Understand memory, design better vaccines

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Naive lymphocytes have a finite lifespan and are continually replaced by input from generative organs. In contrast, memory cells or their progeny can last a lifetime. The expanded populations of memory cells and their more widespread distribution provide protection against recurrent infection.

At the population level, previous exposure to a pathogen or a form of vaccine that induces immunological memory results in less morbidity and enhanced survival. At the level of the individual organism, this means changes in the numbers and distribution of immune lymphocytes that provide more effective protection against the pathogen. At the cellular level, immunological memory depends on epigenetic changes in T lymphocytes and B lymphocytes that allow a faster effector response to pathogens. Immunological memory in B cells may also reflect genetic alterations as immunoglobulin M (IgM) antibodies switch to IgA or IgG and somatic mutations enhance antibody affinity. Beyond exposure to antigen *in utero* and the provision of maternal antibody, the newborn is considered immunologically naive. Real-world experience in the form of exposure to microbes, innocuous antigen and vaccination soon impinges, however, as the innocent neonate gains experience. Lymphocyte populations begin to change. Some lymphocyte clones activated in the first year of life will survive for the lifetime of the individual organism, and the circumstances that surround the first response to antigen will influence the quality of the response to subsequent encounters. This issue of *Nature Immunology* presents a collection of articles on the changing dynamics of the immune system as a consequence of encountering the real world.

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Real or imagined memory?

Naive and memory T cells are distinguished by their surface markers and by the presence of pre-formed mRNAs and permissive chromatin marks in memory cells that allow them to turn on effector functions more rapidly. Not all memory T cells defined in this way may be the result of previous encounter with foreign antigen, however. As reviewed by Sprent and Surh[1], the circulating lymphocyte pool contains T cells of the memory phenotype even before birth. They argue that many such cells are generated by the normal process of lymphocyte homeostasis when naive cells encounter higher than normal concentrations of common γ-chain cytokines, such as interleukin 7 and interleukin 15, working in concert with weak triggering of the T cell antigen receptor (TCR) by self antigen to stimulate division. Although it flies in the face of conventional wisdom about the restricted repertoire diversity of the memory pool, this fits with results obtained by high-throughput sequencing in experiments comparing the TCR diversity of naive and memory T cell pools[2]. Contrary to the widely held belief that the memory TCR repertoire is restricted (that is, it contains large expanded clones with correspondingly low overall diversity), results have shown that a 21-year-old, healthy, cytomegalovirus-negative human subject had TCR diversity in the memory pool almost as great as that in the naive pool. Only 2–7% of memory clones seemed to be expanded. For this person, either previous antigen challenges left behind few memory cells or most memory cells were the result of slow homeostatic turnover with conversion to memory phenotype. This does not contradict the fact that as humans age and acquire certain persistent infections and T cell clonal expansion, the memory repertoire does narrow considerably.

The best defense

The best defense is to set up multiple lines of defense against the reappearance of a pathogenic microbe. The adaptive immune system actually does this at the level of both B cells and T cells (**Fig. 1**). A first encounter with antigen frequently results in the generation of long-lived plasma cells that home to bone marrow niches or take up residence at mucosal sites and constitutively secrete antibody in an antigen-independent fashion to maintain serum and mucosal concentrations of antibody. The T cell correlate of this is the differentiation of effector memory cells, especially those that home to and then spend extended periods of time in barrier sites (such as the skin and mucosa). After re-entry of antigen or pathogen, as well as antigen processing and presentation, these on-site effectors can work quickly to deal with the breach even without requiring proliferation. If the infection spreads beyond the initial site of entry, circulating central memory T cells and memory B cells encounter antigen in central lymphoid organs. Here T cells will respond with rapid proliferation, making sufficient effector progeny to find pockets of infection anywhere in the body. Memory B cells, if they can capture antigen, may also interact with T cells to produce more plasmablasts that secrete antibody of higher affinity.

The best defense against many infections and the predominant participant in many of the most effective vaccines is pre-existing antibody. This is probably true for vaccines against smallpox, yellow fever and measles. Work reviewed by Pulendran and Ahmed[3] has been aimed at understanding the innate signals and the ensuing adaptive immune responses to the highly successful live attenuated vaccines (such as those mentioned above), as well as to nonliving vaccines that must be 'served up'

with an adjuvant. There is reason to expect that combinations of adjuvants that target multiple Toll-like receptors and various other intracellular alarm systems will prove to be most effective in the goal of achieving long-lived antibody and T cell immunity. Because most human vaccines approved at present depend to a large extent on humoral immunity, it is appropriate that much attention has been focused on the generation of B cell (antibody) memory and the fascinating interactions of activated B cells with antigen-specific $CD4^+$ helper T cells. The fact that B cells and T cells specific for epitopes on the same molecular complex must meet to fully differentiate the antibody response has been known since the days of the so-called 'carrier effect'. There is an antigen bridge between B cells and T cells, even though the links in the carrier protein bridge have been broken by the B cell and displayed on the cell surface as complexes of peptide and major histocompatibility complex. As discussed by Nutt and Tarlinton[4], antigen-activated B cells and T cells meet in secondary lymphoid organs at the border of the B cell follicle and T cell areas and rapidly provide early IgM^+ memory B cells and plasmablasts. Some B cells continue to divide in the follicle and set up new structures called 'germinal centers'. This requires that some of the antigen-specific $CD4^+$ T cells, prodded by their interaction with B cells, also undergo differentiation into follicular helper T cells (T_{FH} cells) and migrate into the germinal center. Antigen held by specialized follicular dendritic cells and handed off to the B cells allows T_{FH} cells to drive clonal expansion and mutation of immunoglobulin variable regions. Events in germinal centers make this one of the most interesting and dynamic structures in the immune response. Both switched (IgG^+ and IgA^+) and nonswitched (IgM^+) memory B cells are produced and can contribute to secondary responses, although in this case the memory B cell response must compete with pre-existing serum antibody continually produced by long-lived plasmablasts. It is notable that in seasonal influenza episodes, some immunoglobulin variable regions may have as many as 50 mutations from the germline sequence[5], which reflects the fact that previously switched memory B cells with fewer variable-region mutations have been recruited once more into a germinal center reaction to the new influenza variant. Because memory B cells must compete for antigen, this is aided by the likely possibility that, for seasonal influenza virus, pre-existing antibody may have low affinity for the new variant.

Unlike B cells, $CD4^+$ T cells do not undergo class switching or somatic hypermutation, but their differentiation into stable memory cells is not a simple process. Depending on the innate stimuli that accompany the foreign antigen, during the primary response $CD4^+$ effector cells may appear as T helper type 1 (T_H1) cells, T_H2 cells or interleukin 17–producing helper T cells. Additionally, for a good humoral response, the $CD4^+$ T cell response must also provide T_{FH} cells. Pepper and Jenkins[6] discuss the relationships between these functional lineages and debate the stability of lineage-committed $CD4^+$ memory T cells. They propose that after bacterial infection, T_H1 effector cells expressing the transcription factor T-bet and $CD4^+$ effector cells positive for the chemokine receptor CCR7 but without T-bet expression are produced simultaneously. T_H1 cells migrate from the central lymphoid organs in search of the antigen source and eventually form stable T_H1 effector memory cells, whereas T_{FH} cells remain in the lymphoid organ to help B cells and exist only as long as the germinal center lasts. It is unclear whether T_{FH} cells then die off or instead convert into uncommitted central memory cells with the plasticity to respond to subsequent antigen challenge by differentiating into a range of effector types as demanded by innate signals.

In many cases, unraveling the role of memory T cells in acquired protection is tricky. One salient feature of protective memory highlighted by Sheridan and Lefrancois[7] is location. Many infections occur at body surfaces, such as the skin, lungs, intestinal tract and genital tract. Notably, the vast majority of effector memory T cells are present at these sites, far outnumbering memory T cells in the circulation and all internal organs combined (**Fig. 1**). How these regional or mucosal memory cells do their job cannot be measured in terms of their proliferative response to re-entry of the pathogen. Like pre-existing antibody, on-site effector memory cells may function by rapidly killing a neighboring infected cell or by recognizing antigen and sounding the alarm to recruit blood-borne innate effector cells without themselves undergoing rapid clonal expansion. In the case of latent infection, especially infection with herpes virus, which might recrudesce at previously infected epithelial, mucosal and neuronal sites, memory $CD8^+$ T cells can remain on site as tissue-resident memory T cells that are separate from the recirculating memory pool[8]. This makes the primed, antigen-experienced immune system seem to be a patchwork of resident effector memory T cells grouped by previous antigen encounter. Resident memory may explain the remarkable phenomenon of 'fixed drug eruptions' that are seen as lesions at reproducible sites on the skin when an inciting drug is taken systemically.

Infections that are rapidly cleared, as well as those that result in small amounts of persistent or sporadically recurring latent antigen, leave the memory T cells functionally intact. However, in situations of ongoing high viral replication during chronic infection, such as those that occur with variants of lymphocytic choriomeningitis virus in mice and

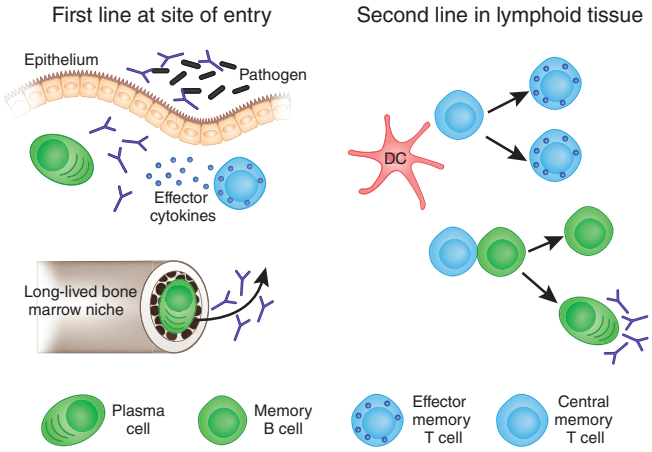

Figure 1 Immunological memory and lines of defense. To provide robust protection, prior exposure to a pathogenic microbe or vaccination with a live attenuated strain or a nonliving form of vaccine should establish adaptive immunity appropriate for the pathogen. This simplified model shows how immediate effector cells of the immune system, such as plasma cells that constitutively secrete antibody and effector memory T cells, provide protection at the site of pathogen entry such as mucosal or skin surfaces (first line at site of entry). Plasma cells may be local, releasing antibody at the mucosal surface or distant in bone marrow niches. Effector T cells can recognize infected cells and accomplish their killing or cytokine-secreting function immediately at the site of infection without cell division. If the infection is not controlled and antigen reaches the draining lymphoid organ, then, with the aid of dendritic cells (DC), extensive proliferation of central memory T cells will ensue, resulting in the production of more effector cells that traffic to sites of infection via the circulation (second line in lymphoid tissue). In addition, memory T lymphocytes and B lymphocytes act together, which results in further enhancement of the antibody specificity.

with human immunodeficiency virus and hepatitis B and C in humans, effector CD8$^+$ T cells can gradually lose functionality as they become exhausted. As discussed by Wherry[9], the kinetics and degree of exhaustion are closely correlated with the amount of antigen the T cells encounter, with a large amount of epitope presentation leading to rapid exhaustion. In these cases, true antigen-independent memory cell differentiation is not achieved, as the effector cells become 'addicted' to TCR triggering for survival. The exhausted T cells are marked by many surface receptors that transmit negative signals, and new treatments that block these signals have shown promise in reversing exhaustion, thereby promoting viral clearance.

Into the unknown

In addition to B lymphocytes and T lymphocytes, natural killer (NK) lymphocytes, as discussed by Paust and von Andrian[10], have also been accredited with the ability to confer adaptive, antigen-specific memory. NK cells lack the expression of RAG recombinase proteins, which are essential for assembly of the genetic elements of B cell antigen receptors and TCRs. NK cells do have a highly complex pattern of expression of the polygenic and polymorphic Ly49 family of innate receptors (and their KIR counterparts in humans). These receptors bind major histocompatibility complex class I molecules or major histocompatibility complex class I–like molecules, which sets up a balance of inhibitory and activating signaling that is established as these cells mature. In C57BL/6 mice, NK cells that express Ly49H, which binds to a mouse cytomegalovirus–encoded molecule, can undergo considerable population expansion after recognizing infected cells, becoming more potent killers and cytokine producers that go on to survive in the long term in greater numbers[11]. Their activity is very much like that of T cells. Painting the skin with a variety of chemically reactive haptens or subcutaneous immunization with purified viral proteins from a range of viruses is also reported to generate specific NK cell memory[12]. The molecular basis of this recognition is unknown. It is conceivable that chemical haptenization of major histocompatibility complex molecules on target cell surfaces results in an imbalance of the inhibitory-activation signals received by a subset of NK cells, and these provide specificity and memory. Explaining how NK cells recognize matrix proteins or group antigen proteins from viruses is very difficult. Because the recognition is RAG independent, in this case it is almost necessary to invoke an undiscovered receptor system like the clonally distributed variable lymphocyte receptors used by lamprey and hagfish.

Concluding remarks

The goal of vaccination is to prevent infectious disease. Many notable, highly effective vaccines have been designed in an empirical way, with full understanding of how and why they work only arriving later. In many ways, the successes have been vaccines against the 'easy' pathogens—those that do not vary much, hide from or rapidly disable the immune system. The science of immunology has progressed so far recently and has provided an understanding of the innate immune signals that serve as adjuvant targets and the diversity of homing and functional subsets of lymphocytes that the time is now ripe for newer successes against the more stubborn infections.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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