Lymphocyte Life-Span and Memory

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Differentiation of immature T and B cells in the primary lymphoid organs gives rise to a pool of long-lived lymphocytes that recirculate through the secondary lymphoid tissues. On the basis of their surface markers, T and B cells comprise a mixture of naïve and memory cells with differing life-spans. Immunization (and vaccination) causes naïve lymphocytes to proliferate and differentiate into effector cells and memory cells. Whether the survival of memory cells is innate or requires persistent contact with residual antigen is controversial. Resolving this issue may be crucial for designing optimal vaccines.

Before 1960, small lymphocytes were regarded as a drab population of short-lived cells of obscure function. These cells had no known role in the immune response—although it was widely thought that small lymphocytes might act as "trephocytes," allowing themselves to be eaten by other cells as a source of nutrients (*1*). Such quaint ideas were dispelled in the early 1960s by the discovery that most small lymphocytes have a long life-span and recirculate continuously between blood and lymph through defined regions of the lymphoid tissues (*2*). With the subsequent discovery that lymphocytes comprise two functionally distinct lineages, T and B cells, it was rapidly appreciated that typical small lymphocytes are the central players in the immune response. By acting as precursors of effector cells, small T and B cells control nearly all types of antigen-specific immune responses, including responses elicited by vaccines.

Small lymphocytes arise in the primary lymphoid organs: T cells are formed in the thymus (*3*) and B cells arise principally in the bone marrow (BM) in mammals (*4*) and in the bursa of Fabricius in birds (*5*). Lymphocytes released from the primary lymphoid organs are considered to be immunologically naïve, that is, the cells have had little or no contact with foreign antigens. The relation of these virgin cells to the typical lymphocytes found in the secondary lymphoid tissues—spleen, lymph nodes (LNs), and Peyer's patches—is controversial. Some lymphocytes in the secondary lymphoid tissues are thought to be the progeny of cells responding to various environmental antigens. Whether these "memory" lymphocytes comprise the majority of typical small lymphocytes or only a minor subset of these cells has long been a contentious issue.

Similar uncertainty applies to the life-span of lymphocytes. Some lymphocytes are known to have a long life-span, but whether these long-lived cells are naïve cells or memory cells or a mixture of the two has been a topic of debate for many years. This question is central to the issue of how the immune system generates a state of long-lived immunological memory after vaccination with defined antigens. Until recently, most of the cells controlling memory responses were considered to be quintessential long-lived resting lymphocytes residing within the recirculating lymphocyte pool. Now, however, there is evidence that the longevity of memory may be a reflection of continuous contact with antigen persisting from the time of initial priming.

In the context of the issues raised above, this article gives a brief overview of the life history of naïve and memory T and B cells. As a prelude, it is important to consider the specificity of T and B cells and the factors that shape this specificity during early differentiation.

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Lymphocyte Production in the Primary Lymphoid Organs

The specificity of mature T cells is directed to peptide fragments of protein antigens bound to major histocompatibility complex (MHC) molecules (*6*). T cells generally respond more effectively to foreign peptides bound to self rather than to nonself MHC molecules. T cells thus appear to have covert specificity for self MHC molecules. This "physiological" specificity for self MHC is a reflection of positive selection in the thymus to MHC molecules expressed on thymic epithelial cells (TECs).

Thymocyte differentiation begins with the production of large numbers of $CD4^+CD8^+$ cells from $CD4^-CD8^-$ precursor cells in the cortex (*6*). This transition in phenotype is associated with extensive proliferation and rearrangement of T cell receptor (TCR) α and β genes. Cell division in the thymus ceases at about the stage when $CD4^+CD8^+$ cells express TCR αβ heterodimers on the cell surface (*7–9*). Most TCR^+ $CD4^+CD8^+$ thymocytes lack specificity for the self MHC-peptide complexes expressed on TECs, and these cells die in interphase within a few days. Positive selection signals the cells to down-regulate CD4 or CD8 molecules and mature into "single-positive" (SP) $CD4^+$ and $CD8^+$ cells. Except during the earliest stage of selection (*9*), cell division during this transition is minimal, and positive selection can be viewed as a device for keeping self MHC-restricted T cells alive: These cells are protected and allowed to mature, whereas the remainder of the $CD4^+CD8^+$ cells in the cortex succumb to programmed cell death. Some thymocytes have strong reactivity to MHC-peptide complexes, and these potentially autoreactive T cells are destroyed by negative selection (*6, 7–9*). This process takes place in both the cortex and medulla and reflects contact with self antigens displayed on TECs and bone marrow–derived cells; these latter cells control tolerance induction to circulating self antigens. Through the combined effects of positive and negative selection, the T cells leaving the thymus are tolerant to self MHC-self peptide complexes but strongly reactive to self MHC-foreign peptide complexes. Few thymocytes fall into this category, and the vast majority of thymocytes (95%) are destroyed before export.

T cells surviving negative selection enter the medulla as SP cells. These cells differentiate into mature T cells by reducing expression of the heat-stable antigen (HSA) and increasing expression of L-selectin, Qa-2, and high molecular weight restricted (R) isoforms of the CD45 molecule (CD45RA,B,C) (*10*). As a reflection of the relatively slow tempo of T cell maturation after positive selection, the SP cells found in the medulla display considerable heterogeneity in terms of their surface markers. On the basis of the rate of incorporation of DNA precursors such as [^{3}H]thymidine (^{3}HTdR) or bromodeoxyuridine (BrdU), cell turnover in the medulla is comparatively slow, and some cells can remain in the medulla for a week or more without dividing (*7–9*). A proportion of T cells in the medulla appear to represent immigrant mature T cells entering the thymus from the periphery (*11*). However, the vast majority of medullary T cells are probably the direct descendants of young maturing T cells entering from the cortex.

T cells leave the medulla through veins and lymphatic vessels and immediately join the recirculating lymphocyte pool (*12*). The vast majority of T cells released from the thymus are SP $CD4^+$ and $CD8^+$ cells, but these cells require a period of several

days to reach the fully mature phenotype of the typical naïve T cells found in the extrathymic environment (*12, 13*). In terms of cell numbers, T cell release from the thymus is conspicuously low, about 1×10^6 cells per day in young mice (*14, 15*).

The above scheme applies to αβ T cells. T cells with γδ receptors do not display classic MHC restriction, and except in rare cases, intrathymic differentiation of these cells does not seem to involve positive selection (*16*). Most γδ T cells are $CD4^-CD8^-$, and this phenotype is maintained throughout thymic differentiation. Export of γδ T cells from the thymus is very low, about 2×10^4 cells per day in young mice (*17*). The function of γδ T cells is largely obscure, and these cells will not be discussed further.

B cell differentiation in mammals takes place in the bone marrow and involves a series of steps in which proliferating precursor cells rearrange immunoglobulin (Ig) genes and express IgM molecules (*4*). Proliferation ceases at about the stage when B cells become surface IgM^+. As for T cells, B cells appear to undergo positive selection, but whether the ligands for positive selection are of endogenous or exogenous origin is unclear (*18*). Like T cells, B cells tend to be tolerant to circulating self antigens but not to tissue-specific antigens (*19*). For the most part, B cell tolerance is restricted to cell-surface antigens and is induced in the bone marrow during early differentiation. B cell tolerance is largely a reflection of clonal deletion, though clonal anergy can occur under defined conditions (*20*).

The total numbers of surface IgM^+ B cells generated in the marrow is quite large, about 2×10^7 cells per day in young mice (*4*). The proportion of B cells that are exported, however, is unclear (*21*). The prevailing view is that death of B cells in the marrow is limited and that most newly formed IgM^+ B cells are allowed to exit to the secondary lymphoid tissues: The majority of these cells die within a few days and only a small proportion are selected for entry into the pool of mature recirculating B cells. Despite the popularity of this scheme, direct evidence that the marrow exports large numbers of new B cells is sparse. Moreover, in certain other species, namely the sheep, B cell production in the primary lymphoid organs (Peyer's patches) is associated with extensive cell death in situ (*22*).

Like T cells, the B cells released from the primary lymphoid organs undergo a period of maturation for several days before acquiring a fully mature phenotype. In mice, B cell maturation is associated with partial down-regulation of HSA expression (*23*). Typical mature B cells express intermediate (int) levels of the HSA marker, and these HSA^{int} cells account for the majority of B cells in spleen and LN. Newly formed B cells in marrow are HSA^{hi}, and cells with this phenotype account for 10 to 15% of B cells in spleen. Consistent with their recent formation from stem cells, most of the HSA^{hi} B cells in marrow and spleen have a rapid turnover (*23*). Similar data apply in the rat for $Thy\text{-}1^+$ B cells (*24*); Thy-1 expression is high on newly formed rat B cells but is low or absent on mature B cells.

Turnover and Fate of Mature T and B Cells

T cells. Mature $CD4^+$ and $CD8^+$ T cells recirculate continuously from blood to lymph and migrate through defined areas of the spleen, LN, and Peyer's patches. On the basis of surface marker expression, mature T cells comprise two distinct subsets, naïve and memory cells (*25*). Naïve T cells are $CD45R^{hi}$, L-$selectin^{hi}$, $CD44^{lo}$ and are presumed to be the direct descendants of newly formed T cells released from the thymus. Most memory T cells express the $CD45R^{lo}$ (RO^{hi}), L-$selectin^{lo}$, $CD44^{hi}$ phenotype of activated T cells and are thought to be the progeny of naïve T cells responding to environmental antigens (*26*). Although both T cell subsets reside in the recirculating lymphocyte pool, their migratory properties are somewhat different (*27*). Naïve T cells express the LN-homing receptor, L-selectin, which enables naïve T cells to enter LNs from the blood. Memory T cells lack this receptor and reach the LN through afferent lymphatics rather than blood.

As a population, mature T cells have an almost indefinite life-span (*21*). This is apparent from the finding that, in mice, total numbers of T cells decline only very slowly after adult thymectomy (ATx) (*28*) or after T cell transfer to severe combined immunodeficient (SCID) hosts (*29*). The lifespan of naïve and memory T cells is more controversial. Many workers have the view that, after leaving the thymus, naïve T cells have an abbreviated life-span and die within a few weeks unless the cells encounter antigen (*21*); contact with antigen rescues

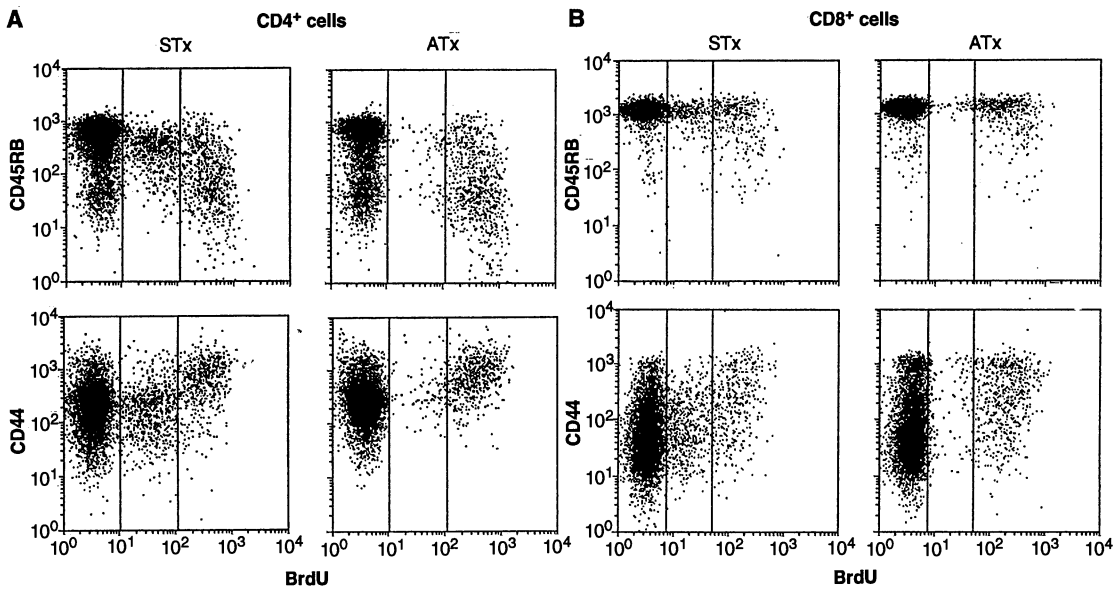

Fig. 1. Cell surface phenotype of recent thymic emigrants and mature T cells after in vivo labeling with BrdU. Adult thymectomized (ATx) or sham thymectomized (STx) C57BL/6 mice were given BrdU (0.8 mg/ml) in their drinking water for 9 days. This was sufficient to label more than 98% of $CD4^+CD8^+$ thymocytes and approximately 70% of mature thymocytes, all of which were $BrdU^{lo}$ (*15*). Three-color staining was used to analyze pooled LN cells for (i) CD45RB or CD44, then for (ii) CD4 or CD8, followed after fixation by (iii) nuclear staining for BrdU incorporation. The data show the expression of CD45RB and CD44 on BrdU-labeled $CD4^+$ cells (**A**) and $CD8^+$ cells (**B**). The vertical lines divide the BrdU-labeled cells into $BrdU^{lo}$ and $BrdU^{hi}$ cells. The $BrdU^{lo}$ cells are largely absent in ATx mice and consist mainly of $CD45RB^{hi}$ and $CD44^{lo}$ cells, consistent with $BrdU^{lo}$ cells being naïve cells of recent thymic origin. The $BrdU^{hi}$ cells are mature T cells present in both ATx and STx mice and are mostly $CD45RB^{lo}CD44^{hi}$ for $CD4^+$ cells and $CD45RB^{hi}CD44^{hi}$ for $CD8^+$ cells. The data for L-selectin expression are not shown. Although most recent thymic emigrants ($BrdU^{lo}$ cells) are L-$selectin^{hi}$, some cells, especially $CD8^+$ cells, are L-$selectin^{lo}$. [Data adapted from (*15*) by copyright permission of The Rockefeller Univ. Press]

the cells and causes a switch to memory cells. The fact that the proportion of memory T cells tends to increase in old age, especially in thymectomized hosts, could be said to favor this idea (*25*). However, naïve T cells rarely disappear in advanced age. In fact, in rodents maintained in a pathogen-free environment, T cells with a naïve phenotype dominate the immune system for months or years, even after thymectomy (*30*). For this reason one can make the case that naïve T cells are potentially very long-lived cells. Because a number of infectious microorganisms release powerful mitogens such as superantigens, the situations in which naïve T cells disappear rapidly and are replaced by memory cells could be attributed to constant exposure to infectious agents. Switching of naïve T cells to memory cells might also reflect exposure to cytokines (*31*).

With regard to T cell turnover, chronic infection could explain the report that short-term treatment of mice with hydroxyurea (a drug that kills dividing cells) causes total numbers of extrathymic T cells to decrease by 40% within 2 to 3 days (*32*). This finding—which is cited as evidence that most T cells are "short-lived"—is at variance with the observations of many groups on the rate of T cell labeling during chronic administration of ^{3}HTdR or BrdU (*21*). Here, the general finding is that most T cells in adult rats and mice divide infrequently, the time required to label 50% of T cells being on the order of weeks or months.

For naïve T cells, the turnover of these cells depends on whether normal or thymectomized hosts are studied. When the thymus is left in place, the turnover of mature extrathymic T cells is obscured by the continuous release of newly formed T cells from the thymus. When mice are given BrdU in their drinking water, T cells released from the thymus are all BrdU labeled and express the typical CD45R^{hi}, L-selectin^{hi}, CD44^{lo} phenotype of naïve T cells (*15*) (Fig. 1). Because of massive cell death in the thymus and local release of DNA precursors, BrdU-labeling of recent thymic emigrants is characteristically much lower than for T cells dividing in extrathymic sites. Thus, BrdU-labeled cells in spleen and LN of thymectomized mice consist almost entirely of BrdU^{hi} cells, whereas a mixture of BrdU^{hi} and BrdU^{lo} cells are found in normal euthymic mice. Counting total numbers of BrdU^{lo} cells in euthymic versus athymic mice has been used to quantitate the rate of T cell export from the thymus (*15*).

With regard to extrathymic T cells, the rate of labeling of naïve-phenotype T cells in thymectomized mice is very slow (*15*). Thus, depending on the surface marker studied, a 4-week course of BrdU administration labels only 5 to 10% of naïve-phenotype T cells. This applies to normal mice. For TCR transgenic mice specific for the HY antigen, the turnover of postthymic T cells is almost undetectable (*33*).

In contrast to naïve T cells, extrathymic T cells with a memory phenotype have a rapid turnover. On the basis of chromosome marker studies, naïve-phenotype (CD45RA$^+$) T cells of humans can remain in interphase for years, whereas memory-phenotype (CD45RO$^+$) T cells divide more frequently (*34*). Rapid turnover of memory T cells also applies to sheep and mice (*21*). For mice, administration of BrdU for 5 weeks labels 70 to 80% of memory-phenotype cells (*15*) (Fig. 2).

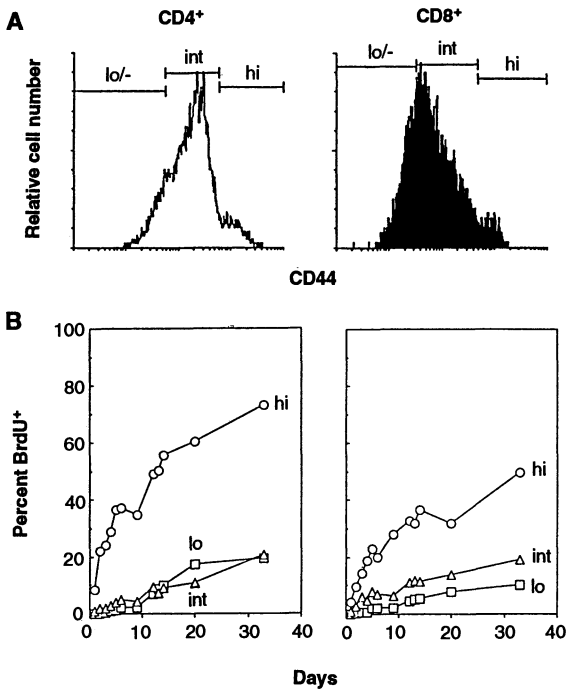

Fig. 2. Turnover of peripheral T cells expressing naïve versus memory markers. Groups of ATx B6 mice were given BrdU in their drinking water for various periods. Pooled LN cells from these mice were then stained for the expression of surface markers and for BrdU incorporation (see Fig. 1). (**A**) CD44 staining profile for CD4$^+$ and CD8$^+$ LN T cells, showing the arbitrary definition of high (hi), intermediate (int), and low (lo) staining. (**B**) Percent BrdU labeling of CD4$^+$ and CD8$^+$ LN T cells expressing lo, int, and hi levels of CD44. BrdU labeling refers to total (BrdU^{lo} plus BrdU^{hi}) staining. [Data adapted from (*15*) by copyright permission of The Rockefeller Univ. Press]

The fact that 20 to 30% of memory-phenotype T cells remain unlabeled during prolonged BrdU administration indicates that a proportion of memory cells are long-lived resting cells. Hence memory cells and activated T cells cannot be equated. Pulse-chase experiments with BrdU-labeled cells have shown that some memory-phenotype T cells can eventually reacquire a naïve phenotype, implying that some memory cells can masquerade as naïve cells (*15*) (Fig. 3). Conversely, some T cells can divide without losing their naïve phenotype. T cells with a naïve phenotype are therefore not necessarily immunologically naïve. In fact many of the naïve-phenotype T cells found in old age could be the progeny of cells responding to antigen in young life. Despite this caveat, as discussed below, cells carrying memory for defined antigens generally display a memory phenotype rather than a naïve phenotype.

The finding that most T cells have a prolonged life-span and divide infrequently only applies to hosts containing normal numbers of T cells. Thus, when T cell numbers are reduced, the residual pool of T cells undergoes considerable expansion (*35*). Such expansion is especially prominent when small numbers of T cells are transferred to immunodeficient (SCID) hosts. In this situation the injected T cells proliferate extensively and give rise to large numbers of memory-phenotype cells. On the basis of studies with T cells from TCR transgenic mice, the expansion of T cells in immunodeficient hosts is antigen specific and is probably a reflection of a chronic response to various environmental antigens and pathogens (*33*).

B cells. Because the ligands for positive selection of B cells are still unknown (*18*), it is unclear whether typical B cells in the secondary lymphoid organs should be regarded as naïve cells selected through contact with undefined self antigens or memory cells primed by various environmental antigens. Nevertheless, in terms of surface marker expression, the majority population of mature B cells is quite different from the typical memory B cells that control secondary responses. In particular, unlike memory B cells, normal unprimed B cells express both IgM and IgD and have not undergone somatic hypermutation (*4*). For simplicity, these cells will be regarded as "naïve." Like T cells, naïve B cells migrate through defined areas of the lymphoid tissues and recirculate from blood to lymph. Although B cells recirculate more slowly than T cells, the vast majority of mature B cells can be mobilized by thoracic duct cannulation over a period of 1 week (*36*). The size of the B cell pool is about 1.5×10^8 cells in mice, which is only slightly less than for T cells (2.0×10^8 cells).

Adoptive transfer experiments and studies with hydroxyurea in mice have led some workers to conclude that most mature B cells have a life-span of only a few days (*32*). The bulk of evidence, however, suggests that B cells have a prolonged life-span and divide infrequently (*21*). Thus, during repeated injection of ^{3}HTdR nearly all B cells in LN and thoracic duct lymph of mice label quite slowly, and it takes about 3 weeks to label 50% of these cells (*21, 36*). Some B cells remain in interphase for even longer periods. Thus, during continuous administration of BrdU, 30 to 40% of spleen B cells can remain in interphase (fail to incorporate BrdU) for up to 3 months (*37*). Similarly, only 20 to 30% of IgM^+D^+ HSA^{int} B cells incorporate BrdU over a 4-week labeling period (*23, 38*). Comparable findings have been reported for mature (Thy-1^-) B cells in rat LN (*24*). A slow rate of B cell turnover also applies to the minor subset of $CD5^+$ B cells in the peritoneal cavity (*39*).

The above data apply to typical naïve IgM^+D^+ HSA^{int} B cells. The HSA^{hi} subset of B cells in spleen has a rapid turnover (*23*), and most of these cells are probably newly formed B cells released from the marrow. However, some HSA^{hi} B cells appear to be the progeny of mature B cells. These mature HSA^{hi} B cells persist after transfer to SCID hosts and show a rapid turnover (*38*). These cells may be the counterpart of memory-phenotype T cells. However, information on the surface phenotype of proliferating mature B cells is still incomplete. Some of these cells are IgM^+D^-, but others may express other Ig isotypes and thus represent "true" memory cells. Interestingly, some mature B cells can divide in the periphery without changing their naïve IgM^+D^+ phenotype (*38*). Thus, as for T cells, many B cells with an apparently naïve phenotype may not be immunologically naïve, especially in old age.

Memory Cells for Defined Antigens

Primary responses to T-dependent antigens, including antigens used for vaccination, generally lead to a state of long-lived immunological memory (*40*). Secondary responses to antigen occur more rapidly than primary responses and are usually more intense. In most situations, memory responses are associated with an increase in precursor frequency. In addition, memory T and B cells generally have higher affinity for antigen than unprimed cells. In considering the generation of memory cells to defined antigens, it is important to discuss the fate of lymphocytes participating in primary responses.

Primary responses to T-dependent antigens involve a proliferative response by naïve T and B cells (*40*). These cells then mature and differentiate into effector cells. Under the control of newly synthesized homing receptors, activated T and B cells leave the recirculating lymphocyte pool and migrate throughout the body, especially to mucosal surfaces, and mediate their effector functions. Primary responses to infectious agents such as viruses are usually of short duration and lead to rapid elimination of the agent concerned; antigen becomes limiting and the response wanes. Most of the cells participating in the response are then eliminated (*41*). Disappearance of these cells seems to reflect a combination of cell death in the lymphoid tissues and irreversible homing to mucosal tissues. Various mechanisms including overstimulation ("exhaustion") and loss of contact with growth factors have been invoked to explain the death of effector cells.

The elimination of effector T cells at the end of the primary response applies to diverse antigens, including superantigens, histocompatibility antigens, and viruses (*41*). Eliminating these cells may be a device to prevent the repertoire of naïve T and B cells from being swamped with primed cells. With high doses of antigen, the elimination of effector cells can be extreme and lead to functional tolerance. The more usual finding, however, is that elimination of specific lymphocytes is incomplete: Some cells survive and differentiate into long-lived memory cells. The factors controlling cell survival leading to memory are still poorly understood. For $CD8^+$ T cells, generation of memory cells seems to reflect preferential survival of high-affinity cells. Such affinity maturation also applies to B cells. In contrast to T cells, however, affinity maturation of B cells is a reflection of somatic hypermutation (*4, 42*). Curiously, naïve and memory B cells may represent different lineages (*43*).

Because immune responses to viruses encountered in young life generally lead to life-long immunity, it has been assumed for many years that memory is carried by long-lived cells that revert to resting cells after antigen is cleared (*44*). In accordance with the view that memory cells are not in cell cycle, long-term memory cells are resistant to destruction by "suicidal" doses of ^{3}HTdR

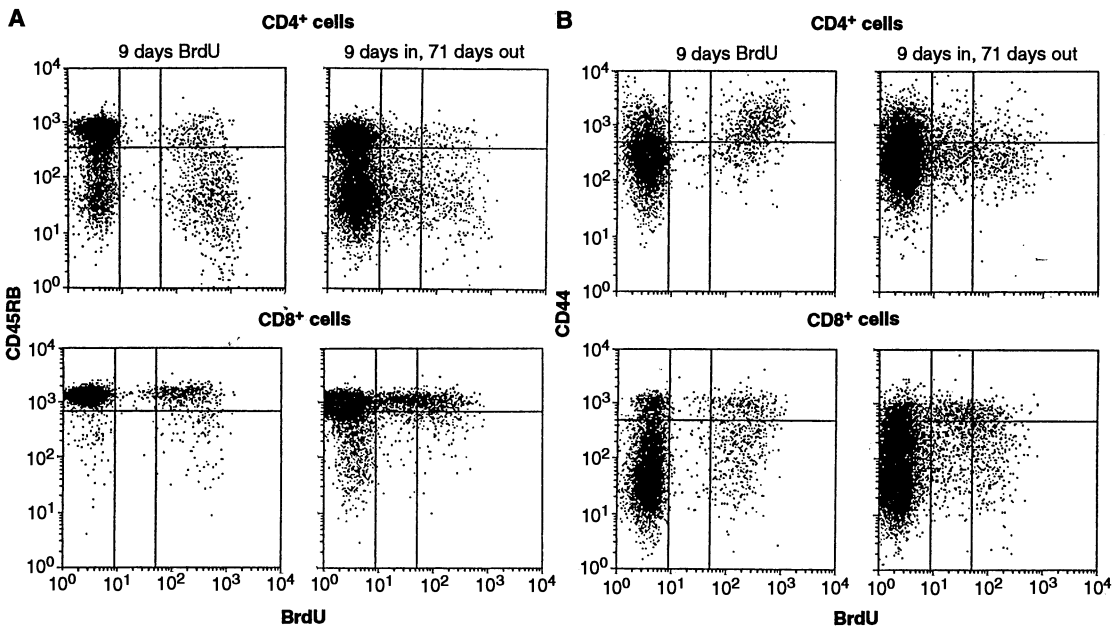

Fig. 3. Decay of BrdU labeling in T cells from ATx mice after discontinuation of BrdU administration. This pulse-chase approach is possible because BrdU is not reutilized. ATx B6 mice were placed on BrdU water for 9 days and then on normal water for a further 71 days. The data show the level of CD45RB (**A**) and CD44 (**B**) expression on BrdU-labeled $CD4^+$ cells (top) and $CD8^+$ cells (bottom). Although there is a marked switch from $BrdU^{hi}$ to $BrdU^{lo}$ cells, implying extensive division during the chase period, some of the cells remained $BrdU^{hi}$, indicating very limited division of these cells. Although both $BrdU^{lo}$ and $BrdU^{hi}$ cells comprised a mixture of memory-phenotype and naïve-phenotype cells after the chase period, there was a definite switch from a $CD44^{hi}$ to $CD44^{lo/int}$ phenotype among $CD4^+$ cells; this was much less apparent for $CD8^+$ cells. [Data adapted from (*15*) by copyright permission of The Rockefeller Univ. Press]

(45) and, at least for B cells, show only a slow rate of labeling during chronic administration of BrdU (46). Antigen-specific memory T cells generally express the same $CD45R^{lo}$, L-selectin^{lo}, $CD44^{hi}$ phenotype as the subset of "memory-phenotype" T cells found in normal animals (40) (a proportion of which are resting cells). For B cells, memory cells generally differ from naïve cells in expressing Ig isotypes other than IgM. Memory B cells also differ from naïve B cells in being HSA^{lo} and $CD44^{hi}$.

Despite the evidence that most long-term memory cells appear to be noncycling cells, memory cells share many of the surface markers of activated cells. This raises the question of whether the maintenance of long-term memory reflects continuous contact with residual antigen persisting from the time of initial immunization. The evidence on this question is conflicting (47). Some workers have reported that, both for T and B cells, memory responses decay rapidly when primed cells are transferred adoptively in the absence of specific antigen (48). These workers therefore conclude that survival of memory cells requires continuous exposure to antigen, perhaps in the form of immune complexes bound to the follicular dendritic cells of germinal centers. Despite these findings, two studies on long-term memory cells have shown that purified $CD8^+$ T cells primed to viral antigens fail to disappear after adoptive transfer in the apparent absence of specific antigen (49). In view of this finding, the authors conclude that maintenance of memory does not require persistence of antigen. The authors point out, however, that survival of memory cells may reflect cross-reactive specificity for environmental antigens or even for self antigens.

In light of the above data, drawing broad conclusions on the role of specific antigen in maintaining memory is difficult. As suggested elsewhere (47), one can make the case that constant exposure to antigen is only crucial for early memory cells (many of which display overt signs of activation). The central issue is whether some form of antigenic stimulus, either from specific antigen or environmental antigens, is required for the survival of late memory cells. Despite the evidence that most late memory cells are in interphase, long-term memory $CD8^+$ cells do not appear to lose their memory phenotype, for example, high CD44 expression (49). Because this phenotype is shared by activated T cells, the survival of long-term memory cells might require a covert form of stimulation that is sufficient to maintain expression of activation markers but not to push the cells into cycle. Alternatively, up-regulation of surface markers such as CD44 might be irreversible and not require constant stimulation of the cells. Resolving this issue is clearly important for vaccine design.

Implications for Vaccine Design

In considering the most effective methods for inducing the production of memory cells, it is worth reiterating that most of the antigen-specific lymphocytes participating in primary immune responses differentiate into short-lived effector cells and are rapidly eliminated. Only a small proportion of the cells survive to become memory cells. Indeed, it was mentioned earlier that in certain situations the elimination of the responding cells can be near complete and lead to functional tolerance. Designing effective vaccines for memory cell generation thus begs the question of how lymphocytes avoid being eliminated during the normal immune response.

The elimination of lymphocytes can occur during different stages of the immune response. In typical responses, lymphocytes proliferate extensively and disappear only after the responding cells have differentiated into effector cells and cleared the antigen concerned; elimination of the responder cells is only partial, and appreciable numbers of cells survive to become memory cells. However, in certain situations lymphocytes are eliminated or clonally inactivated at very early stages of the immune response. This is exemplified by the finding that T cell responses to bacterial superantigens can lead to selective disappearance of some of the responding cells within the first 24 hours of the response, that is, well before the onset of proliferation (50). This phenomenon may be related to "high-zone tolerance," a form of rapid-onset unresponsiveness induced by immunization with large doses of soluble antigen (51). The mechanism of high-zone tolerance is still poorly understood, largely because the precursor frequency of antigen-specific lymphocytes in normal animals is too low to trace the fate of tolerized cells. This problem can be avoided by use of TCR transgenic mice as hosts. As with normal animals, priming TCR transgenic mice with soluble peptide antigens mixed with adjuvant leads to a strong initial proliferative response and then subsequent elimination of most of the proliferating cells (52). When peptides alone are injected, however, the T proliferative response is weak and leads to rapid onset of clonal elimination or induction of anergy.

Although the mechanism of tolerance induced by soluble antigens has yet to be resolved, there is general agreement that generation of long-lived memory to soluble antigens requires the presence of adjuvant (53). Adjuvants are presumed to promote immunogenicity by trapping antigens in sites accessible to reactive lymphocytes and also by inducing antigen-presenting cells (APCs) to express co-stimulatory molecules such as B7. Arguably, the main function of adjuvants, however, is to allow the immune system to store antigen for prolonged periods and thereby provide a continuous stimulus for memory cells. But here one has to come to grips with the issue of why the survival of at least some types of memory cells requires contact with persisting antigen.

In considering this question, it is notable that the late elimination of specific lymphocytes in normal primary responses is much less marked in *bcl-2* transgenic mice (54). Because up-regulation of *bcl-2* expression makes lymphocytes relatively resistant to apoptosis, one can envisage that up-regulation of *bcl-2* is a prerequisite for the generation of memory cells (55). The survival of memory cells may also depend on a failure to up-regulate apoptosis-inducing molecules, such as Fas (56). Regulation of such molecules as bcl-2 and Fas could be heavily influenced by the precise conditions encountered by lymphocytes during and after antigen priming. In the case of priming with soluble antigen without adjuvant, the responding lymphocytes are exposed to high concentrations of antigen for only a brief period. These conditions might stimulate rapid up-regulation of Fas but not bcl-2, thus causing the progeny of the responding cells to quickly succumb to apoptosis. Conversely, by trapping antigen for extended periods, adjuvants prolong lymphocyte contact with antigen; in addition, the antigen-trapping activity of adjuvants may limit antigen uptake by APCs and thus guard against excessive stimulation of T cells. These conditions could be crucial for bcl-2 up-regulation and thus facilitate long-term lymphocyte survival and differentiation into memory cells. Though largely hypothetical, this scenario is relatively easy to test in TCR transgenic mice.

As argued above, designing optimal vaccines for memory cell generation may hinge on reaching a clear appreciation of how different forms of antigen presentation influence the expression of death and survival genes in naïve lymphocytes and their progeny. Because the properties of the three main subsets of memory cells—$CD4^+$ cells, $CD8^+$ cells, and B cells—are distinctly different, vaccines will probably have to be tailor made for each subset.

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