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Early Self-Regulatory Mechanisms Control the Magnitude of CD8\(^+\) T Cell Responses Against Liver Stages of Murine Malaria\(^1\)

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Following immunization with *Plasmodium yoelii* sporozoites, the CD8\(^+\) T cell population specific for the SYVPSAEKI epitope expressed in sporozoite and liver stages of this malaria parasite revealed the existence of a short term Ag presentation process that translated into a single clonal burst. Further expansion of this CD8\(^+\) T cell population in conditions of sustained Ag exposure and additional supply of naive cells was inhibited by regulatory mechanisms that were developed as early as 24–48 h after priming. Studies using mouse models for Plasmodium or influenza virus infections revealed that this mechanism is Ag specific and is mediated by activated CD8\(^+\) T cells that inhibit the priming of naive cells. This interference of the priming of naive cells appeared to result from limited access to Ag-presenting dendritic cells, which become disabled or are eliminated after contact with activated cells. Thus, concomitantly with the development of their effector antimicrobial capacity, CD8\(^+\) T cells also acquire a self-regulatory role that is likely to represent one of the earliest mechanisms induced in the course of an immune response and that limits the magnitude of the early expansion of CD8\(^+\) T lymphocytes reactive to microorganisms. *The Journal of Immunology*, 2003, 171: 964–970.

The induction of CD8\(^+\) T cell responses against different infectious pathogens is a process that follows a distinct program that is initiated just a few hours after CD8\(^+\) T cells recognize TCR ligands on the surface of APCs (1–6). Studies using the rodent malaria parasite *Plasmodium yoelii* together with TCR transgenic (Tg)\(^3\) CD8\(^+\) T cells specific for an epitope located in the circumsporozoite protein of this parasite revealed that one of the first signs marking the onset of CD8\(^+\) T cell activation is the development of effector functions in this T cell population that are detectable as early as 24 h after i.v. immunization with sporozoites. This is followed 24–48 h later by an accelerated process of clonal CD8\(^+\) T cell expansion, which reaches a peak 4–5 days after Ag exposure. On days 6–8, a phase of sudden contraction is evident that involves the death of ~90% of the activated cells, probably due to apoptosis. After day 8 the parasite-reactive CD8\(^+\) T cell population is stabilized, and memory populations begin to be established (7).

As shown in this model, the magnitude of the resulting CD8\(^+\) T cell response is solely defined by the initial events of Ag recognition and clonal burst that occur immediately after i.v. immunization with sporozoites and the display of the SYVPSAEKI epitope bound to H2K\(^{\dagger}\), a process that takes place on the surface of Ag-presenting dendritic cells (DCs) (8, 9). In this system the in vivo Ag presentation lasts <96 h, and whereas the magnitude of the CD8\(^+\) T cell response depends on the immunizing dose, a sustained delivery of sporozoites does not enhance the magnitude of the developing CD8\(^+\) T cell clonal burst (8). This situation is quite similar to that found in normal mice that are immunized via bites of sporozoite-infected mosquitoes every 48 h, which mimics the parasite exposure experienced by humans living in certain malaria endemic areas. Under this condition of immunization, these mice develop a primary response after the first or second exposure to parasites, and the magnitude of this response does not change even after receiving multiple additional immunizations (8). The inability of CD8\(^+\) T cells to expand further, despite increased exposure to Ag during the development of primary responses, resembles the situation found in humans living in malaria-endemic areas. These individuals develop a rather limited CD8\(^+\) T cell response to plasmodial Ags expressed in sporozoites or liver stages despite being constantly exposed to parasites throughout their lifetimes (10, 11).

These findings are also consistent with observations made in an experimental model of *Listeria monocytogenes* infection in which the continued presence of Ag, due to bacterial survival several days after priming, does not affect the magnitude of the developing CD8\(^+\) T cell response (12, 13). Moreover, in this system once a primary CD8\(^+\) T cell response is established, it is extremely difficult to induce an in vivo expansion unless an extremely large dose of the same Ag source, reaching 50–100 times the priming dose, is used for boosting (3, 14).

Taken together, the studies in mice and humans strongly suggest that immediately after the first clonal burst and together with the swift development of CD8\(^+\) T cell populations expressing effector functions, inducible regulatory mechanisms are switched on to prevent the activation of naive CD8\(^+\) T cells after additional exposure to the same TCR ligand. While these regulatory mechanisms may limit the overall magnitude of an antimicrobial primary response,
they may also play a key physiological role by preventing a mas-
sive T cell activation that could overwhelm the immune system and
produce deleterious effects in secondary lymphoid organs and
nonlymphoid tissues infected by microorganisms.

In this report we describe studies using mouse models of infec-
tions by Plasmodium and influenza virus that are aimed at char-
acterizing the mechanisms regulating the in vivo expansion of
CD8+ T cell populations and discuss evidence indicating that this
is a self-regulatory mechanism mediated by activated CD8+ T
cells.

Materials and Methods

Mice

Five- to 8-wk old BALB/c (Thy1.2+) or CB6F1 mice were obtained from
the National Cancer Institute (Frederick, MD). The generation of TCR Tg
mice specific for the H2Kb-restricted epitope SYVPSAEKI of the circum-
sporozoite protein of Plasmodium yoelii, or malTg, has been described
previously (7). Mice were maintained in the BalB/c (Thy1.2+ and
Thy1.1+) and CB6F1 backgrounds. The clone 4 TCR Tg (15) or fluTg mice
specific for the H2Kb-restricted epitope SYTVAASL of the influenza virus
hemagglutinin protein (fluTg) in BalB/c (Thy1.2+) background were
obtained from Dr. L. Sherman (The Scripps Institute, La Jolla, CA). Unless
otherwise specified, the majority of the experiments were performed using
the BalB/c (Thy1.2+) background. Normal BalB/c mice expressing
Thy1.1 Ag were obtained from Dr. H. Shen (University of Pennsylvania,
Philadelphia, PA).

Parasites and viruses

P. yoelii (17X NL strain) parasites were maintained, and sporozoites
were dissected as previously described (16). Immunizations were performed
by i.v. injection of 3–4 × 107 radiation-attenuated (gamma source, 20 krad)
parasites or as otherwise specified in the figure legends. Wild-type influen-
za virus A (WSN/H1N1 strain) was provided by Dr. A. Garcia-Sastre
(Mt. Sinai School of Medicine, New York, NY) and used at 1–2 × 105
PFU/mouse for i.v. immunizations.

ELISPOT assay

The in vivo IFN-γ ELISPOT assay for the quantification of the number of
SYVPSAEKI-specific CD8+ T cells in immunized mice was performed as
previously described (17, 18). MHC-compatible A20.2J target cells were
loaded with the SYVPSAEKI peptide and were incubated with mouse lym-
phocytes for 20–24 h. Anti-mouse IFN-γ (R4) and biotinylated anti-mouse
IFN-γ (XMGl.2) were obtained from BD Pharmingen (San Diego, CA).

Flow cytometric analysis

SYVPSAEKI-specific H2Kb tetramers (subsequently designated Tetramer)
were either obtained from the National Institute of Allergy and Infectious
Diseases, National Institutes of Health, Tetramer Facility or prepared as
previously described (3, 19). In the absence of a reliable tetramer system to
detect activated fluTg CD8+ T cells, Thy1.2+ fluTg CD8+ T cells were
used for adoptive transfer into normal Thy1.1+ mice. These cells were
detected by gating on CD8+ Thy1.2+ T cells. FITC-, PE-, and allophyco-
cyanin-labeled mAbs to mouse CD8 (53-6.7), Thy1.1 (OX-7), and Thy1.2
(53-2.1) were obtained from BD Pharmingen. Stained cells were analyzed
using FACS Calibur and CellQuest software (BD Immunocytometry Sys-
tems, San Jose, CA).

CFSE staining

Staining of Tg cells with CFSE was performed based on the manufactur-
er’s instructions (Molecular Probes, Eugene, OR). The number of CFSE-la-
beled CD8+ Tetramer- or CD8+ Thy1.2+ cells undergoing division in the
spleen was calculated following the methodology described previously
(20).

Adoptive transfer

Spleen cells from Tg mice (malTg or fluTg), unlabeled or CFSE-labeled,
containing ~2 × 109 naive Ag-specific CD8+ T cells, were injected i.v.
to syngeneic recipient mice. For experiments in which primed spleen
cells were used for adoptive transfer, Plasmodium parasite-primed spleen
cells were obtained from mice that received 2 × 106 malTg CD8+ T cells
and 3–4 × 105 sporozoites 48 h earlier. Influenza virus-primed spleen
cells were obtained from mice that received ~2 × 106 fluTg CD8+ T cells and
1–2 × 105 PFU influenza virus 72 h earlier. For experiments in which a
particular subset was either depleted or purified from primed spleens, dis-
sociated spleens were stained with FITC-conjugated mAbs (CD8 (53-6.7),
CD4 (RM4-4), NK1.1 (PK136), γ6 TCR (GL4), or CD45R/B220 (RA3-
6B2; all from BD Pharmingen)), followed by anti-FITC-conjugated mag-
netic beads. Depletion by negative selection or enrichment by positive
selection was then performed following the instructions of the manufac-
turer (Miltenyi Biotec, Auburn, CA). Depletion of NK1.1+ cells was per-
formed using spleens from primed CB6F1 mice.

Preparation of DCs

Mature DCs were prepared as previously described (21). Briefly, bone
marrow was removed from the leg bones of normal mice, and the precu-
sors were cultured in conditioned medium containing 30% of the super-
natant from the myeloma cell line Ag8563 (provided by Dr. A. Rodriguez,
New York University, New York, NY), as a source of murine GM-CSF.
The conditioned medium was replaced every 2 days. Seven or 8 days later,
matureation of the DCs was induced by addition of 1 μg/ml Salmonella
tenterica LPS (Sigma-Aldrich, St. Louis, MO) to the cultures. Twenty-four
hours after LPS addition, DC maturation was verified by flow cytometry
based on the expression and up-regulation of mouse CD11c (HL3), CD40
(3/23), CD80 (16-10A1), CD86 (GL-1), and MHC class I (SF1-1.1; all from
BD Pharmingen) compared with noninduced cells. Mature DCs were then
washed thoroughly and incubated with the SYVPSAEKI peptide (10
μg/ml) for 2.5 h. After incubation, the SYVPSAEKI-loaded DCs were
washed again before transfer at 2.5 × 106 cells/mouse.

Results and Discussion

Further activation of CD8+ T cells is inhibited immediately
after priming with malaria sporozoites

Previous studies using Tg CD8+ T cells specific for an H2Kb-
restricted epitope (SYVPSAEKI) expressed in sporozoite and liver
stages of P. yoelii (malTg CD8+ T cells) indicated that the CD8+
T cell response induced by immunization with sporozoites is the
result of a single clonal burst induced by a short term Ag presen-
tation that lasts <96 h. The supply of additional Ag does not en-
hance the magnitude of the CD8+ T cell response, indicating the
development of a state of refractoriness (8).

That this refractory state is established just a few hours after
priming was indicated when we compared the CD8+ T cell re-
sponse in groups of normal BALB/c mice that received malTg
CD8+ T cells and were immunized with 2 × 105 P. yoelii para-
sites. Groups of mice received an additional dose of 8 × 104 para-
sites 24, 48, 72, or 96 h after priming, and the resulting CD8+ T
response was evaluated by ELISPOT 14 days later. The mag-
nitudes of the responses in mice receiving two immunization doses
was almost identical with that in mice receiving only the initial
2 × 104 parasite dose (Fig. 1). The response levels in mice re-
cieving two doses were all lower than that in mice that received
1 × 105 parasites in a single initial dose. Therefore, a regulatory
mechanism that inhibits the additional activation of CD8+ T cells
is established as early as 24 h after priming, and the magnitude of
the immune response depends on the initial dose. The immuniza-
tions were performed using radiation-attenuated sporozoites, which are
capable of invading hepatocytes, but are unable to pro-
liferate and generate blood stage parasites.

Activation of naive CD8+ T cells is inhibited in mice previously
primed with sporozoites

The existence of a mechanism limiting the development of sec-
condary clonal bursts also became evident in experiments in which
we studied the in vivo proliferative activity of naive CD8+ T cells.
In these experiments CFSE-labeled naive malTg CD8+ T cells
were transferred into normal BALB/c mice that were subsequently
immunized i.v. with sporozoites. Seventy-two hours after immu-
nization, flow cytometric analysis was performed to evaluate the
dilution of the CFSE stain in CD8+ Tetramer+ T cells as previ-
ously described (7, 20). As expected, in the absence of sporozoite
T cell responses against malaria liver stages.

To further characterize the nature of this inhibitory mechanism, we performed adoptive transfer experiments using primed spleen cells obtained from mice that had received matTg CD8+ T cells and sporozoites. In these experiments spleen cells obtained 48 h after priming were transferred into normal mice that subsequently received naive CFSE-labeled matTg CD8+ T cells and were then immunized with parasites. The CFSE dilution profile of the labeled cells, evaluated by flow cytometry 72 h later, revealed a major inhibition of proliferation of naive matTg CD8+ T cells when primed spleen cells were transferred into mice (Fig. 3A). In contrast, no effect was observed when normal spleen cells were transferred into mice. These results clearly indicated that the mechanism inhibiting the activation of naive CD8+ T cells was transferable and mediated by primed spleen cells. Quantification of the CFSE proliferation peaks further confirmed the inhibitory effect of primed spleen cells on the proliferation of labeled CD8+ T cells (Fig. 3B).

In view of these results we next examined whether the regulatory mechanism observed in the plasmodial system might also develop in other microbial systems. For this purpose we performed experiments using influenza virus and Tg CD8+ T cells specific for the H2Kb-restricted IYSTVASSL epitope of the influenza virus hemagglutinin protein (fluTg CD8+ T cells). Immunization with influenza virus was performed i.v., which is known to result in limited or no viral replication (22). CFSE-labeled fluTg CD8+ T cells were transferred into normal mice, and 72 h later the dilution of the CFSE stain was evaluated by flow cytometry. As expected, these CD8+ T cells displayed a strong proliferative response in mice that also received influenza virus (Fig. 4). However, when CFSE-labeled fluTg CD8+ T cells and influenza virus were transferred to mice that had received fluTg CD8+ T cells and virus 72 h...
The proliferation inhibitory activity is present in parasite-primed spleen and is transferable. A. The proliferation profile of labeled naive CD8 \(^+\) T cells was assessed 72 h after the transfer of 2 \times 10^6 CFSE-labeled malTg CD8 \(^+\) T cells and 4 \times 10^4 parasites into normal mice (i), normal mice that received parasite primed spleen cells generated as described in Fig. 2 (ii), or normal mice that received nonprimed spleen cells (iii). Flow cytometry CFSE histograms were obtained after gating on CD8 \(^+\) Tetramer \(^{-}\) T cells. B. The percentage of CFSE-labeled CD8 \(^+\) Tetramer \(^{-}\) T cells in the spleen undergoing division in each of the above groups was estimated based on previous data (19). Results represent the mean of three mice \(\pm SD\).

FIGURE 3. The proliferation inhibitory activity is present in parasite-primed spleen and is transferable. A. The proliferation profile of labeled naive CD8 \(^+\) T cells was assessed 72 h after the transfer of 2 \times 10^6 CFSE-labeled malTg CD8 \(^+\) T cells and 4 \times 10^4 parasites into normal mice (i), normal mice that received parasite primed spleen cells generated as described in Fig. 2 (ii), or normal mice that received nonprimed spleen cells (iii). Flow cytometry CFSE histograms were obtained after gating on CD8 \(^+\) Tetramer \(^{-}\) T cells. B. The percentage of CFSE-labeled CD8 \(^+\) Tetramer \(^{-}\) T cells in the spleen undergoing division in each of the above groups was estimated based on previous data (19). Results represent the mean of three mice \(\pm SD\).

earlier, the proliferation of labeled naive CD8 \(^+\) cells was strongly inhibited (Fig. 4ii). Furthermore, the adoptive transfer of spleen cells obtained from mice developing an anti-influenza immune response also strongly inhibited the proliferation of naive CFSE-labeled fluTg CD8 \(^+\) T cells administered together with another dose of the virus (Fig. 4iii). These results are strikingly similar to those observed in the malaria system and therefore suggest that this regulatory phenomenon represents a general mechanism that critically constrains the activation and proliferation of naive CD8 \(^+\) T cells against microorganisms.

The existence of similar inhibitory mechanisms in these different infection models allowed us to investigate the Ag specificity of this phenomenon. Specifically, we examined whether Plasmodium sporozoite-primed spleen cells could inhibit the proliferation of naive fluTg CD8 \(^+\) T cells in response to viral immunization and vice versa. Spleen cells obtained from influenza-primed mice did not prevent the proliferation of naive fluTg CD8 \(^+\) T cells in response to parasite immunization (Fig. 5A), and conversely, spleen cells from parasite-primed mice did not prevent the proliferation of naive fluTg CD8 \(^+\) T cells in response to influenza virus immunization (Fig. 5B). The inhibitory activity was therefore not only transferable, but also Ag specific. Just as importantly, these results strongly suggested that primed/activated Ag-specific T cells themselves do contribute to the mechanism that prevents activation of additional naive CD8 T lymphocytes sharing the same TCR.

Activated CD8 \(^+\) T cells inhibit the activation of naive CD8 \(^+\) T cells

To identify the inhibitory cell population, spleen cells from parasite-immunized mice were systematically depleted of different T cell populations before transfer into normal mice. The recipient mice then received CFSE-labeled naive malTg CD8 \(^+\) T cells and parasites, and the dilution profile of labeled cells was evaluated at 72 h by flow cytometry. Depletion of NK cells, \(\gamma\delta\) T cells, or CD4 \(^+\) T cells did not affect the inhibitory capacity of the primed spleen compared with controls (Fig. 6, i–v). However, depletion of CD8 \(^+\) T cells allowed for a normal proliferative profile by the malTg CD8 \(^+\) T cells (Fig. 6vi). Confirmatory evidence that these CD8 \(^+\) T cells were indeed mediating the inhibitory activity was obtained in adoptive transfer experiments. CD8 \(^+\) T cells purified from spleens of parasite-immunized mice inhibited the activation of naive malTg CD8 \(^+\) T cells as efficiently as the whole primed spleen (Fig. 6vii). Similar results were obtained in the influenza system wherein purified activated influenza-specific CD8 \(^+\) T cells inhibited the activation of naive fluTg CD8 \(^+\) T cells (data not shown).

Activated and naive CD8 \(^+\) T cells compete for a limited number of APCs

These results indicated that activated CD8 \(^+\) T cells regulated the activation of naive CD8 \(^+\) T cells, suggesting the possible existence of a competitive relationship between these two cell populations of identical epitope specificity. This possible competitive relationship was further characterized in experiments that measured the in vivo Ag-driven expansion of naive CD8 \(^+\) T cells in the presence of
increasing numbers of activated cells. For this purpose, mice received a constant number (1.5 x 10^5) of naive Thy1.1^+ malTg CD8^+ T cells plus different numbers of activated Thy1.2^+ malTg CD8^+ T cells, ranging from 180–400,000. These mice were then immunized with parasites. Seven days later, the expansion of naive CD8^+ T cells was assessed by flow cytometry with gating on CD8^+ T cells and not that of transferred activated cells that bear the Thy1.2 marker. The expansion of Thy1.1^+ CD8^+ T cells diminished proportionally as the numbers of transferred Thy1.2^+ activated cells increased (Fig. 7). It is noteworthy that activated cells are highly efficient in competing for APCs, as indicated by the results obtained when transferring equal numbers of naive and activated CD8^+ T cells. In this situation the proliferation of naive CD8^+ T cells is inhibited by 90% or more, thus supporting the idea that activated/memory T cells have higher avidity for Ag presented by APCs (23).

The preceding results demonstrating the existence of competition between naive and activated CD8^+ T cells raised the question of the nature of the mechanism mediating this phenomenon. Recent in vitro and in vivo studies indicate that activated T cells may severely affect the function of professional Ag-presenting DCs by stripping Ag from their surface or by direct killing (24, 25). In view of these observations it has been suggested that competition for DCs could negatively affect the induction of T cell responses (26, 27). To determine whether DCs played a role in the self-regulatory activity of CD8^+ T cells, we evaluated the effect of introducing large numbers of peptide-loaded DCs on the apparent in vivo competition among CD8^+ T cells. For this purpose, mature DCs derived from bone marrow precursors were pulsed with the SYVPAEQI peptide and transferred into normal mice along with primed spleen cells, CFSE-labeled naive malTg CD8^+ T cells, and parasites, as described in the preceding experiments. The transfer of peptide-pulsed DCs overcame the inhibitory effect exerted by activated cells on naive CD8^+ T cells (Fig. 8). These findings

**FIGURE 5.** The inhibitory mechanism induced after immunization is Ag specific. The Ag specificity of primed parasite- or influenza-primed spleen was assessed by evaluating the proliferation of Tg CD8^+ T cells. A, CFSE-labeled naive malTg CD8^+ T cells and 4 x 10^6 sporozoites (2 x 10^6 each) were injected into normal mice (i), normal mice that received influenza-primed spleen (ii), or normal mice that received parasite-immune spleen cells (iii). Parasite-primed and influenza-primed spleen cells were obtained from primed mice generated as described in Figs. 2iii and 4i, respectively. The proliferation of the labeled naive CD8^+ T cells was assessed by flow cytometry 72 h after transfer into mice by evaluating the dilution of the CFSE stain gating on CD8^+ Tetramer^+ T cells. B, CFSE-labeled naive Thy1.2^+ fluTg CD8^+ T cells (2 x 10^5) and 2 x 10^8 PFU influenza virus were injected into normal Thy1.1^+ mice (i), normal Thy1.1^+ mice that received Thy1.2^+ parasite-primed spleen cells (ii), or normal Thy1.1^+ mice that received Thy1.2^+ influenza-primed spleen cells (iii).

**FIGURE 6.** Activated CD8^+ T cells inhibit the activation and proliferation of naive CD8^+ T cells. To identify the T cell subset mediating the inhibitory activity on naive cells, 2 x 10^6 CFSE-labeled naïve malTg CD8^+ T cells and 4 x 10^6 parasites were injected into normal mice (i), normal mice that also received parasite-primed spleen cells generated as described in Fig. 2iii (ii), and mice that received parasite-primed spleen cells depleted of NK1.1^+ T cells (iii), γδ T cells (iv), CD4^+ T cells (v), or CD8^+ T cells (vi). Mice of group vii received 2 x 10^6 CFSE-labeled malTg CD8^+ T cells, parasites, and activated CD8^+ T cells purified from parasite-primed spleens. The proliferation of the labeled naive CD8^+ T cells was assessed by flow cytometry 72 h after transfer into mice by evaluating the dilution of the CFSE stain gating on CD8^+ Tetramer^+ T cells.
expressed as total numbers of CD8+T cells. Indeed, the effect of the microbes was detectable. This mechanism could also be a self-regulatory mechanism controlling the magnitude of CD8+T cell responses against microbial pathogens before any pathogenic effect of the microbes was detectable. This mechanism could also serve to prevent an excessive activation and expansion of CD8+T cells that might overwhelm the immune system, leading to undesirable effects and potential pathological sequelae. This self-regulatory activity is likely to represent one of the earliest mechanisms induced in the course of an immune response, developed to control the magnitude of the CD8+T cell response. It is worth emphasizing that the inability of an established CD8+T cell response to expand after repeated Ag exposure is not a phenomenon observed only in experimental systems using Tg cells. Indeed, the first evidence indicating the presence of early self-regulatory T cell-mediated mechanisms was strongly suggested in previous studies using normal mice (8). However, the low precursor frequency of Ag-specific T cells in normal mice does not permit the labeling of Ag-specific T cells to perform flow cytometric analyses as described in the present study.

It is striking that the time needed to develop this self-regulatory capacity is quite rapid, i.e., within 24–72 h after delivery of the source of antigenic peptides. However, it is noteworthy that previous studies in mice immunized with Plasmodium sporozoites indicated that CD8+T cells displayed clear differentiation signs and development of effector mechanisms as early as 24 h after priming (7). In the influenza system there is also evidence indicating that influenza-specific CD8+T cells become activated 24–72 h after immunization with the virus (28). An important issue that remains to be elucidated is the effect that factors such as virulence, tissue specificity, and ability to replicate may have on the development of these self-regulatory mechanisms.

While the present study establishes that competition between naive and activated CD8+T cells at the level of APCs is an early regulatory mechanism, it is nevertheless likely that additional regulatory mechanisms are subsequently developed as the immune response evolves. In fact, CD4+T cells have been shown to be important in sustaining the development of CD8+T cell responses after clonal expansion. In Plasmodium and influenza systems, mice depleted of or lacking CD4+ cells have very diminished or compromised responses against the microbes (29–31).
Finally, our findings indicating the existence of Ag-specific CD8\(^+\) T cells that exert an inhibitory effect on the immune responses raise the issue of whether this represents the activity of a specialized T cell subset, similar to the CD8\(^+\) T suppressor cells that many years ago were the subject of extensive studies and were proposed to be regulators of the immune response. While some of the inhibitory phenomena described in those early studies may concur with the regulatory activity of CD8\(^+\) T cells described here, our present findings suggest that there is no need to postulate the existence of a specialized class of suppressor CD8\(^+\) T cells. Therefore, we favor the idea of a dual function for effector CD8\(^+\) T cells, wherein activated CD8\(^+\) T cells both perform their antimicrobial functions and also regulate the magnitude of the CD8\(^+\) T cell response, using the same or similar effector mechanisms.

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