The Instructive Role of Innate Immunity in the Acquired Immune Response

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Innate immunity has been considered only to provide rapid, incomplete antimicrobial host defense until the slower, more definitive acquired immune response develops. However, innate immunity may have an additional role in determining which antigens the acquired immune system responds to and the nature of that response. Knowledge of the molecules and pathways involved may create new therapeutic options for infectious and autoimmune diseases.

Two general systems of immunity to infectious agents have been selected during evolution: innate, or natural immunity, and acquired, (adaptive), or specific immunity. The former is phylogenetically older, with some forms presumably present in all multicellular organisms, whereas the latter evolved about 400 million years ago and is found only in cartilaginous and bony fish, amphibians, reptiles, birds, and mammals (*1*). The essential difference between the two systems is the means by which they recognize microorganisms.

Definitions of Innate and Acquired Immunity

Innate immune systems use proteins encoded in the germ line to identify potentially noxious substances. These proteins, whether they are cell surface receptors or soluble, seem usually to recognize carbohydrate structures. For example, macrophages endocytose particles or soluble glycoconjugates that are bound by the mannose receptor, a C-type lectin with broad carbohydrate specificity (*2*). Macrophages also have a receptor for lipopolysaccharide (LPS). This common constituent of Gram-negative bacterial outer membranes signals the presence of infection by stimulating the synthesis of chemicals and cytokines, such as interleukin-1 (IL-1), IL-6, IL-12, and tumor necrosis factor (TNF), that induce the acute phase response, enhance the microbicidal functions of macrophages and other cells, and promote the development and growth of helper T cells (discussed below) (*3*). Some natural killer (NK) cells also have lectin-like membrane receptors that are involved in the recognition of target cells destined for cytolysis (*4*). The major soluble protein effector of innate immunity, complement, is activated when either its alternative pathway interacts with carbohydrate-rich particles lacking sialic acid (*5*), or its classical pathway is triggered by the binding of collectin to certain carbohydrates (*6*). Thus, innate immunity has divided the universe into innocuous and potentially noxious substances according to their particular carbohydrate signatures. Recognition of carbohydrates may have evolved because these common constituents of microbial cell walls have functions, and thus structures, that are distinct from those of carbohydrates of eukaryotic cell surfaces.

In contrast to this "hard-wired," relatively inflexible system is the almost infinitely adaptable acquired immune system of lymphocytes. Using products of the *RAG1* and *RAG2* genes, B and T lymphocytes somatically rearrange the V, D, and J elements of their immunoglobulin (Ig) and T cell receptor (TCR) genes to create as many as 10^{11} different clones of B and T lymphocytes that express distinct antigen receptors (*7*). The receptors on B lymphocytes recognize conformations of native antigen, which may be protein, carbohydrate, or simple chemical groups, whereas the receptors of most T lymphocytes recognize only peptides, which are derived from protein antigens, that are bound to cell surface proteins termed major histocompatibility complex (MHC) class I (*8*) and class II (*9*). A peptide-based system of recognition provides a broader range of molecular structures for immune responses than carbohydrates and enables T cells to detect nonglycosylated, virally encoded proteins derived from the cytosol of infected cells. Clones of lymphocytes that have receptors of adequate affinity are triggered by antigen to proliferate and develop into effector cells. After elimination of an infection, the antigen-specific clones remain expanded as "memory" lymphocytes that provide a more rapid response to a second exposure to the antigen (*10*). This quality of memory enables acquired immunity to construct, by selective processes that have an immediate rather than evolutionary time scale, hard-wired responses that are appropriate for contemporary infectious agents.

In adopting this strategy for coping with the genetic variability of microorganisms, however, acquired immunity has sacrificed a cardinal characteristic of innate immunity; the inherent ability to distinguish between potential pathogens that require an immune response, and innocuous substances for which an immune-response is either unnecessary or, in the example of self-antigen, injurious (*11*). Here we show that cellular and soluble components of innate immunity (Table 1) provide instruction that enables the acquired immune response to select appropriate antigens and the strategies for their elimination (Fig. 1).

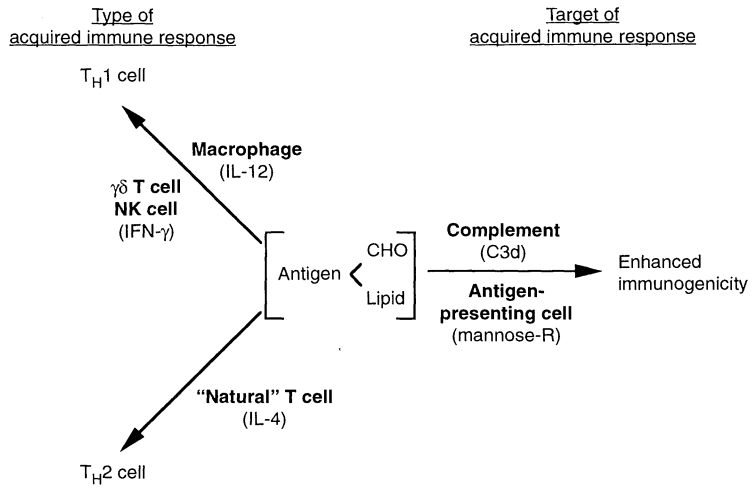

Fig. 1. Components of innate immunity (in boldface) that recognize carbohydrates (CHO) and lipids and instruct the acquired immune response to the protein antigens with which they are associated.

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Innate Immunity and Selection of Antigens for Acquired Immune Responses

Helper T lymphocytes (T_H cells) orchestrate an acquired immune response by promoting intracellular killing by macrophages, antibody production by B lymphocytes, and clonal expansion of cytotoxic T lymphocytes. The TCRs of T_H cells trigger cellular activation when they are bound by complexes between peptides and MHC class II membrane proteins on antigen-presenting cells (APCs). The peptides, which are generated by the breakdown of proteins present in endosomal compartments, including those inhabited by certain intracellular parasites and mycobacteria, are loaded onto newly synthesized class II proteins in a post-Golgi vesicular compartment. The complex is then delivered to the plasma membrane (9). A second signal is also required from the APC that binds CD28, a membrane protein of T_H cells that together

Table 1. Major recognition molecules of the innate immune system. Abbreviations: CRP, C-reactive protein; SAP, serum amyloid protein; MBP, mannose binding protein; CRD, Ca^{2+}-dependent carbohydrate recognition domain; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; PMN, polymorphonuclear leukocytes; C3, complement factor 3; SRCR, scavenger receptor cysteine-rich domain; and SCR, short consensus repeat.

Molecule	Structure	Location	Ligands	Function
		Humoral		
CRP	Pentraxin; Ca^{2+}-dependent lectin	Primary synthesis in liver; part of acute phase response; increases from 1 μg/ml to >1 mg/ml in plasma	Microbial polysaccharides	Activates complement; enhances phagocytosis
SAP	Pentraxin; Ca^{2+}-dependent lectin	Primary synthesis in liver; normal levels 30 μg/ml in plasma	Extracellular matrix proteins; microbial cell wall carbohydrates	Enhances phagocytosis; stabilizes extracellular matrix proteins
MBP	Collectin; contains 18 CRD sites per molecule on helical collagenous domains	Primary synthesis in liver; normal levels vary with allelic variants; up to 10 μg/ml	Microbial cell wall saccharides	Binds collectin (C1q) receptor; activates complement; promotes phagocytosis; modulates CD14-induced cytokine production
LBP	Lipid transferase	Primary synthesis in liver; normal levels <0.5 μg/ml, increase to 50 μg/ml with acute phase response	Catalytically transfers LPS to CD14 and from CD14 to serum lipoproteins	Enhances sensitivity to LPS; system for inactivating LPS
sCD14	Leucine-rich protein	Plasma protein, 3 μg/ml; presumed shed from myelomonocytic cells	LPS; numerous microbial cell wall components	Enhances sensitivity to LPS 100- to 10,000-fold; complex with LPS binds to receptor on endothelium, PMNs, and macrophages
C3	Disulfide-linked dimer	Primary synthesis in liver; normal levels 1 mg/ml; induced by acute phase response	Forms ester linkage to OH-groups on carbohydrates and proteins	Attachment of ligand for receptors such as CD21 and CD35
		Cellular receptors		
Mannose receptors				
Macrophage mannose receptor	8 CRDs	Tissue macrophages; hepatic endothelial cells	Multiple carbohydrates	Potentially targets antigens to class II–loading compartment
DEC-205	Mannose-type receptor; 10 CRDs	Dendritic cells; thymic epithelium	Multiple carbohydrates	Potentially targets class II–loading compartment
Scavenger receptors				
Type I	Type II trimeric transmembrane protein with helical collagenous stalk domain and terminal SRCR domain	Tissue macrophages; hepatic endothelial cells; high endothelial venules	Bacterial and yeast cell walls	Clearance of LPS and microbes; adhesion
Type II	Alternatively spliced form missing the terminal SRCR domain			
MARCO	Extended form resembling type I	Marginal zone of spleen; medullary lymph node; macrophages	Bacterial cell walls	Bacterial clearance
Lipopolysaccharide receptor				
CD14	Lipid-anchored glycoprotein; leucine-rich protein	Monocyte-macrophages; PMNs	LPS; numerous microbial cell wall components	LPS sensitivity; clearance of microbes; proinflammatory cytokine induction
Complement receptors				
CD35 (CR1)	30 SCRs	Monocyte-macrophages, PMNs, and lymphocytes	C3b, C4b	Enhances C3b and C4b cleavage
CD21 (CR2)	15 SCRs	B lymphocytes; follicular dendritic cells	iC3b, C3dg, C3d	Augments B cell activation by antigen
CD11b, CD18 (CR3)	Integrin	Monocyte-macrophages; PMNs, NK cells	iC3b, LPS, fibrinogen	Adhesion; LPS clearance

with the TCR costimulates the transcription of the gene encoding IL-2 and stabilizes IL-2 messenger RNA (mRNA) (*12*). This second signal is provided by the expression of the CD28 ligands, B7.1 (CD80) and B7.2 (CD86), on APCs (*13*). Therefore, processes that select proteins for endocytosis by APCs, or augment the expression of B7.1 or B7.2, determine which antigens activate T_H cells.

Dendritic cells are the most potent APCs for T_H cells (*14*). After their development in the bone marrow, they transiently reside in nonlymphoid organs where they are extremely active in bulk, fluid phase macropinocytosis. Internalized proteins are degraded, and the resulting peptides associate with newly synthesized class II molecules to form complexes that are expressed at the plasma membrane. In response to inflammatory stimuli, such as LPS, these precursor dendritic cells migrate to T cell zones of lymph nodes and mature into effective APCs by ceasing endocytosis, stabilizing expression of class II–peptide complexes, and increasing expression of B7.1 or B7.2. Dendritic cells select potential T cell antigens by taking up microbial glycoconjugates through specialized receptors. A mannose receptor [relative molecular mass (M_r), 175,000] on cultured human dendritic cells mediates the endocytosis of greater than 10^5 molecules of mannosylated protein per cell per hour (*15*). A membrane protein (M_r, 205,000) of murine dendritic cells that is structurally homologous to the macrophage mannose receptor internalizes ligand via coated pits and vesicles for delivery to a multivesicular endosomal compartment containing MHC class II proteins (*16*). This process increased by 100-fold the efficiency of antigen presentation to T_H cells. Macrophages also are important APCs, and targeting an antigen to their scavenger receptors increases the antigen's immunogenicity (*17*).

Innate immunity also can shape the antibody response of acquired immunity. On the plasma membrane of B cells is a complex that contains two proteins: CD19, which is a component of the acquired immune system, and CD21, a receptor for the C3d protein of complement and, therefore, of innate immunity (*18*). CD19 is required for a normal antibody response to antigens that are dependent on the interaction of B cells with T_H cells (*19*), and amplifies signaling by membrane immunoglobulin (mIg) through its capacity to bind intracellular proteins, such as phosphoinositol 3-kinase and Vav (*20*). This function necessitates the cross-linking of CD19 to mIg, and complement can serve this purpose by covalently attaching the C3d fragment of the C3 protein to microbial carbohydrate antigens (*21*). In a model system, the threshold dose for immunizing mice with hen egg lysozyme was reduced by a factor of 10,000 by the attachment of three copies of C3d to the antigen (*22*). Activation of complement in a nonimmune host can occur via "natural" IgM, the acute phase reactant, C-reactive protein (Table 1), collectins, or the alternative pathway.

Innate Immunity and the Type of Acquired Immune Response

Different microbes require different types of responses for their elimination. Type 1 responses are mediated primarily by macrophages and involve the phagocytosis and intracellular killing of microorganisms; type 2 responses are macrophage-independent and are mediated by noncytotoxic antibodies, mast cells, and eosinophils. We will focus on the MHC class II–restricted, $CD4^+$, $\alpha\beta$ T_H1 and T_H2 cells that mediate these distinct responses, although $CD8^+$, MHC class I–restricted $\alpha\beta$ T cells (*23*), and $\gamma\delta$ T cells (*24*) also can participate in type 1 or 2 responses.

T_H1 cells promote type 1 responses by secreting cytokines, such as interferon γ (IFN-γ), lymphotoxin, and TNF-α (*25*). These cytokines induce nitric oxide (NO) synthase in macrophages to increase their microbicidal activity, and IFN-γ causes murine B cells to switch their Ig isotype to IgG2a (in humans, IgG1), which promotes phagocytosis by activating the classical pathway of complement and binding to Fc receptors on macrophages. T_H1 cells also express the Fas ligand, which induces contact-mediated apoptosis of Fas-positive cells (*26*). T_H2 cells mediate type 2 responses by secreting the cytokines IL-4, IL-5, IL-6, IL-10, and IL-13, which collectively mediate the growth and activation of mast cells and eosinophils, direct murine B cell Ig switching to IgE and IgG1 (in humans, IgG4), and inhibit macrophage activation. Although interest in the T_H2 cells has been directed at their protective role in helminthic infections (*27*) and pathogenic role in allergy, they may have important regulatory functions in countering the tissue-damaging effects of T_H1 cells and macrophages (*28*).

T_H1 and T_H2 cells can develop in response to signals derived from the innate immune system (*29*). Activation of tissue macrophages through cell surface pattern receptors or by CD14 to which LPS has bound causes the secretion of IL-12 and TNF-α. IL-12 induces the differentiation of naïve T_H cells to the T_H1 phenotype through its ability to maximize IFN-γ and curtail IL-4 production by stimulated naïve T_H cells (*30*). LPS also causes macrophages to produce IFN-γ–inducing factor, which may have similar effects (*31*). By virtue of their ability to take up and present antigen, macrophages may also bias the development of T_H1 cells. T_H cells responding to antigen express CD40 ligand, which may cross-link CD40 on macrophages to stimulate secretion of IL-12 and TNF-α (*32*). These two cytokines synergize with IL-2 from T cells, or with IL-15 from the activated macrophages themselves (*33*), to induce production of IFN-γ by NK cells. IFN-γ, in turn, augments IL-12 secretion and activity through its capacity to activate transcriptionally both the inducible p40 component of the heterodimeric IL-12 protein by macrophages and the second component of the IL-12 receptor on T and NK cells. Thus, IFN-γ and IL-12 comprise an autocrine positive feedback system that amplifies the levels of IFN-γ for macrophage activation and IL-12 for the proliferation and activation of NK and T_H1 cells (*34*).

Some T lymphocyte subpopulations with specialized functions express a restricted set of V(D)J genes and may be considered to share with innate immunity the characteristic of being hard-wired. T cells that have rearranged predominantly the $V_\gamma2V_\delta2$ (also called $V_\gamma9V_\delta2$) genes can be activated by antigens containing prenyl pyrophosphate (components of microbial membrane involved in carbohydrate attachment) that do not require intracellular processing or association with MHC proteins (*35*). These T cells secrete IFN-γ and TNF-α to induce T_H1 differentiation. Other cytokines produced by activated macrophages and NK cells, particularly granulocyte-macrophage colony-stimulating factor, cause macrophages to express CD1a, -b, and -c. These nonpolymorphic MHC proteins can present nonpeptide mycolic acids and lipoglycans from microorganisms to subpopulations of $CD4^-CD8^-$ (double negative) and $CD8^+$ $\alpha\beta$ T cells to result in IFN-γ production and cytolytic activity (*36*). Thus, the nonrandom V(D)J rearrangement in some T cells may reflect selection for genes that are specific for products of microorganisms that are best eliminated by type 1 responses.

The development of T_H2 cells requires IL-4 during priming of naïve T_H cells (*37*), and the cellular sources of this cytokine, other than T_H2 cells, may be as varied as are the sources for IL-12 and IFN-γ for type 1 responses. APCs, through their expression of B7.2 (*38*) or the ligand for CD30 (*39*), preferentially induce IL-4 from responding T cells. Members of the C-C chemokine family produced by stimulated macrophages tend to recruit type 2 effector cells (*40*), and their production in response to different pathogens must be systematically examined. A subpopulation of T cells that express the NK cell markers NKR-P1 and Ly-49 are $CD4^+$ or $CD4^-CD8^-$ and have a relatively invariant TCR comprised of a canonical α chain (in the mouse, $V_\alpha14J_\alpha281$; in humans,

$V_\alpha 24J_\alpha Q$) paired with a restricted population of rearranged β chains (predominantly $V_\beta 8$ in the mouse and $V_\beta 11$ in humans) (*41*, *42*). These natural T cells, or NT cells, rapidly produce large amounts of IL-4 upon binding of the nonclassical MHC class I protein, CD1 (the homolog of human CD1d) (*43*). It is not clear whether activation of these cells is regulated by induced expression of CD1d in inflammatory loci, binding of nonpeptide microbial products analogous to those identified for CD1a, -b, and -c, or recognition of hydrophobic peptides bound in the narrow cleft of CD1 (*44*). Expression of CD1d by intestinal epithelial cells may contribute to the propensity for type 2 responses with oral immunization.

Pathologic Consequences of Altered Innate Immunity

The propensity for pathologic viruses to capture genes that target cytokines produced by cells of the innate immune system illustrates the survival value of interrupting communication between the innate and the acquired immune system (*45*). Soluble receptors for IL-1, TNF-α, and the type I interferons have been described; in some instances, these viral receptors have broader species specificity and greater affinity for the cytokine ligand than the mammalian receptors (*46*) and would be capable of competing with specific cytokine receptors, thus abrogating their activation.

Differences in resistance to pathogens among inbred strains of mice has led to uncovering genes involved in innate resistance. Positional cloning identified one such gene, *Nramp1*, that is involved in resistance to several intracellular organisms, including *Mycobacterium bovis*, *Leishmania donovani*, and *Salmonella typhimurium*, perhaps by compartmentalizing or concentrating substrates for NO synthase (*47*). A family of *Nramp* genes has been defined and shown to be conserved among organisms ranging from *Drosophila* to humans, but the role of Nramp in determining susceptibility to human infectious diseases remains to be established. Intriguingly, Nramp1 controlled the rejection of hematopoietic tumor cells by murine T cells that expressed $V_\gamma 1.1$, the most common γδ T cell population in peripheral lymphoid organs of the mouse ($V_\gamma 9$ in humans) (*48*), linking innate resistance to tumor surveillance.

Viruses also have coopted aspects of the complement system to enhance infectivity. Epstein-Barr virus and measles virus use two membrane proteins of the complement system, CD21 (*18*) and CD46 (*49*), respectively, as cellular receptors for attachment. Vaccinia encodes a protein that has the structure and function of a family of complement regulatory proteins (*50*). Helminthes also have been shown to synthesize proteins that suppress activation of complement (*51*).

Inherited deficiencies of complement proteins also are associated with impaired defense against microbial infection. However, the occurrence of the autoimmune disease systemic lupus erythematosus in patients deficient in the proteins of the classical pathway of complement provides perplexing evidence for a role of complement in tolerance to self (*52*). Rare individuals with total deficiencies of C1, C4, or C2 have a 30 to 75% chance of developing this disease. A function of the classical pathway may be to remove self antigen-antibody complexes that are normally formed. In the absence of this pathway, these complexes accumulate, activate the alternative pathway, are coated with C3d, and become sufficiently immunogenic to break self-tolerance.

Therapeutic Considerations

Administering antigens with IL-12 engenders strong T_H1 responses that have been shown to provide protective immunity (*53*). The capacity of IL-12 to suppress IL-4–mediated activities has also been used as an "anti-pathology" vaccine to block allergic or fibrosing syndromes (*54*). Antigens from pathogens that elicit IL-12 release from macrophages or dendritic cells should prove powerful adjuvants for eliciting T_H1 responses (*55*). Additional approaches could involve the preferential blockade of B7.1- or B7.2-derived costimulatory signals (*56*) and the use of Schiff base–forming drugs that potentiate T_H1 responses (*57*). Strong T_H1 responses, however, result in tissue-destructive, autoimmune syndromes (*36*, *58*), and an inverse relation between the numbers of IL-4–producing NT cells and the incidence of autoimmune disease has been proposed (*59*). Thus, upregulation of CD1d to create ligands for NT cells may suppress autoimmune diseases mediated by type 1 responses. Proteins that microorganisms use to subvert innate immunity, such as soluble cytokine receptors and complement inhibitors, are potential targets for acquired immune responses. Effective vaccines may be developed by attachment of C3d or carbohydrates to these antigens to target them to receptors on B cells, macrophages, and dendritic cells.

Conclusion

The essence of innate immunity is the detection of molecules that are unique to infectious organisms. This capability allows the innate immune system to guide the selection of antigen by B and T lymphocytes, and the secretion by helper T lymphocytes of cytokines that promote an appropriate host response to the infection. Therefore, mammalian innate immunity is not merely a vestige of ancient antimicrobial systems that have been made redundant by the evolution of acquired immunity. Rather, it dictates the conduct of the acquired immune response.

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60. Supported by the Wellcome Trust (D.T.F.), National Institutes of Health (grant AI26918 and AI30663) (R.M.L.), and the Burroughs Wellcome Fund (R.M.L.).

Immunological Memory and Protective Immunity: Understanding Their Relation

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The immune system can remember, sometimes for a lifetime, the identity of a pathogen. Understanding how this is accomplished has fascinated immunologists and microbiologists for many years, but there is still considerable debate regarding the mechanisms by which long-term immunity is maintained. Some of the controversy stems from a failure to distinguish between effector and memory cells and to define their roles in conferring protection against disease. Here the current understanding of the cellular basis of immune memory is reviewed and the relative contributions made to protective immunity by memory and effector T and B cells are examined.

An 18th-century natural experiment that occurred on the remote Faroe Islands provided rare insight into the mechanism of protective immunity against infectious diseases. This experiment began in 1781 with a measles outbreak. During the ensuing 65 years, the Faroes remained measles-free until a major outbreak in 1846 that affected 75 to 95% of the population. Ludwig Panum, a Danish physician, investigated this epidemic and made the interesting observation that "of the many aged people still living on the Faroes who had had measles in 1781, not one was attacked a second time" (*1*). This natural experiment had also provided Panum with a control group, and he astutely noted that "all the old people who had not gone through with measles in earlier life were attacked when they were exposed to infection." Panum's classic study made two points: first, that immunity to measles was long-lived; and second, that reexposure to the virus was not essential for maintaining long-term protective immunity. The first point was important but perhaps not that surprising; after all, the Greek historian Thucydides, describing the plague of Athens in 430 B.C., had noted that "the same man was never attacked twice" (*2*). It was Panum's second point, that protective immunity could be sustained in the absence of reexposure to measles virus, that provided a critical insight into the nature of immunological memory. This observation, which was supported by observations made during epidemics of yellow fever in Virginia (*3*) and polio among Eskimo villages in Alaska (*4*), showed that the immune system could remember an encounter that occurred many years ago and that there are inherent (endogenous) mechanisms for sustaining this long-term memory.

The subject of immune memory has been extensively studied, but there is still considerable debate regarding the mechanisms by which protective immunity is maintained (*5*). In this article, we will review our current understanding of the cellular basis of immunological memory and then examine the relative contributions of memory and effector T and B cells to protective immunity. We will also consider differences between protection against mucosal versus systemic infections—a critical issue that is often ignored. We hope this review will provide a framework for trying to understand why protective immunity is long-lived in some situations and of shorter duration in others.

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T Cell Memory

It is well established that T cell memory, as assessed by accelerated recall responses in vivo, is long-lived (*5*). However, the nature of T cell memory has remained controversial, with debate centered around two opposing views (*5*). One view postulates that memory is due to long-lived memory cells that do not require contact with specific antigen for their survival. The other envisions long-term memory as the result of continuous stimulation of T cells by persisting antigen. Although these are interesting and useful hypotheses, they have imposed constraints on the understanding of the cellular basis of T cell memory. For instance, it is conceivable that memory T cells may be antigen-independent but still cycle because of some nonspecific stimulus. It is also plausible that memory cells are long-lived but need antigen stimulation to maintain a state of readiness. Instead of trying to distinguish between the two opposing hypotheses, we will examine memory within the broad framework of the following questions: What is the cellular basis for accelerated recall responses? Are memory T cells distinct from effector cells? What is the lineage of memory T cells? Is specific antigen necessary for the maintenance of T cell memory? What is the life-span of memory T cells? Do these cells cycle? If so, what is the stimulus? Before addressing these questions, it is useful to review the kinetics of primary T cell responses.

Both CD4 and CD8 T cell responses can be broken down into three distinct phases: (i) activation and expansion, (ii) death, and (iii) stability or memory. During the initial phase, which typically lasts about a week, antigen-driven expansion of the specific T cells and their differentiation into effector cells occur. In several viral systems, between 100- and 5000-fold expansion of virus-specific $CD8^+$ T cells takes place (*6*–*13*). Substantial expansion of $CD4^+$ T cells has also been reported for several antigenic systems (*14*, *15*) and a recent study (*16*) has documented up to 1200-fold expansion of $CD4^+$ T cells responding to pigeon cytochrome c (PCC). A period of death then ensues (between days 7 and 30), during