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VIEWPOINT

Arrested Differentiation, the Self-Renewing Memory Lymphocyte, and Vaccination

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Vaccination for persistent viral or bacterial infections must program the immune system for a lifelong need to generate antigen-specific effector lymphocytes. How the immune system does this is not known, but recent studies have shown that a subset of B lymphocytes, the germinal center B cell, is capable of self-renewal because it expresses a transcriptional repressor, BCL6, that blocks terminal differentiation. If a similar mechanism for arresting differentiation exists for long-lived, antigen-selected lymphocytes, a stem cell–like capacity for self-renewal could be the basis for the continual generation of effector lymphocytes from the memory pool. Understanding how to regulate the terminal differentiation of lymphocytes will improve immunotherapeutic approaches for chronic infectious diseases and cancer.

Vaccination is the attempt to mimic certain aspects of an infection for the purpose of causing an immune response that will protect the individual from that infection. Usually vaccination is performed for prophylaxis, but it may also have a therapeutic application, as, for example, in the treatment of patients with chronic infections or cancer. Empirical approaches to the development of vaccines have served us well in the past, but the "easy pickings" are over, and to meet current challenges requires a better understanding of the immune system. The starting point of this overview is a description of the aims of an immune response, as these are the endpoints for vaccination.

The immune system must accomplish three goals to protect the host from infectious disease. First is the generation of effector lymphocytes, such as plasma cells to secrete antibody, helper T cells to secrete cytokines and stimulate other immune cells by expressing CD40 ligand, and cytotoxic T lymphocytes (CTLs) to kill virally infected cells. Second is the development of the ability to generate rapidly these effector lymphocytes when antigen is encountered again in the future, a function that is ascribed to "memory" lymphocytes. Third is less explicit but is evident when one considers infections that are chronic, such as those caused by herpes viruses, hepatitis B, human immunodeficiency virus, *Mycobacterium tuberculosis*, etc. These require an ability to generate effector cells continually and over long periods, perhaps for the lifetime of the host. Here, we focus on recent studies that bear on the question of how the immune system might generate effector lymphocytes for the lifetime of the host, and on the possible relation of this process to what has been termed immunological memory.

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The Antigen-Dependent Phase of Lymphocyte Development and Continual Generation of Effector Cells

The adaptive immune system has antigen-independent and antigen-dependent phases of development. During the antigen-independent first phase, the immune repertoire of the immunologically naïve host is created by the generation of clones of B and T lymphocytes, each having a unique antigen receptor. For the most part, these antigen receptors appear not to have been selected for antimicrobial specificity; instead, they provide the host with a vast array of clonally distributed potential antigen-binding specificities. Thus, it becomes virtually certain that all infectious microorganisms will express antigens during some phase of their life cycle that will be recognized by at least a few of these clones. In humans, this phase of B cell development continues relatively unabated into adulthood, whereas the generation of new T cells is drastically reduced because of thymic involution. One might suspect, then, the existence not only of mechanisms to preserve naïve T cells, but also—and of particular relevance to this review—of mechanisms to maintain lymphocyte clones that have been selected during the antigen-dependent phase of development by microbial antigens. This would be important because such cells may be irreplaceable.

During this second phase of differentiation, lymphocytes bind antigen and, with the innate arm of the immune system promoting responses to antigens of microbial origin, initiate complex intracellular and intercellular processes leading to cellular proliferation and differentiation. The proliferation phase is an especially daunting task because the host starts with relatively few antigen-reactive clones in its naïve repertoire, but requires millions (or billions, depending on the size of the host) of terminally differentiated effector lymphocytes just to control the initial infection. This need, which becomes even greater if the infection persists into a chronic phase, is particularly stringent for T

cells because many of their effector functions are executed on a cell-to-cell basis.

In organ systems in which nonreplicating, terminally differentiated cells must be replaced continually (such as the skin, gastrointestinal tract, and blood), a relatively less differentiated stem cell having a high replicative capacity maintains the supply of differentiated cells. By analogy, antigen-dependent lymphocyte development may include a phase in which terminal differentiation is prevented and from which effector cells can be generated indefinitely. The phase that may offer such a function may be represented by the memory lymphocyte, whose presence offers a means of protecting the host from future infections. The ability of the memory lymphocyte to undergo additional rounds of replication and production of effector cells suggests that it also may be the stage at which the replicative potential of antigen-selected lymphocyte clones is preserved. If so, then the memory lymphocyte not only protects against future reinfection, but also maintains the long-term production of effector lymphocytes in chronic, persistent infections. Here, we propose that this function is based on the active suppression of terminal differentiation in these cells, giving the memory lymphocyte a self-renewing capability like that of stem cells in other organ systems.

A Stem Cell–Like, Self-Renewing Function for Memory Lymphocytes

A model for the prolonged and continual generation of effector cells from relatively few initial cells is found in hemopoietic systems of differentiation. A remarkably small number of pluripotent stem cells reconstitutes most, if not all, of the hemopoietic lineages by giving rise to committed progenitor cells that are irreversibly destined to differentiate further to one or a few blood cell types. The committed progenitor cells rapidly replicate a limited number of times before terminally differentiating to effector cells, which have a limited life-span. Because terminal differentiation is coupled to the loss of replicative function, effector cell numbers can be maintained only if one of the daughter cells of a replicating stem cell remains in a nondifferentiated, self-renewing state. There is evidence to suggest that memory lymphocytes might resemble stem cells in having a self-renewing capability.

The evidence suggesting a stem cell–like function for memory lymphocytes comes from two apparently unrelated sets of studies: one analyzing antigen-dependent B cell differentiation, the other characterizing cell surface markers and tissue homing behavior of antigen-activated T cells (Fig. 1). In the B cell lineage, memory cells are derived from the germinal center, in which rapidly proliferating, antigen-stimulated clones of B cells are undergoing an iterative process of proliferation, somatic mutation of their rearranged immunoglobulin (Ig) genes, exit from the cell cycle, and selection by antigen for higher affinity antibody variants (*1*). Eventually, after a number of iterations sufficient to create the mutations necessary for high-affinity antibody, terminal differentiation to a plasma cell is allowed. It is assumed that because the B cell cannot predict how many times it must mutate to create high-affinity antibody for every antigen, there cannot be a programmed number of cellular divisions before terminal differentiation. Therefore, it was proposed that a mechanism exists to suspend terminal differentiation until high-affinity antibody variants have been created (*2*).

BCL6, a transcriptional repressor (*3–5*) that is expressed by germinal center B cells but not by naïve B cells or plasma cells, is required for the germinal center reaction (*6–8*). It suppresses terminal differentiation of the B cell (*2, 9*) by preventing the expression of Blimp-1, a transcription factor that drives the development of plasma cells (*10*). Thus, BCL6, as long as it is expressed, gives the germinal center B cell a stem cell–like capacity for self-renewal. With respect to this function, it is interesting that BCL6 structurally resembles Tramtrack, a *Drosophila* transcriptional repressor that blocks neuronal differentiation. BCL6 may have an additional role during B cell development that is related to maintaining replicative potential. Because germinal center B cells have been selected by antigen, they are the component of the total repertoire that is relevant to host defense. Accordingly, after an infection has been resolved, a few of these cells are retained in a resting, pre–terminally differentiated state known as memory cells. Upon rechallenge with antigen, memory B cells reestablish germinal centers and can undergo up to an estimated 20 additional rounds of replication (*11*). Therefore, the memory B cell has a stem cell–like function and, perhaps not surprisingly, expresses BCL6 (*12*).

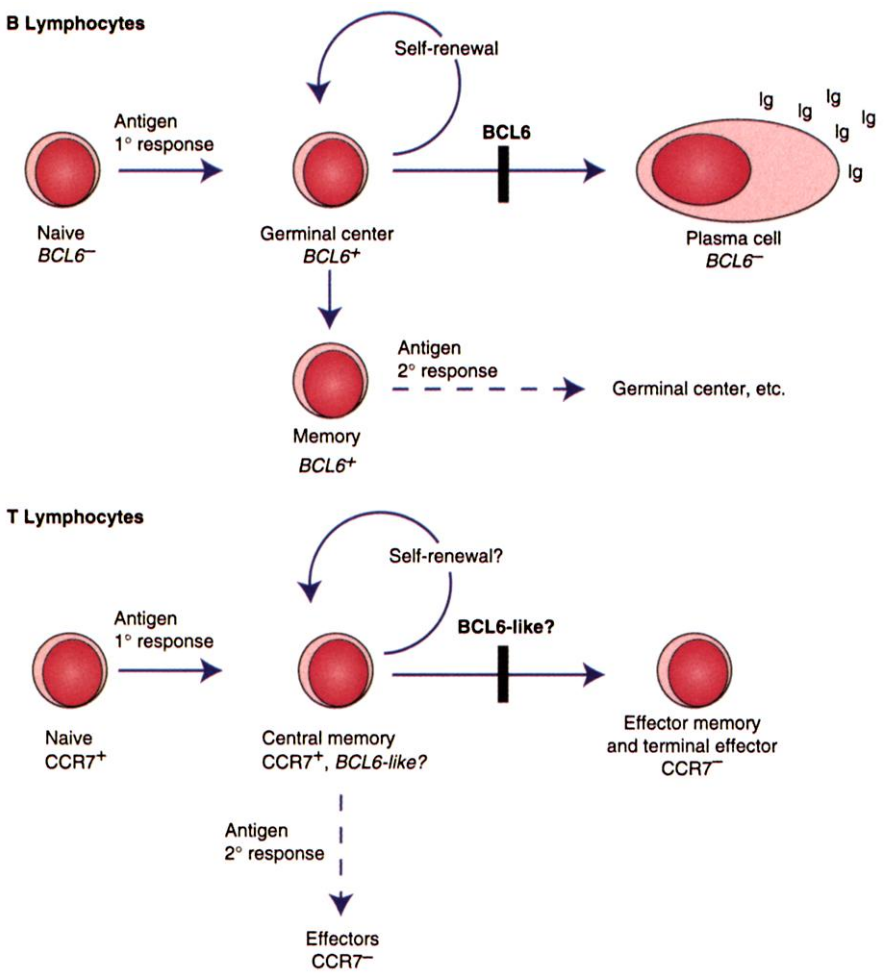

Fig. 1. Antigen-dependent pathways of differentiation of B and T cells. The naïve B cell is induced by antigen to become a rapidly cycling germinal center cell that expresses BCL6 to prevent terminal differentiation. The memory cell maintains expression of BCL6 and can reenter the germinal center phase of development during a secondary response. The naïve T cell is stimulated by antigen to become a $CCR7^+$ central memory T cell, which is proposed to have a stem cell–like self-renewal capability and to express a *BCL6*-like gene. The $CCR7^-$ effector memory T cell differentiates from the central memory T cell and is proposed to undergo a limited number of divisions before terminally differentiating to an effector cell with no replicative function. Some studies suggest that dedifferentiation of effector to memory cells may occur in the T, but not B, lineage.

Defining the stages of antigen-dependent development of T cells has been more difficult because the terminally differentiated T cell is not as morphologically distinct as the plasma cell, and, in contrast to the plasma cell, it may be able to revert to earlier stages of differentiation (*13*). Several studies have begun to resolve this problem by correlating the loss of two cell surface receptors, CD27 and CD28, with terminal differentiation of $CD8^+$ T cells (*14, 15*). Another study (*16*) has defined the developmental stages of T cells on the basis of the expression pattern of CCR7 (a chemokine receptor) and CD45 isoforms. For antigen-activated $CD4^+$ T cells, these stages are (i) naïve cells that are $CD45RA^+$ $CCR7^+$, (ii) "central memory" cells that are $CD45RO^+$ $CCR7^+$, and (iii) "effector memory" cells that are $CD45RO^+$ $CCR7^-$ (Fig. 1). The expression of CCR7 on the central memory set predicted homing of its cells to secondary lymphoid organs, whereas the absence of CCR7 on effector memory cells predicted their homing to inflamed, peripheral tissue sites of infection; these predictions have been confirmed (*17*). Phenotyping of $CD8^+$ T cells showed a similar pattern, with an additional $CD45RA^+$ $CCR7^-$ subset that was considered to be a terminally differentiated effector population, consistent with their lacking CD27 (*16*). As with the $CD4^+$ T cells, the predicted central and peripheral homing patterns of $CCR7^+$ and $CCR7^-$ antigen-experienced $CD8^+$ cells also have been confirmed (*18*). Taken together, these studies are most compatible with a linear pathway of antigen-dependent T cell development from naïve T cells to central memory T cells (both expressing CCR7) to effector memory T cells lacking CCR7 and, in the instance of $CD8^+$ T cells, to effector cells having a reversion to the CD45RA isoform and lacking CCR7. It was not evident from these studies, however, why there might be a need for two subsets of memory T cells.

By analogy to the antigen-dependent pathway of B cell development, we suggest that $CCR7^+$ central memory T cells represent stem cell–like T cells with a self-renewal capability, whereas $CCR7^-$ effector memory T cells represent committed progenitor cells that terminally differentiate after a limited number of cell cycles (Fig. 1). Five predictions arise from this suggestion, some of which have been met. The first is that $CD45RO^+$ $CCR7^+$ T cells are less differentiated than are $CD45RO^+$ $CCR7^-$ T cells; indeed, $CD4^+$ and $CD8^+$ effector memory T cells produce effector cytokines, express perforin, and have cytolytic activity (*16, 19*), whereas central memory T cells do not. Second, a developmental distinction based on the expression of CCR7 suggests that the signals required to maintain the self-renewing state require the microenvironment of secondary lymphoid organs where lymphocytes encounter antigen on specialized dendritic cells. Whether this is correct is not known, but homing to inflamed peripheral sites where antigen is present on target cells is allowed only for $CCR7^-$ T cells that have been permitted to terminally differentiate. Third, after control of the infection, most effector cells should be eliminated (a finding that has been amply confirmed), whereas the self-renewing population should be retained. The fate of the latter cells has not been analyzed as yet, unless these are represented by the transgenically marked subpopulation of memory CTLs that persist after resolution of an acute viral infection in the mouse (*20*). Fourth, ascribing a stem cell–like function to $CD45RO^+$ $CCR7^+$ cells is consistent with the relatively small fraction of the total antigen-specific T cell pool that these cells represent (*19*). Fifth, the $CD45RO^+$ $CCR7^+$ T cells should express a transcriptional repressor with a function analogous to that of BCL6 in B cells. This is not yet known, although there is at least one candidate (*21*). In summary, the antigen-dependent phase of $CD4^+$ and $CD8^+$ T cell differentiation may have a stem cell–like stage of development analogous to the $BCL6^+$ memory B cell, and this phase may enable the life-long generation of antigen-specific effector T cells.

Clinical Implications of the Resemblance of Memory Lymphocytes to Stem Cells

The thesis that memory lymphocytes preserve replicative potential by expressing a transcription factor that arrests terminal differentiation is relevant to developing vaccines for chronic diseases. First, as suspected by the investigators who described exhaustive deletion of antigen-specific CTLs by overwhelming viral infection (*22–24*), this phenomenon might be caused by the differentiation of the entire stem cell–like pool of antigen-stimulated T cell clones to effector cells. Knowing the signals required to induce and maintain a state of arrested differentiation of memory B cells and central memory T cells could allow vaccines to be modified so that they selectively expand this population to provide larger numbers of effector lymphocytes upon subsequent boosting or infection. Perhaps a process similar to this underlies the impressive effects of "prime-boost" vaccines (*25, 26*). Conversely, expanded populations of antigen-specific T cells that lack effector function have been described in patients with viral infections or cancer (*19, 24, 27–29*). With the finding that BCL6 blocks the terminal differentiation of B cells, one can ask whether other transcription factors exist in T cells that suppress effector functions, and whether their expression is dysregulated in these pathological states.

Finally, recent findings confirm beyond any doubt the ability of the immune system to influence the evolution of tumors, and, conversely, of tumors to alter the immune system by rendering potentially protective clones of lymphocytes unable to respond ("anergic" or "tolerant") (*30*). Until antigen-dependent lymphocyte differentiation is sufficiently understood to enable therapeutic manipulation of memory and effector lymphocytes in vivo, adoptive immunotherapy represents the best opportunity to examine the potential utility of these manipulations (*31*). For example, freezing a patient's T cells in vitro at a self-renewing stage by introducing vectors that reversibly express transcription factors that suppress terminal differentiation could enable highly efficient expansion of antigen-specific CTLs. This may permit additional in vitro manipulations that might be too risky if performed in vivo, such as the depletion of $CD25^+$ regulatory T cells (*32*) to unleash high-affinity, tumor antigen–specific T cell clones from anergic suppression. Thus, as with research directed at self-renewing cells in other developmental systems, a focus on stem cell–like memory lymphocytes arising from the antigen-dependent phase of lymphocyte differentiation allows one to consider new opportunities for therapeutic advances. Our further understanding of these cells is warranted.

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