Glimpsing the real CD4+ T cell response

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The present views of how CD4+ T cells respond to antigen are based largely on artificial systems. A highly sensitive approach that allows normal T cell responses to be monitored in physiological conditions overturns some existing ideas about the differentiation of CD4+ T cells.

A vital feature of adaptive immune responses is the ability to remember encounters with previous infections, thereby resulting in stronger and faster anamnestic responses. Effective recall responses require the generation and maintenance of antigen-specific memory B lymphocytes and T lymphocytes that persist for many years after elimination of the pathogen concerned. For T cells, much of the understanding of immunological memory has come from studies of mouse CD8+ T cells. After being activated, these cells undergo a programmed course of population expansion, differentiation and population contraction that allows only a small fraction of newly generated effector cells to survive and enter the stable pool of long-lived memory cells[1]. The generation of memory CD4+ T cells is broadly similar to that of CD8+ T cells, although CD4+ T cell memory gradually decreases with time, whereas CD8+ T cell memory persists almost indefinitely. In this issue of *Nature Immunology*, Pepper *et al.* use a very sensitive method to monitor the immune responses of endogenous antigen-specific polyclonal CD4+ T cells to a typical pathogen in normal mice[2]. The approach thus examines immune responses in their natural physiological state and avoids the pitfalls associated with experiments involving adoptive transfer of T cells and the use of noninfectious antigens. This aspect is a crucial point, because CD4+ T cell responses are highly plastic and can result in differentiation into many types of effector cells depending on the particular conditions encountered during T cell priming[3]. This work of Pepper *et al.* brings a sharper focus to the complexity of the CD4+ T cell response[2], providing direct support for some existing ideas about these cells but questioning other ideas.

Tracking the immune responses of T cells to antigen *in vivo* is now routinely done by transfer of T cell antigen receptor–transgenic T cells into mice, followed by immunization of the hosts with antigen, either as a protein or peptide with adjuvant or carried by an infectious agent[4]. If only small numbers of T cells are injected, this approach works well and has generated much valuable information about quantitative and qualitative aspects of the primary T cell response. To examine normal physiological immune responses, however, it is obviously an advantage to monitor the fate of endogenous polyclonal T cells rather than adoptively transferred T cell antigen receptor–transgenic cells. Tetramers of peptide and major histocompatibility complex can be used to identify endogenous antigen-specific polyclonal T cells, but the low sensitivity of this approach makes it unsuitable for the detection of small numbers of antigen-specific T cells. Jenkins and co-workers have added an enrichment step to greatly increase the sensitivity of the tetramer-staining approach, thus making it possible to detect even the very low numbers of antigen-specific polyclonal T cells present before immunization[5].

Pepper *et al.* have now used tetramer staining to monitor the primary response of endogenous CD4+ T cells to antigen in normal mice[2]. The system involves immunizing C57BL/6 (B6) mice by intravenous or intranasal infection with *Listeria monocytogenes* expressing a foreign peptide, an I-Eα peptide variant designated 2W1S. Naive B6 mice have a total of only ~200 CD4+ T cells specific for 2W1S (ref. 6). Here, the authors show that these cells undergo population expansion of about 500-fold at the peak of the response in the secondary lymphoid organs after intravenous infection with 2W1S-expressing *L. monocytogenes*. They find a lesser (~100-fold) expansion after intranasal infection, but this may represent an underestimation of the degree of expansion, because many of the T cells responding to intranasal infection are known from other studies to migrate to nonlymphoid tissues, which are not examined in this study. Nevertheless, even with inclusion of cells diverted to nonlymphoid tissue, the magnitude of the CD4+ T cell population expansion seems to be only ~10% as much as the massive population expansion of endogenous CD8+ T cells seen during a typical viral infection, as measured by others using the same tetramer staining approach[4]. Thus, CD4+ T cells seem to be generally less responsive to antigen than are CD8+ T cells[7].

In the present study, it is notable that the proliferating CD4+ T cells rapidly acquire the phenotype of effector cells, but most of the cells fail to synthesize cytokines, which confirms published work with T cell antigen receptor–transgenic cells[8]. These findings contrast sharply with results obtained with CD8+ T cells, most of which readily produce a range of cytokines soon after stimulation. The restricted differentiation of CD4+ T cells during infection is in line with the view that these cells have stringent requirements for differentiation into discrete subsets of effec-

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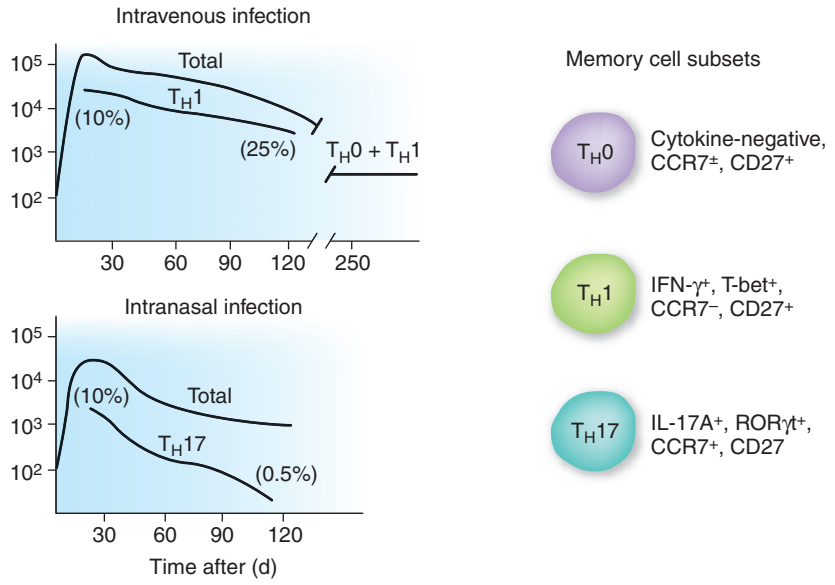

Figure 1 Endogenous CD4+ T cell responses to intravenous and intranasal infection with *L. monocytogenes*. A tiny pool (~200) of antigen-specific T cells undergoes better population expansion after intravenous than intranasal infection; only ~10% of the expanded cells differentiate into cytokine producers. Nearly all of the cytokine-producing cells after intravenous infection are IFN-γ+T-bet+ T_H1 cells, whereas only IL-17A+RORγt+ T_H17 cells are generated by intranasal infection. T_H1 cells are CD27+ and convert into memory cells just as efficiently as T_H0 cells; in contrast, T_H17 cells are CD27− and disappear within 4 months. T_H1 and T_H0 memory cell numbers decrease slowly over time, but persist stably after ~8 months in numbers near to those found in naive mice. The T_H1 and T_H17 cells have the phenotype of effector memory and central memory T cells, respectively, and thus do not fit easily into the present paradigm of the generation of memory CD4+ T cells. Numbers in parentheses (left) indicate the fraction of total cells that produce T_H1 or T_H17 cytokines at the peak of the response and 4 months later.

tor cells, such as T_H1, T_H2, T_H17 and induced regulatory T cells.

Notably, although it is limited, cytokine synthesis in the study of Pepper *et al.* varies according to the infection route[2]. Thus, intravenous infection leads to the sole emergence of T_H1 cells (positive for interferon-γ (IFN-γ)), whereas intranasal infection induces only T_H17 cells (positive for interleukin 17 (IL-17)). This influence of the infection route fits nicely with the observation that antigen-presenting cells in the spleen and mucosal tissues produce different cytokines, with splenic antigen-presenting cells being skewed to the production of IL-12 and mucosal antigen-presenting cells being skewed to the production of IL-6 and transforming growth factor-β. These cytokines are known to promote the selective differentiation of T_H1 and T_H17 cells, respectively[3].

After the expansion phase, death of activated CD4+ T cells in the ensuing contraction phase occurs in a biphasic manner. After the peak of the response, the population contraction of the cells was rapid for ~2 weeks but then continued gradually for the next 7–8 months, which confirms a published report that antigen-specific memory CD4+ T cell numbers decrease slowly with time[9]. Nonetheless, in the present study, a small pool of memory CD4+ T cells persists stably after 8 months, with the total numbers of these cells being close to the small fraction of cells present in unprimed mice. How a tiny pool of memory CD4+ cells is maintained in constant numbers late after infection is an interesting question that will require further investigation. It is notable that most of the memory CD4+ T cells are found in the secondary lymphoid organs rather than bone marrow. This finding contrasts with a published report showing that memory CD4+ T cells reside mostly in the bone marrow when a large inoculum of transgenic CD4+ T cells is primed with peptide plus adjuvant after adoptive transfer[10]. The conditions in that study were clearly less physiological than those in the present work.

A striking finding in the experiments of Pepper *et al.* is that T_H1 and T_H17 memory cells show distinct differences in their lifespans[2]. Thus, whereas T_H1 memory cells, like T_H0 cells, have a relatively long lifespan, T_H17 memory cells disappear rapidly and are mostly gone from secondary lymphoid organs by 4 months (**Fig. 1**). The possibility that IL-17+ cells migrate from secondary lymphoid organs into the mucosal tissues is not examined, although this scenario is unlikely because most of the memory IL-17+ cells express CCR7, the homing receptor for secondary lymphoid organs. Moreover, the IL-17+ cells have features characteristic of short-lived cells. In particular, they lack expression of CD27, which is typical of short-lived memory CD4+ and CD8+ T cells[11]; in contrast, long-lived IFN-γ+ T_H1 cells are mostly CD27+. The CD27− memory CD4+ cells also have lower expression of CD122 (IL-2Rβ) and the antiapoptotic protein Bcl-2 than do their CD27+ counterparts. The lower expression of CD122 presumably lowers their responsiveness to IL-15, thus accounting for the abbreviated lifespan of the T_H17 cells[8]. The clinical implication, therefore, is that protective immunity mediated by T_H17 cells is probably quite brief.

It is worth noting that the characteristics of the T_H1 and T_H17 memory cells found in the study of Pepper *et al.* differ from those of canonical T_H1 and T_H17 cells[2] (**Fig. 1**). The bulk of the T_H1 cells in this study are IFN-γ+T-bet+ effector memory T cells, as defined by low expression of CCR7. However, these cells are long-lived and responsive to homeostatic cytokines such as IL-15, which contrasts with the view that effector memory T cells are short-lived and poorly sensitive to cytokines[12]. Similarly, the T_H17 cells in this study are mostly IL-17+RORγt+CCR7+ and thus resemble central memory T cells, yet the cells are short-lived and cytokine unresponsive. These findings are clearly at variance with the present paradigms of CD4+ T cell differentiation, which are based largely on the results of stimulating CD4+ T cells with antigen under nonphysiological conditions.

The present data provide an important first look at how endogenous CD4+ T cells respond to an infectious agent under normal physiological conditions *in vivo*. The results confirm certain known features of the primary response of CD4+ T cells but shed important new light on how these cells differentiate into subsets of memory cells. Yet many questions remain unanswered. For example, why are most effector and memory cells in an undifferentiated T_H0 state, and do these cells differentiate further after secondary immunization? Likewise, do T_H1 cells, T_H17 cells and other subsets of memory cells show plasticity and differentiate into other polarized subsets after the appropriate restimulation? These and other related questions about the normal physiological immune response can now be addressed with this system of Pepper *et al.*[2].

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Defensins keep the peace too

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The mammalian intestine contains a large number of commensal bacterial strains. New work suggests that antimicrobial peptides used for defense against pathogenic bacteria are also used to adjust the balance among bacterial populations and to control intestinal homeostasis.

The principle of 'live and let live' is the cornerstone of any form of coexistence. It fully applies to the intestinal environment, in which the host epithelium normally gets along with a complex and large population of luminal microorganisms that represent a constant threat yet at the same time accomplish a multitude of beneficial roles for the host. Hidden within such tolerant behavior, the intestinal epithelium retains the ability to deal with most insults in a swift, albeit controlled, way. The epithelium's major dilemma at any given time is how to maintain a healthy equilibrium between its passive and aggressive behaviors and ensure that the homeostatic balance is preserved. When things go awry and the 'good neighbor' policy is abandoned, pathological disorders such as Crohn's disease may develop. To guarantee a peaceful and mutually beneficial state of affairs, the intestinal epithelium and the commensal microbes must communicate with each other constantly and go through continual adjustments. In this issue of *Nature Immunology*, Salzman *et al.* shed light on how this communication actually happens and the sort of responses that can be mutually evoked[1]. They provide strong experimental evidence supporting the idea of the involvement of enteric α-defensins in shaping the composition of the ileal microbiota and, with it, the immunological landscape of the intestinal mucosa.

Enteric α-defensins are part of the antimicrobial arsenal produced in the mammalian small intestine by Paneth cells, a specialized lineage of secretory cells that localize to the bottom of intestinal crypts, where they are exposed and respond to the luminal microflora[2]. Mice express many isoforms of enteric α-defensins, but humans express only two: human defensin 5 (HD5; encoded by *DEFA5*) and HD6. The antimicrobial activity of Paneth cell α-defensins is associated with protection against enteric *Salmonella typhimurium* infection *in vivo*[3]. On the other hand, functional defects and lower expression of α-defensins have been linked to greater susceptibility to enteric infection and the onset of ileal Crohn's disease[4,5]. Paneth cell α-defensins are synthesized as precursors that are processed into active forms by trypsin in humans and matrix metalloproteinase 7 (MMP-7) in mice. After synthesis, they are stored in secretory granules that are eventually discharged into the intestinal lumen.

Salzman *et al.* have made a detailed analysis of the small intestine microbiota of two genetically modified mouse models previously developed for the study of enteric α-defensin function[1]. Mice homozygous for a transgene encoding HD5 (*DEFA5*-transgenic) produce physiological quantities of this human α-defensin in their Paneth cells[3], and mice lacking MMP-7 ($Mmp7^{-/-}$ mice) are unable to process endogenous α-defensins into their mature, functional forms[5]. Salzman *et al.* report that both strains of genetically modified mice show striking differences in their ileal microbiota composition relative to that of their corresponding wild-type littermates. Although the total bacterial cell numbers are unchanged, there are reciprocal microbial composition shifts in the homozygous *DEFA5*-transgenic and $Mmp7^{-/-}$ intestines. The ratio of Firmicutes to Bacteroidetes is inverted in mice lacking and overexpressing α-defensins, mostly because of changes in the numbers of *Clostridium* and *Bacillus* bacteria. In the *Clostridium* group, the *Eubacterium rectale–Clostridium coccoides* group is more prevalent in the homozygous *DEFA5*-transgenic mice, whereas *Clostridium leptum* is more prevalent in $Mmp7^{-/-}$ mice.

Next Salzman *et al.* focus on the finding that the relative amounts of segmented filamentous bacteria (SFB), a ubiquitous colonizer of vertebrates, are particularly diminished in homozygous and hemizygous *DEFA5*-trangenic mice, which suggests that SFB are very sensitive to the presence of HD5 and indicates that poor colonization by SFB is a phenotypic trait directly associated with the presence of the *DEFA5* transgene. Moreover, the F_1 offspring of hemizygous (*DEFA5*-transgenic) parents lack SFB regardless of their genotype, which indicates poor SFB transmission from hemizygous *DEFA5*-transgenic parents to offspring. The intestinal presence of SFB is restored in the wild-type mice but is not restored in either hemizygous or homozygous *DEFA5*-transgenic offspring by oral gavage with feces containing SFB or by interbreeding with other wild-type mice. Thus, mice lacking the *DEFA5* transgene are still permissive to SFB colonization. A series of wild-type × homozygous *DEFA5*-transgenic crossing experiments in which the hemizygous *DEFA5*-transgenic offspring are exposed to maternal feces only confirms that wild-type but not homozygous *DEFA5*-transgenic mothers are able to transmit SFB to their litters. These are important findings for the general understanding of α-defensin function because they establish a direct connection between the presence of a particular enteric α-defensin and the persistence of a bacterial group in the intestinal microbiota.

It should be noted that both models used by Salzman *et al.* show extreme deviations from the normal enteric α-defensin makeup of mice. Moreover, given the tight interaction of SFB with the intestinal epithelium, is not totally surprising that SFB are uniquely affected by altered amounts of α-defensins. It would be

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