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T Memory Cells: Quality not Quantity

Dispatch

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Immunological memory to pathogens is associated with clonal expansion and heightened responsiveness of antigen-specific T cells. Recent work emphasizes that, for efficient protective immunity, qualitative changes in T memory cells are vitally important.

Exposure to infectious microorganisms generally leads to a powerful immune response by specific T and B cells [1,2]. Contact with the pathogen in the lymph nodes and spleen causes specifically reactive T and B cells to proliferate extensively and differentiate into effector cells. These cells then home to the sites of infection and, via a combination of cellular and humoral (antibody-mediated) immunity, induce rapid destruction of the pathogen. Being now redundant, most effector T and B cells are destroyed. However, some of these cells survive and differentiate into long-lived memory cells (Figure 1).

Immunological memory is often lifelong and results in a heightened response to the pathogen upon re-infection [3–6]. Since memory at the T cell level is associated with a marked increase in the frequency of antigen-specific lymphocytes, the simplest explanation for the efficiency of secondary (memory) immune responses is that there are more reactive cells available to attack the pathogen than in the primary response. But is an increase in precursor frequency alone sufficient to account for memory? According to a paper published recently in *Science* by Lauvau *et al.* [7], the answer seems to be no. In this paper the authors found that priming mice with either live or heat-killed bacteria led to intense proliferation of antigen-specific T cells followed by survival of a proportion of these cells as memory cells. Surprisingly, when the mice were later injected with live bacteria, protective immunity to the pathogen was seen only after priming with live bacteria. Priming with killed bacteria was conspicuously ineffective.

To study the requirements for induced protective immunity, Lauvau *et al.* [7] examined memory in mice injected intravenously with *Listeria monocytogenes*, a Gram-positive facultative intracellular bacterium (Table 1); immunity to this bacterium is known to be controlled largely by $CD8^+$ T cells. To follow the response of antigen-specific $CD8^+$ cells to the bacterial antigens, the authors utilized the finding that $CD8^+$ cells react to antigenic peptides bound to class I major histocompatibility complex (MHC) molecules and that peptide-specific $CD8^+$ cells can be detected via binding of soluble MHC class I tetramers plus specific peptide [6].

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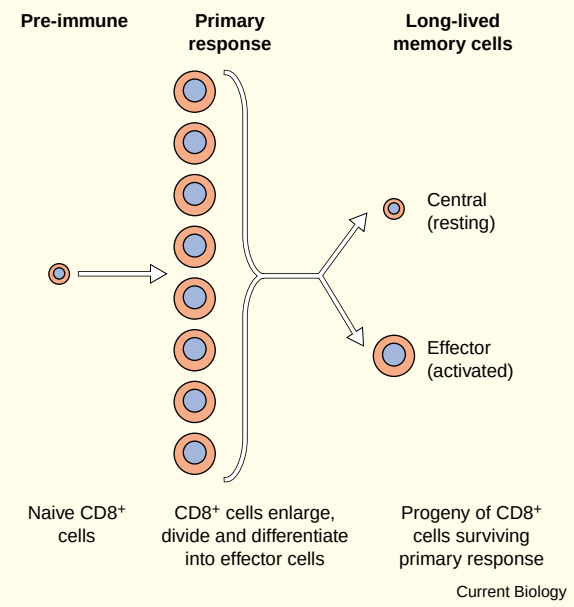

Figure 1. A scheme for differentiation of naive $CD8^+$ T cells into memory cells.

During the primary response, naive T cells recognize MHC class I–peptide complexes on professional antigen-presenting cells (dendritic cells) in spleen and lymph nodes and undergo conspicuous clonal expansion followed by differentiation into effector cells. After destroying the pathogen, most effector cells are eliminated, but some of these cells (about 10%) survive to form long-lived memory cells. Effector memory cells persist in an activated state and are poised to give very rapid responses to pathogens upon re-infection. Central memory cells are resting cells and need to be reactivated before expressing effector function.

As expected, priming mice with live bacteria led to marked clonal expansion of specific $CD8^+$ cells in the primary response and even greater expansion in the secondary response. Based on T cell binding of three different peptide–tetramer complexes, the precursor frequency of $CD8^+$ cells for the bacterial antigens rose from undetectable levels before priming to 2–3% of $CD8^+$ cells at the height of the primary response (day 7) and then to about 25% during the secondary response (day 5). Testing the mice 21 days after initial injection of live bacteria showed strong protective immunity. Thus, when the mice received a second intravenous injection of live bacteria, counts of viable bacteria in the spleen measured 3 days later were far lower than were found at this time in the primary response.

These results refer to priming with live bacteria. When mice were primed with heat-killed bacteria, clonal expansion and persistence of specific $CD8^+$ cells was as high as for priming with live bacteria. Thus, when mice were primed with killed bacteria and then challenged with live bacteria 21 days later, 27% of $CD8^+$ cells in the spleen were antigen-specific on day 5 after challenge (compared with 24% for mice primed – and challenged – with live virus). Despite this

Table 1. A description of the model used by Lauvau *et al.* [7] (see text).

	Primary response		Secondary response after challenge with live bacteria	
Bacteria used for priming	Clonal expansion	Effector function	Clonal expansion	Elimination of bacteria
Live	++++	++++	++++	++++
Killed	++++	–	++++	–

It should be noted that, in the secondary response, precursor frequencies of antigen-specific $CD8^+$ cells were measured only on day 5; data on precursor frequencies just prior to challenge with live virus were not presented. Hence, prior to challenge, total numbers of specific memory cells could have been lower in the group primed with killed bacteria, thus delaying the onset of effector cell generation.

extremely high proportion of antigen-specific cells, however, there was no protective immunity: on day 3 after challenge with live bacteria, counts of live bacteria in the spleen were very high, in fact as high as in primary infection of naive mice.

Since immune responses to infectious agents involve $CD4^+$ T cells as well as $CD8^+$ cells [1,2], the lack of immunity in mice primed with killed bacteria could have reflected a failure to stimulate $CD4^+$ T cells, the T cell subset that leads to humoral immunity via stimulation of antigen production by B cells. In the model studied, however, generation of protective immunity seemed to be controlled only by $CD8^+$ and not $CD4^+$ cells. Thus, protective immunity was abolished in mice lacking $CD8^+$ cells but retained in mice lacking $CD4^+$ cells.

In light of this finding, the authors were faced with a paradox: when challenged with live bacteria after priming with killed bacteria, immunity in the spleen was undetectable even though the spleen contained very large numbers of specific $CD8^+$ cells. The authors' explanation for this puzzle is that the specific $CD8^+$ cells failed to express effector function. In support of this idea, adoptive transfer studies with T cell receptor transgenic mice specific for one of the bacterial peptides showed that differentiation of $CD8^+$ cells into cytotoxic T lymphocytes and interferon-γ-producing cells was conspicuous in hosts primed with live bacteria but almost undetectable in hosts given killed bacteria. With the latter, the failure of the transgenic $CD8^+$ cells to differentiate into effector cells correlated with a small (twofold) reduction in clonal expansion and impaired downregulation of a lymph node homing receptor, CD62L. To explain these findings, the authors suggested that live and killed bacteria are phagocytosed by different antigen-presenting cells, namely by dendritic cells ('professional' antigen-presenting cells) for live bacteria and macrophages for killed bacteria. The assumption here is that both types of antigen-presenting cell elicit clonal expansion of $CD8^+$ cells but only dendritic cells promote differentiation into effector cells. This interesting idea has yet to be proved.

At face value, these data would seem to provide strong support for the authors' conclusion that the lack of protective immunity after priming with killed bacteria reflected a failure of memory $CD8^+$ cells to differentiate into effector cells. However, in the T cell receptor transgenic model, effector function was studied only in the primary and not the secondary response. This is an important omission because the live bacteria used in the secondary response were presumably highly immunogenic; here, one would expect presentation of antigen by professional antigen-presenting cells to not only induce extensive proliferation of memory cells but also drive these cells to differentiate into effector cells. The only direct evidence presented in the paper on this issue, however, is that bacterial counts measured in the spleen on day 3 were not reduced. This is good evidence for a lack of protective immunity, but it does not prove that the memory cells failed to form effector cells. Perhaps effector cells were formed, but more slowly than in mice primed with live bacteria (see Table 1 legend)?

In considering this possibility, it is important to bear in mind that there are two broad subsets of memory T cells, termed effector and central memory cells [8]. Effector memory cells lack CCR7 and CD62L lymph node homing receptors and are scattered throughout the body. These cells are maintained in an overtly activated state and thus display very rapid responses upon secondary contact with antigen. Because of their activated status, some workers view effector memory cells as the 'real' memory cells controlling protective immunity [4]. Unlike effector memory cells, central memory cells are relatively quiescent and resemble naïve T cells in expressing CCR7 and CD62L receptors. Being resting cells, central memory cells need to be reactivated, probably by professional antigen-presenting cells, in order to express effector function. Therefore, in secondary infection, the response of central memory cells is delayed relative to effector memory cells. Some workers maintain that this delay is crucial and makes central memory cells much less useful than effector memory cells for protective immunity [4]. Others disagree, arguing that the delay in the response of central memory cells is quite short and that, being numerous, these cells are highly important for protective immunity [3].

In the experiments of Lauvau *et al.* [7], the memory $CD8^+$ cells found in mice primed with killed bacteria were $CD62L^+$ and thus had features of central memory cells. Hence, challenging the primed mice with live bacteria may have induced strong differentiation of the memory cells into effector cells, but with delayed kinetics (relative to mice primed with live bacteria). If so, bacterial counts in the spleen may have declined precipitously, but only in the later stages of the response. Since bacterial counts were measured only on day 3, this possibility was not ruled out.

But suppose the authors tested this idea and found no evidence of protective immunity and no induction of cytotoxic T lymphocytes? Such a finding would be most intriguing and suggest that the memory $CD8^+$ cells generated after priming with killed bacteria were functionally anergic: the memory cells proliferated extensively when challenged with live bacteria, but, because of aberrant priming, were unable to

differentiate into effector cells. A precedent for this idea comes from the finding that memory $CD8^+$ cells in patients with AIDS [9] and mice chronically infected with lymphocytic choriomeningitis virus [10] display only very limited cytotoxic T lymphocyte activity. However, in these chronic infections, anergy of $CD8^+$ cells may be largely a reflection of continuous T cell stimulation. By contrast, antigen levels in mice primed with killed bacteria probably decline abruptly within a few days.

Whatever the explanation, the apparent incompetence of memory $CD8^+$ cells generated in response to killed bacteria has important implications for vaccine design. With some notable exceptions, for example the Salk vaccine for poliomyelitis, vaccination with killed microorganisms often affords poor protection and may rely largely on humoral immunity via antibody production [11]. Since effective (live) vaccines generally elicit powerful primary responses of T cells, the limited cellular immunity induced by killed microorganisms is usually attributed to weak priming of T cells. This assumption is clearly questioned by the finding of Lauvau *et al.* [7] that a killed vaccine can induce intense T cell proliferation but provide poor protection. Defining precisely why the memory cells in this situation are incompetent could be vital for improving vaccine design.

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