.2 recipients 20 hours before injecting H-Y-DCs carrying a GFP fusion protein of MHC-II-Ab.\textsuperscript{21} By day 2, the activated Marilyn T cells had captured a considerable amount of MHC-II from the DCs (Figure 4Ai) and were displaying these captured molecules on the cell surface, as evidenced by the staining with anti-Ab monoclonal antibody (Figure 4Aiii,iv), although approximately 50-fold less per cell than did the DCs (Figure 4Av), measured by GFP mean fluorescence (MFI). The T cells did not pick up GFP-MHC-II molecules in the contralateral LN (Figure 4Aii,iv), to which the injected DCs did not travel (Figure 4Avi).

To determine whether the T cells displaying MHC-II were those that had been recently activated, we injected CFSE-labeled CD45.1 Marilyn T cells into CD45.2 recipients 20 hours before injecting H-Y-DCs carrying a GFP fusion protein of MHC-II-A\textsubscript{b}.\textsuperscript{21} By day 2, the activated Marilyn T cells had captured a considerable amount of MHC-II from the DCs (Figure 4Ai) and were displaying these captured molecules on the cell surface, as evidenced by the staining with anti-A\textsubscript{b} monoclonal antibody (Figure 4Aii,iv), although approximately 50-fold less per cell than did the DCs (Figure 4Av), measured by GFP mean fluorescence (MFI). The T cells did not pick up GFP-MHC-II molecules in the contralateral LN (Figure 4Aii,iv), to which the injected DCs did not travel (Figure 4Avi).

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Marilyn cells into CD45.2 hosts 20 hours before injecting WT H-Y-DCs. Two days later, all the activated Marilyn T cells displayed MHC-II molecules (Figure 4Bii). This suggested that, if capture and presentation of H-Y by Marilyn cells were the mechanism of inhibition, the inhibitory effect should be apparent very early in the response. To test this, we injected a second cohort of naive or memory Marilyn T cells at various times after initiating the primary immune response (Figure 4C). The inhibition of memory T-cell proliferation reached a maximum as soon as day 2. Thus far, these results indicate that responding CD4 T cells can capture MHC-II complexes during a primary immune response in vivo, and this capture correlates kinetically with the inhibition of memory T-cell recruitment.

Activated Marilyn CD4 T cells can capture and present MHC-II/peptide complexes in vitro

To test directly whether Marilyn T cells can capture and present H-Y peptides and inhibit memory T-cell proliferation, we first established an in vitro model. We incubated naive Marilyn cells overnight with H-Y peptide–loaded CFSE-labeled, LPS-matured BMDCs (H-Y-DCs), and then purified the T cells by FACS. Less than 0.02% contaminating DCs remained (Figure 5Ai), and most of these T cells expressed MHC-II molecules (“H-Y T cells,” Figure 5Aii). We then fixed the purified H-Y T cells to prevent any chemokine or cytokine secretion that might induce Ag-independent T-cell proliferation38 and used them as APCs for naive or memory CFSE-labeled Marilyn T cells. We measured CD69 expression (Figure 5B), at 24 hours as an early marker of T-cell activation, and proliferation at 3 days (Figure 5C). The captured MHC-II complexes did indeed contain the H-Y Ag, as the H-Y T cells stimulated both naive and memory Marilyn cells to up-regulate CD69 (Figure 5Biii,iv). Anti–MHC-II antibodies blocked this activation (Figure 5Aiv), showing that the presentation was MHC dependent. As controls, we used naive Marilyn T cells that had not encountered H-Y-DCs (Figure 5Bi,ii), or OT-II T cells that had captured MHC-II/OVA peptide complexes (Figure 5Bi), both of which were nonstimulatory.

Although the early induction of CD69 expression was similar for naive and memory T-cell responders, the consequences were different. For naive responders, stimulation by Ag-bearing T cells did not lead to efficient day-3 proliferation, although it prevented death (Figure 5Ci vs 5Cix), and allowed them later to respond to H-Y-DCs (Figure 5Cii-iv). In contrast, memory T cells were strongly inhibited, and died (Figure 5Cv vs 5Cxi) even in the presence of additional H-Y-DCs (Figure 5Cvi-viii vs Figure 5Cxx-xxii). The suppression depended on specific Ag presentation, as it was not induced by T cells that had not captured the H-Y Ag (unpulsed T cells; Figure 5Cxiv-xvi). Thus, in vitro, Ag-bearing T cells compete with Ag-loaded DCs and inhibit proliferation of memory CD4 T cells.

To determine whether the dominant inhibition over proliferation induced by new H-Y–loaded DCs is also true in vivo, we set up an ongoing immune response with a first cohort of T cells plus peptide-loaded DCs, and then injected fresh peptide-pulsed DCs into the original footpad, or into the contralateral foot at the same time as the second cohort of naive or memory Marilyn cells. Although the new H-Y-DCs rescued most of the proliferative capacity of late-arriving naive T cells (Figure 5D), they did not rescue memory cells, either in the original or in the distant node. Thus, the inhibitory effect dominates both in vitro and in vivo, and as expected for a T cell–mediated effect, it also circulates to distant nodes, although with a slight delay, allowing a few cells to escape for a small number of divisions.

Model for the regulation of CD4 T-cell expansion during an immune response

Figure 6 shows one way to account for all the experimental data: (1) At the onset of the immune response, naïve CD4 T cells (and also memory cells during secondary responses) are recruited and stimulated by activated Ag-bearing APCs. (2) These CD4 T cells capture their specific MHC-peptide complexes. (3) As the number of CD4 T cells bearing captured MHC-peptide complexes increases, they begin to outnumber the APCs, thereby increasing the probability that newly arriving T cells (and any T cells that have just been activated and need a second hit to continue proliferating39) will encounter them before meeting a proper professional APC.40 (4) The T-T interaction leads to inhibition of Ag-experienced but not naïve T cells.

Direct inhibition of memory T-cell proliferation by Ag-bearing T cells

To directly test the in vivo inhibitory capacity of T cells that have captured their specific Ag, we allowed Marilyn T cells to capture H-Y/MHC complexes from DCs in vitro, as in Figure 5B, sorted them to obtain pure T cells, and transferred them by footpad injection into adoptive hosts into which CFSE-labeled naïve or memory Marilyn T cells had been transferred beforehand (Figure 7A). As controls for any potential nonspecific effects of infusing activated T cells, we also used Marilyn cells that had been stimulated with anti-TCR, as these can capture MHC class II (Figure 5Aii) but not H-Y peptide. After a 6-day period to allow for T-T interactions in vivo, we injected fresh H-Y peptide–loaded DCs into the same footpad. Six days later, we examined T-cell proliferation (Figure 7B). Whereas naïve T cells proliferated in all conditions, memory T cells proliferated if they had interacted with anti-TCR–activated Marilyn T cells, and not with H-Y–bearing Marilyn cells. As the number of anti-TCR–activated T cells in the lymph node was approximately 3-fold higher than that of H-Y–activated cells, (Figure 5A) these results demonstrate that it is the presence of MHC-peptide complexes on the activated T cells that inhibits Ag-experienced T-cell proliferation.

Discussion

Altogether the data demonstrate that an ongoing immune response establishes conditions that preferentially inhibit the proliferation of Ag-experienced T cells. The inhibition is Ag specific and occurs very early in the immune response. Although late-arriving naive cells proliferate somewhat less because of some Ag loss from the APCs and because of intraclonal competition, effector/memory cells in the same LN and during the same time period are stopped by an active phenomenon, even in the presence of persisting or newly arriving Ag-presenting APCs.

This is not due to APC education27,41 because the inhibition is Ag specific and allows the DCs to present Ags to other memory T cells (Figure 3B), nor is it due to classical regulatory T cells, whose effects are not Ag specific.10 Neither, for 3 reasons, is this likely to be due to simple competition for Ag.11,12,42 First, competition should be most effective against naïve, rather than memory, T cells. Second, the inhibition is set up too quickly (by day 2; Figure 4C), when the original number of proliferating cells is still
low. Third, the injection of a new set of Ag-loaded DCs should increase the number of stimulatory APCs sufficiently to overcome the competition. Although it does increase the proliferation of naive CD4 T cells (Figure 5D), it does not for Ag-experienced cells. All of our data fit with the scenario that the inhibition is due to the capture of MHC/Ag complexes by the responding T cells and presentation of these complexes to new T cells. The capture is clearly measurable by day 2 after the initiation of a primary
response (Figure 4A), at which time the inhibition of memory cell proliferation is also clearly visible (Figure 4C). At this time, new T cells entering the lymph node would encounter 2 types of cells bearing their Ag: the APCs that entered from Ag-loaded tissues and the T cells that have captured MHC/Ag complexes from those and earlier APCs (Figure 6).

There are several ways in which experienced CD4 T cells might be preferentially inhibited. First, the Ag-presenting T cells might express inhibitory ligands for which only experienced T cells have receptors. Alternatively, because the Ag-bearing T cells present less Ag than do APCs, effector/memory T cells may more easily recognize them and be inhibited for lack of proper costimulation, or by the presence of general inhibitory signals. Importantly, this inhibition is likely operative on both Ag-experienced T cells newly entering the LN and on any previously activated naive T cells that require another hit to continue dividing and differentiating.8,39

This type of regulation is analogous to the Ag-specific feedback loop that stops B cells from responding once there is enough circulating antibody (a B cell is inhibited if it recognizes Ag already bound by free antibodies whose Fc portions bind to the FcγRIIB on the B-cell surface).24,43,44 Because T cells do not usually secrete their Ag-specific receptors, they need another way to communicate with each other to regulate their numbers. The capture and presentation of Ag is a unique Ag-specific feedback loop that is completely proportional to the number of responding T cells and the number of Ag-presenting APCs. Until the responding T cells begin to outnumber the APCs, proliferation continues. But as APC numbers drop (perhaps signaling the end of the infection) or as responding T cells proliferate (signaling that there are sufficient numbers), proliferation stops. This scenario is compatible with recent results showing an inverse relationship between CD4 cell frequency and proliferation/differentiation.13

Why should the inhibition preferentially target Ag-experienced T cells? There might be several biologic advantages. First, it allows for the continuous generation of a diverse immune repertoire, as new naive CD4 or CD8 T cells can still be recruited into the immune response at late time points and display good effector functions.12,45 Second, it allows for the recruitment of naive T cells able to recognize mutating pathogen epitopes. Third, it may allow a small number of initial responders to expand to a useful number before switching off proliferation and switching on differentiation into fully fledged effectors. Finally, a late-responding T cell needing a second hit to keep proliferating might become an effector cell upon encountering an Ag-bearing APC, or a memory cell upon encountering an Ag-bearing T cell.13

We do not know whether such an Ag-capturing quorum-sensing mechanism might also apply to CD8 T cells, as their activation requirements may be different from those of CD4. For example, CD8 T cells have been reported to be able to divide autonomously after their first encounter with Ag,46-48 whereas CD4 T-cell division requires the continuous presence of Ag.8,39 Perhaps differences in the life span of presented MHC-I versus MHC-II Ag complexes49,51 or the ability of CD8 T cells to kill their APCs7 might lead to the requirement for a negative feedback loop for one population of T cells (CD4 T cells) but not the other (CD8 T cells). Alternatively, a similar negative feedback loop could also be operating for CD8 T cells through fratricidal killing.33
For CD4 T cells, the rapidly occurring preferential inhibition of Ag-experienced T cells appears to be a built-in, Ag-specific, quorum-sensing mechanism operating at the site of the immune response to fine-tune the response intensity to the amount of presented Ag and the number of responding T cells.

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References


Authorship

Contribution: J.H. and A.J. performed most of the experiments with the help of N.J., I.G., and G.D. for some of them; A.K. and B.M. generated the Lat-DTR mouse; P.M. suggested key experiments and concepts and helped with writing; O.L. designed and supervised the work; and J.H., P.M., and O.L. wrote the paper.

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Antigen-specific T-T interactions regulate CD4 T-cell expansion

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