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THE REGULATION AND MATURATION OF ANTIVIRAL IMMUNE RESPONSES

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In this chapter, we review how the immune response to viral infection is regulated, and how the effector arms of the response mature over the course of infection and beyond. The complexity of the antiviral immune response is great, and requires that we be selective in the topics that we discuss. Consequently, we focus almost entirely on the adaptive (antigen-specific) immune response, and refer only briefly to innate immunity; our discussion of adaptive immunity, although covering both antibodies and T cells, favors T cells. Our overall intent is to describe the adaptive immune response, focusing on both quantitative and qualitative changes that occur over the course of a viral infection.

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I. Overview of the Immune Response to Viral Infection

Over the past several thousand years, the urbanization of human society has permitted viral infections to wreak havoc on human health. Led by smallpox, viruses have killed or incapacitated hundreds of millions of people throughout past centuries, but the advent of widespread antiviral vaccination has had dramatic effects: not only has it permitted the eradication of the smallpox virus, and the approaching extirpation of poliovirus, but it also has come close to consigning diseases such measles, mumps, and rubella to the pages of history. Despite this progress, viruses continue to exact a heavy toll in human suffering. Human immunodeficiency virus (HIV) is thought to infect almost one in four Africans (Gregson *et al*., 2002); previously unidentified viruses have emerged to cause substantial harm—most recently exemplified by the novel coronavirus that causes severe acute respiratory syndrome (SARS) (Fouchier *et al*., 2003; Kuiken *et al*., 2003); and old adversaries, such as influenza virus, give sporadic reminders of the threats that they pose (Shortridge *et al*., 1998).

The main bulwark protecting the population from microbial onslaught is the immune system. The efficacy of the antiviral immune response is well established: (i) the majority of infections, even by viruses considered highly pathogenic, are resolved by an immunocompetent host; (ii) this requires an intact immune system, because even normally innocuous virus infections can be fatal in immunosuppressed individuals; and (iii) the enormous benefits of antiviral vaccination rely on the adaptive immune response. The importance of vaccination is well demonstrated by the current reemergence of measles as an important human pathogen. Irrational parental fears of measles vaccine side effects have led to reduced vaccine uptake in some countries, with potentially catastrophic consequences. There have been sporadic outbreaks of measles in areas of the United States with low vaccine uptake (Robbins, 1993), and in the United Kingdom, parental acceptance of the MMR vaccine has dropped to a level that may eventually permit the measles virus to become endemic once again (Jansen *et al*., 2003). Perhaps most striking is that low vaccine coverage has resulted in an explosion of measles in Japan—an estimated 30,000 to 200,000 cases annually (Nakayama *et al*., 2003)—and deaths have numbered in the hundreds. Thus, to address current and future viral challenges, and to further improve the safety and efficacy of available antiviral vaccines, it is imperative that the immune responses to viral infection be fully understood.

The immune response can be classified in several ways, but we consider classification most logical by *antigen specificity.* Thus, immune responses may be termed *nonantigen-specific* or *antigen-specific*. As the name indicates, nonantigen-specific responses do not rely on recognition of specific antigenic motifs; these responses are broad-based and include phagocytes, natural killer cells, type I interferons, and "barrier" defenses, such as skin, lysozyme, and gastric acid. Their actions are exerted very early in the course of combating an infection, and they do not require any form of antigenic "instruction"; consequently, they are termed *innate* immune responses. In contrast, the antigen-specific immune system can learn from experience and thus is termed *adaptive* immunity Upon first encounter with any given antigen, the antigen-specific responses will be somewhat slow to develop, usually becoming detectable only after the innate responses have approached their peak; however—and in contrast to the innate responses—upon second exposure to the same agent, the antigen-specific responses are greatly improved, both in quantity and quality. These enhanced antigen-specific secondary immune responses—termed *anamnestic* (from the Greek word for *recall*)—originate from memory cells that are specific for the antigens previously encountered and that are the cornerstone of all antiviral vaccines. Both the innate and adaptive immune responses play key roles in controlling a viral infection, and it is becoming increasingly clear that these responses are not, as previously supposed, separate and are instead inextricably linked; however, our goal in this chapter is to cover the adaptive response, and we shall provide no further description of the innate immune system herein.

Adaptive immunity relies on lymphocytes, of which there are two classes: T-lymphocytes (derived from the thymus) and B lymphocytes (named for the avian organ, the bursa of Fabricius). B lymphocytes give rise to antibody-producing plasma cells. Antibodies act mainly to diminish the infectivity of free virus, whereas T cells recognize (and often kill) infected cells. Thus, antibodies and T lymphocytes act in a complementary manner. Antibodies neutralize viruses in the fluid phase (e.g., blood, lymph, interstitial spaces), thereby reducing the number of infected cells and easing the T lymphocytes' workload. T lymphocytes kill infected cells before virus maturation has occurred, minimizing the release of an infectious virus and thus easing the load on antibodies. In the following pages, we shall review the biology of B and T lymphocyte responses to virus infection and vaccination.

II. B Lymphocytes and Their Role in Antiviral Immune Responses

Preexisting antibody provides the first line of defense against infection. The potential of serum transfer in the prevention and treatment of infectious disease was first appreciated more than 100 years ago, and serum- or plasma-derived antibody preparations were the only therapeutic resources available prior to the advent of antibiotics or antiviral drugs, and the development of pathogen-specific vaccines. Although highly effective vaccines now play the predominant role in protection against many viral and bacterial pathogens, in recent years the development of safer "humanized" monoclonal antibodies, together with a better understanding of the antimicrobial roles of humoral immunity, have led to a resurgence in the study of antibody-mediated protection against disease. Herein, we describe the antiviral functions of antibody molecules and the role of humoral immunity in a variety of acute and chronic viral infections.

B lymphocytes recognize microbial antigens via the B cell receptor [BcR, more commonly termed immunoglobulin (Ig) or antibody (Ab)], a cell-surface molecule which, as the B cell matures into a plasma cell, is synthesized in secretable form. There are several different classes (isotypes) of Ig (see following paragraphs), and we shall use the most abundant class, IgG, as the basis for our description of structure of antibodies and of the genes that encode them. An IgG molecule comprises four chains, two "heavy" (H) and two "light" (L). The H chain is composed of an N-terminal variable region, followed by three constant domains that are almost identical within any one antibody class (e.g., IgG or IgM) and that define the effector functions of that antibody class. The L chain contains one variable and one constant region. Each L chain is noncovalently paired with one H chain, and the contiguous H and L variable regions together form one antigen recognition site, thereby defining the antigen specificity of the antibody. Two identical H/L chain pairs are themselves noncovalently linked to form the canonical Y-shaped IgG molecule, which has two antigen recognition sites. This Y-shaped structure is common to all antibody classes (Section, II.A.1). The DNA sequences encoding the constant and variable regions are physically separate in the germ line, but undergo rearrangement to form the gene that encodes an H or L chain. Furthermore, the variable regions themselves are generated by the rearrangement of small genetic segments: the variable region of a heavy chain results from the rearrangement of three segments, termed V_H, D_H, and J_H, and the variable region of an L chain is formed by the fusion of a V_L segment and a J_L segment. A variety of V, D, and

J segments are present in the genome, and this combinatorial aspect of antibody gene formation allows a relatively small number of V, D, and J segments to generate a large number of different variable regions, estimated (in humans) at around 300 for L chains and 11,000 for heavy H chains. If H and L chains associate randomly, this provides $\sim 3.3 \times 10^6$ possible combinations (and, thus, antigen specificities) of H/L pairs. In addition, during the process of V–(D)–J rearrangement, small numbers of nucleotides can be added or lost, and this "junctional diversity" increases the number of different H/L specificities to around 10^{11}. Antibody diversity can be further increased by the process of somatic hypermutation, described later.

A. How Antibodies Combat Viral Infections

Antibodies can exert their antimicrobial effects by a variety of different mechanisms (Burton, 2002). For instance, a virus can be "neutralized" prior to infection of its target cell. This can occur by the antibody's binding to the surface of the virus and, by steric hindrance, blocking its ability to bind to its target. Alternatively, some antibodies can be directly virucidal, or they may activate the complement cascade (reviewed in Spear *et al.*, 2001), leading to disruption of the viral membrane. In addition to these mechanisms of direct antibody-mediated killing of the invading pathogen, many types of phagocytic cell express antibody Fc-receptors; antibodies can bind to these receptors, coating the phagocyte and allowing it to recognize, engulf, and destroy microbial pathogens. Although most antibody functions are exerted on free virus particles, in some cases a virus remains vulnerable to antibody-mediated destruction even after it has entered a host cell; if virus proteins are presented on the cell surface and are recognized by the ensuing antiviral antibody response, then destruction of the infected target cell may occur by means of complement-dependent cytotoxicity or by destruction by antibody-dependent cell-cytotoxicity mediated by NK cells or other cell types expressing the appropriate Fc-receptors. For example, when researchers examined the direct *ex vivo* cytolytic response of volunteers immunized with vaccinia, they could not detect a direct *ex vivo* T-cell-mediated lytic response but instead found that direct *ex vivo* lytic activity required NK cells and vaccinia-specific antibodies (Perrin *et al.*, 1977). Interestingly, antibodies do not necessarily need to destroy the infected cell to stop or slow the spread of infection. Some antibodies have been shown to block virus release from infected cells (Gerhard, 2001; Vanderplasschen *et al.*, 1997), interrupt cell-to-cell spread (Burioni *et al.*, 1994; Pantaleo

et al., 1995; Vanderplasschen *et al.*, 1997) or, in the case of some neurotropic viruses such as measles (Fujinami and Oldstone, 1979) or Sindbis virus (Levine *et al.*, 1991), antiviral antibodies may block viral replication without directly resulting in destruction of the infected cell. Together, these studies demonstrate that humoral immunity can result from a multitude of independent and interrelated mechanisms of antiviral activity.

1. Different Antibody Classes and Their Attributes

Antibody is produced in five different classes: IgG, IgM, IgA, IgD, and IgE. These antibody molecules differ in their molecular composition as well as in their biologic functions (Padlan, 1994).

- IgG is the most abundant class of immunoglobulin in the serum (mean adult serum level is ~12 mg/mL) and, in humans, can be organized into four subclasses, IgG1, IgG2, IgG3, and IgG4, which respectively constitute approximately 70%, 15%, 10%, and 5% of total serum IgG. The main effector function shared by all four IgG subclasses is neutralization, although human IgG3 also very effectively activates the complement system. IgG1 is especially effective in opsonization, a process in which the pathogen becomes coated with antibody and the multiple exposed IgG constant domains facilitate internalization by phagocytes expressing Fc receptors. Opsonization is generally more important in countering bacterial, rather than viral, infection.
- IgM is structurally similar to an IgG molecule, but its H chains carry a fourth constant region. It is the first immunoglobulin expressed at the surface of a developing B cell, and as the cell matures, the antibody is secreted into the plasma in the form of a star-shaped multimeric array of five antibodies; plasma IgM molecules therefore contain 10 antigen recognition sites. The multiplicity of antigen-binding sites would seem to make IgM well-suited for neutralization and it is, perhaps, surprising that its main biological role appears to be complement activation.
- IgA is similar in appearance to IgG, but forms a dimer that has the capacity to be actively transferred across epithelial surfaces, allowing its entry into luminal spaces; as a result, dimeric IgA is a key factor in providing barrier mucosal immunity.
- IgE structure is similar to that of a single IgM molecule (with four constant regions in the H chain), but it does not form multimers. Its serum level is orders of magnitude lower than that of other classes, and it is instead found on the surface of mast cells, where it plays a role in allergies (and, perhaps, in immunity to parasites).

• IgD is superficially similar to IgG, but its function remains unknown (although it can substitute for IgM, if genetic defects prevent the synthesis of that antibody class; Lutz *et al.*, 1998). The biological features of the three classes most important for controlling virus infection—IgM, IgG, and IgA—are summarized in Table I.

2. The Efficacy of Antibodies in Controlling a Variety of Human Viral Diseases

There are several possible outcomes of virus infection: some virus families cause acute (i.e., short-lived) infections, whereas others can persist in the host for months or years. It is, therefore, important to understand the role played by antibodies in preventing infection, or disease progression, under these widely disparate circumstances. One school of thought is that antibodies play a key role in controlling acute infections, but not persistent infections (Kagi and Hengartner, 1996). The authors noted that during acute infections, infected cells are rapidly destroyed by the virus, and therefore cellular immunity (which exerts its effect by acting on infected cells) may be of minimal importance; under these circumstances, the host must rely on antiviral antibodies. In contrast, cellular immunity may be more important than antibodies during persistent infection, when the host's goal is to eradicate virus that is "hiding" inside cells. Does this proposal fit the data? The prophylactic and therapeutic efficacy of antibodies against a number of human viruses is summarized in Table II. In support of the hypothesis, there are many instances of lytic viral infections (most notably poxviruses and flaviviruses) that are highly susceptible to antibody-dependent immunity However, there are several exceptions,

TABLE I
SUMMARY OF THE BIOLOGIC FEATURES OF THE THREE IG CLASSES THAT COMBAT VIRUS INFECTIONS

	IgM	IgG	IgA
Appearance time	Early	Later	Later
Location of abundance	Serum	Serum and interstitial spaces	Serum and mucosal secretions
Placenta crossing	No	Yes	No
Antigen recognition sites	10	2	4
Neutralization	+	++	++
Complement activation	+++	++	+

TABLE II

SUMMARY OF ANTIBODY EFFICACY IN THE PREVENTION AND TREATMENT OF A VARIETY OF HUMAN VIRAL DISEASES

		Antibody-mediated protection?	
Virus family	Pathogen	Prevention	Treatment
Arenaviridae	Junin virus	—	Yes[17]
	Lassa fever virus	—	Yes[18]
			Possible[19]
			No[20]
Filoviridae	Ebola virus	Yes[1]	Yes[21]
Flaviviridae	Yellow fever virus	Yes[2]	No[22]
	West Nile virus	Yes[3]	Yes[23]
	Tick-borne encephalitis virus	Yes[4]	Possible[24]
Hepadnaviridae	Hepatitis B virus	Yes[5]	No[25]
Herpesviridae	Cytomegalovirus	Yes[6]	Possible[26]
	Varicella-zoster virus	Yes[7]	Yes[27]
Paramyxoviridae	Measles virus	Yes[8]	Yes[28]
	Respiratory syncytial virus	Yes[9]	—
Picornaviridae	Polio virus	Yes[10]	—
	Hepatitis A virus	Yes[11]	—
Poxviridae	Vaccinia virus	Yes[12]	Yes[29]
	Smallpox virus	Yes[13]	Possible[30]
Retroviridae	Simian human immunodeficiency virus	Yes[14]	—
Rhabdoviridae	Rabies virus	Yes[15]	—
Togaviridae	Chikingunya virus	Yes[16]	No[31]

[1] Gupta *et al.*, 2001; Parren *et al.*, 2002: [2] Monath and Cetron, 2002; Sawyer, 1931: [3] Ben-Nathan *et al.*, 2003: [4] Kreil and Eibl, 1997: [5] Anonymous, 2003: [6] Wittes *et al.*, 1996: [7] Balfour, Jr. *et al.*, 1977; Fisher and Edwards, 1998: [8] Stiehm, 1979: [9] Romero, 2003: [10] Hammon *et al.*, 1953: [11] Ward and Krugman, 1962: [12] Kempe, 1960: [13] Kempe *et al.*, 1961: [14] Nishimura *et al.*, 2002; Parren *et al.*, 2001: [15] Prosniak *et al.*, 2003: [16] Igarashi *et al.*, 1971: [17] Enria and Barrera Oro, 2002: [18] Frame, 1989: [19] McCormick, 1986: [20] White, 1972: [21] Mupapa *et al.*, 1999: [22] Monath, 2003: [23] Ben-Nathan *et al.*, 2003: [24] Kreil and Eibl, 1997: [25] Keller and Stiehm, 2000: [26] Keller and Stiehm, 2000: [27] Ogilvie, 1998: [28] Stiehm, 1979: [29] Kempe, 1960: [30] Peirce *et al.*, 1958: [31] Igarashi *et al.*, 1971.

in which a preexisting antibody response to a typically nonlytic (persistent) virus appears to afford partial and, in some cases, complete protection against infection. For example, some arenaviruses can persist for the lifetime of the host, but it is well established that administration of convalescent serum or plasma results in a significant level of protection against lethal Junin virus infection (Enria and Barrera Oro, 2002), although the results for protection against Lassa fever virus, another member of the Arenavirus family, have been mixed (Clayton, 1977; McCormick, 1986). The prototypic arenavirus, LCMV, can establish lifelong persistence in mice, and immunity against this agent is mediated largely by $CD8^+$ T cells, consistent with the hypothesis. Monoclonal LCMV-specific antibodies, however, can ameliorate disease (Wright and Buchmeier, 1991), and a vaccine that appeared to induce antibodies in the absence of protective levels of $CD8^+$ T cells also could confer protection (Di Simone and Buchmeier, 1995), again indicating that the hypothesis may be an oversimplification.

The results outlined in Table II illustrate an important point; antibodies are generally more effective prophylactically than therapeutically. Preexisting antibody (from acquired immunity or by passive transfer) often can prevent, or at least ameliorate, disease caused by a subsequent virus infection, but the same antibody is less effective when administered after severe, disease symptoms have appeared. This may be due to overwhelming levels of virus that cannot be adequately controlled by a finite amount of transferred antibody. Conversely, there may be very little virus remaining at a time when symptoms are most severe, because many symptoms of viral diseases reflect the immunopathology that occurs during virus clearance (Slifka and Whitton, 2000b) (see Section VI). Nevertheless, antibody-mediated therapies are beginning to gain wider acceptance, especially now that humanized monoclonal antibodies are more easily obtained and can be used in place of convalescent sera. For example, Palivizumab is the first humanized monoclonal antibody licensed for the prevention of respiratory syncytial virus (RSV) infections and, since its introduction in 1998, it has had a significant impact on the number and duration of RSV-associated hospitalizations in susceptible infant populations (Romero, 2003). Other monoclonal antibody formulations are also showing promise; in animal models, a combination of monoclonal antibodies has been shown to be effective in postexposure prophylaxis against rabies virus (Prosniak *et al.*, 2003), and a vaginally applied monoclonal antibody directed against the HIV-1 gp120 molecule protects against mucosal virus transmission (Veazey *et al.*, 2003). Monoclonal antibody therapy has at least two major advantages over

convalescent serum, including (i) low lot-to-lot variation in neutralizing titer, giving a guaranteed standard of therapeutic efficacy and (ii) a significantly decreased risk of contamination with human viruses or other clinically relevant pathogens, a common risk factor encountered when administering convalescent serum or plasma.

B. Memory B Cells Acting as Antigen-Presenting Cells

In addition to producing antibodies to directly combat microbial infections, some B cells—most prominently memory B cells—also help to regulate the immune response by acting as antigen-presenting cells. Memory B cells are detectable in lymphoid organs and the bloodstream within 1 to 2 weeks after acute viral infection, and are maintained at steady-state levels thereafter. These cells do not secrete antibody, and instead maintain cell-surface expression of their immunoglobulin receptors so that they can recognize their specific antigen. Once bound, the antigen is internalized and processed, and the viral epitopes are presented at the cell surface by major histocompatibility complex (MHC) class II molecules; these complexes on the surface of memory B cells are extremely effective triggers of antigen-specific $CD4^+$ T cells responses (Lanzavecchia, 1985), the importance of which is described below. Highly activated memory B cells can also proliferate and differentiate into antibody-secreting plasma cells, the main cell type involved with maintaining antibody levels after vaccination or infection. There appears to be a clear division of labor between these related cell types, in that memory B cells are mainly involved with antigen processing, presentation, and mounting anamnestic immune responses, whereas plasma cells are unlikely to be involved with antigen uptake or presentation, because they are largely deficient in surface immunoglobulin and show little or no MHC class II expression (Abney *et al*., 1978; Halper *et al*., 1978; Slifka *et al*., 1998). Plasma cells instead devote most of their energy to the production and secretion of antibody.

C. The Cells That Serve as the Source of Long-Term Antibody Production

Many viruses, and many vaccines (both live and inert), induce antibody responses that remain detectable for years after antigen exposure and, since the half-life of an antibody molecule is measured in weeks, the longevity of the response must be explained by ongoing antibody synthesis. There is general agreement that long-term antibody levels

are maintained by the combined efforts of two largely distinct cell types, memory B cells and plasma cells, but there is substantial controversy regarding the role(s) played by each. Plasma cells secrete up to 10,000 molecules of antibody per second (Helmreich *et al*., 2003; Hibi and Dosch, 1986), and typically are measured by the ELISPOT technique, which detects spontaneous antibody production by individual cells. Plasma cells accumulate in the spleen or in draining lymph nodes during the early stages of an acute viral infection, but then as the infection is resolved and the local immune response subsides, the majority of virus-specific plasma cells are typically found in the bone marrow compartment (Bachmann *et al*., 1994; Hyland *et al*., 1994; Slifka *et al*., 1995; Youngman *et al*., 2002). For many years, it was believed that plasma cells were very short-lived (a half-life of less than 3 days), thus requiring continuous replenishment by proliferating memory B cells if long-lived antibody responses were to be maintained (Slifka and Ahmed, 1996). As noted above, memory B cells undoubtedly can differentiate into plasma cells, and it was thought that they must do so at a very high frequency to replace the short-lived plasma cells. However, this notion has recently been challenged by studies demonstrating that individual plasma cells can survive for months to years in the absence of proliferation (Manz *et al*., 1997), and without their being reconstituted by resident memory B cells (Slifka *et al*., 1998). In mice, virus-specific plasma cells could be observed more than 500 days after memory B cell depletion, indicating that at least a subpopulation of plasma cells could survive for the life of this host. The life span of plasma cells in humans in currently unknown, and it will be interesting to learn whether plasma cells in larger, more long-lived mammals have a maximal life span of only 1 to 2 years (as found in mice) or whether the life span is extended commensurate with host longevity.

D. How B Cell (Antibody) Functions Mature Over the Course of Infection

B cell responses mature in at least three ways over the course of a viral infection.

- First, B cells produce secreted Ig molecules (antibodies). Naive B cells express IgM, restricted to their cell surfaces. Following an appropriate encounter with cognate antigen, the cells are activated, begin to divide, and produce the secreted, multimeric, form of IgM, which facilitates activation of the complement cascade.

• Second, B cells undergo class switching, alluded to previously. In this process, the variable regions (and, hence, the antigen specificity) of the antibody remain unchanged, but the H chain constant domains are rearranged. For example, the IgM constant domains may be replaced with IgG constant domains. The IgM-secreting B cell will now instead secrete IgG, with identical antigen specificity but with different structure (divalent instead of decavalent) and new effector functions (e.g., enters interstitial spaces, better neutralizing activity) (see Table I).

• Third, during the process of expansion that results from antigen contact, multiple mutations are introduced into the variable regions of the antigen-specific B cells in a process termed *somatic hypermutation*. Somatic hypermutation has (at least) two consequences. One consequence is that many of the changes may reduce or abolish recognition of the triggering antigen, but the resulting B cells and antibodies may now recognize a different antigenic moiety; thus, the process further increases the diversity of the antibody response (to as high as 10^{14}–10^{16} specificities). Another consequence is that some of the mutations will increase the antibody's affinity for the original antigen, and those B cells that express the improved Ig molecules are preferentially expanded by continued antigen contact. As a result, as long as antigen is present to drive the response, the overall affinity of the antigen-specific antibody population will increase and antibodies can reach extremely high affinities (the range of K^d for antibodies begins at about 10^{-7} M and extends as high as about 10^{-12} M). High-affinity antibodies are more specific for their cognate antigen (diminishing the risk of side effects), and the stronger binding enhances their effector functions (e.g., neutralization, complement fixation, etc.). The strong binding between an individual antibody and its antigen may be effectively irreversible; therefore, antibodies should be considered disposable effector molecules. As will be described next, these features distinguish the maturation of antibody responses from the changes in T cell responses that take place during infection.

III. Antiviral T Cells: A Primer

In contrast to antibodies, which generally recognize antigenic moieties on intact molecules, most T lymphocytes recognize short (9–24 amino acid) fragments (epitopes) of foreign proteins that are presented

on the cell surface by host glycoproteins encoded in the MHC. Antigen recognition by a T cell relies on the T cell receptor (TcR), a cell surface molecule present in multiple identical copies, each of which is structurally reminiscent of one arm of the Y in an antibody molecule. The TcR is a heterodimer, with each chain comprising one constant region (common to all T cells) and one variable region (which varies among different T cells). There are two categories of TcR: $\alpha\beta$, and $\gamma\delta$. The function of cells bearing the $\gamma\delta$ heterodimer remains largely unknown, and will not be discussed in this review. The great majority of $CD8^+$ T cells responding to viral infection express $\alpha\beta$ TcR heterodimers, and it is this population that has been intensively studied over the past decade. The paired variable regions in an $\alpha\beta$ TcR determine its antigen specificity and, therefore, the specificity of the T cell itself. $\alpha\beta$ T cells are subdivided into two major classes distinguished by their expression of cell surface proteins termed CD8 and CD4. There are two major types of MHC molecule: class I and class II. In general, $CD8^+$ T cells recognize peptide epitopes presented at the cell surface by MHC class I, and $CD4^+$ T cells recognize peptides presented by MHC class II. During the recognition process, broadly speaking, the TcR recognizes the specific combination of peptide epitope and MHC molecule, thus conferring antigen specificity upon the cell; and the CD4 or CD8 molecules interact directly with conserved areas of their respective MHC molecules (Konig *et al.*, 1992; Salter *et al.*, 1990), increasing the avidity of the interaction and helping to assemble the signal transduction apparatus.

There are (at least) two key differences between the MHC class I and class II molecules, and these define the biologic roles of $CD4^+$ and $CD8^+$ T cells. They are summarized in Table III. First, the molecules differ in their distribution: class I molecules are almost ubiquitous, whereas class II molecules are expressed by a relatively limited number of cells, most of which are specialized antigen presenting cells (APCs) important in the induction of an immune response. Second, the molecules differ in the source of viral peptides that they present. MHC class I molecules present epitopes from proteins made within the cell, thus ensuring that, in general, $CD8^+$ T cells will recognize only cells actively infected with a virus. In contrast, MHC class II molecules present peptides that come from proteins taken up from the extracellular milieu; thus, specialized APCs can be recognized by $CD4^+$ T cells even if they are not actively infected. These differences in MHC distribution and function have profound implications for the biological activities of T lymphocytes. $CD8^+$ T cells can, in principle, recognize (and exert their effects on) almost any somatic cell that is unfortunate

TABLE III
MHC Distribution and Function Define the Biologic Roles of the Two Types of T Lymphocyte

	$CD8^+$ T cells	$CD4^+$ T cells
Recognize epitopes presented by:	MHC class I	MHC class II
Source of antigen presented by MHC and recognized by T cell usually as an:	Endogenous antigen, so $CD8^+$ T cells recognize infected cells	Exogenous antigen, so cells recognized by $CD4^+$ T cells need not be infected
Distribution of MHC class I/II expression allows the related T cells to recognize:	Almost all nucleated cells, with the possible exception of neurons	Specialized antigen-presenting cells (class II negative somatic cells invisible to $CD4^+$ T cells)
T cell functions can:	Usually can kill infected cells and release cytokine, also an important function	Provide "help" to B cells (thereby aiding antibody production) help maintain $CD8^+$ T cell memory, and directly inhibit virus production

enough to become infected; thus, $CD8^+$ T cells can be effective front line combatants against virus infection. In contrast, $CD4^+$ T cells are unable to recognize the majority of infected cells, and therefore are less plausible candidates for the direct control of infection (although they may have some direct effects) (Section V.C.3); however, their interactions with specialized APCs ensure that $CD4^+$ T cells play important roles in marshalling the immune response. As might be expected from their different roles in countering virus infection, the two T cell types differ somewhat in the ways in which they respond to infection. Therefore, we shall consider them separately, beginning with the better-understood $CD8^+$ T cell responses; and in both cases, we shall consider how, over the course of infection, the T cells vary in quantity and in quality.

IV. $CD8^+$ T Lymphocytes and Their Role in Antiviral Immune Responses

Virus-specific $CD8^+$ T cells develop when naive cells carrying an appropriate TcR encounter a specialized APC that is presenting the appropriate peptide via its MHC class I molecules. Elegant work

has shown that, in many cases, the APC itself must first be activated through the CD40/CD40L pathway to provide appropriate stimulation to the naive $CD8^+$ T cells (Bennett *et al.*, 1998; Ridge *et al.*, 1998; Schoenberger *et al.*, 1998).

A. The Kinetics of the Antiviral $CD8^+$ T Cell Response

The kinetics of antiviral $CD8^+$ T cell responses have been extensively studied in a number of animal model systems and, more recently, in humans. Careful quantitation has provided a relatively detailed picture of the numbers of virus-specific (and/or epitope-specific) $CD8^+$ T cells present at all phases of infection. Lymphocytic choriomeningitis virus (LCMV) infection of mice has been widely used in studying many aspects of antiviral immunity, and a quantitative overview of the LCMV-specific $CD8^+$ T cell response is presented in Fig. 1. The response is traditionally considered as having three phases: expansion, contraction, and memory. Although shown in the figure as entirely separate, there is some temporal overlap between the phases.

1. $CD8^+$ T Cells: The Expansion Phase

The expansion phase begins when a naive antigen-specific $CD8^+$ T cell encounters its cognate antigen, which results in triggering of a program that leads to the cell's division and differentiation. A study in mice indicated that there may be approximately 100 to 200 naive cells specific for a given antigen, and, since a mouse has $\sim 2 \times 10^7$ $CD8^+$ T cells in total, the frequency of naive $CD8^+$ T cells of a given antigen specificity would be about 1 in 10^5 (Blattman *et al.*, 2002). If, indeed, a mouse contains $CD8^+$ T cells of only approximately 10^5 different specificities, this is far below the number of antibody specificities that are available to the animal, and may have implications for either the number of epitopes that can be recognized or for the fidelity of TcR-antigen recognition.

Triggering of a naive $CD8^+$ T cell requires that it receives at least two signals: (i) contact with cognate epitope, delivered via the TcR and (ii) costimulatory signals, which come from a variety of receptor-ligand interactions. The widespread expression of MHC class I molecules ensures that most somatic cells can present viral antigen to $CD8^+$ T cells, but only a few cell types—in particular, activated dendritic cells and memory B cells—express the appropriate costimulatory molecules. Consequently, only those cells—located in lymphoid tissues such as the lymph nodes—can trigger naive $CD8^+$ T cells to enter the activation pathway. The interaction between a naive $CD8^+$ T cell and

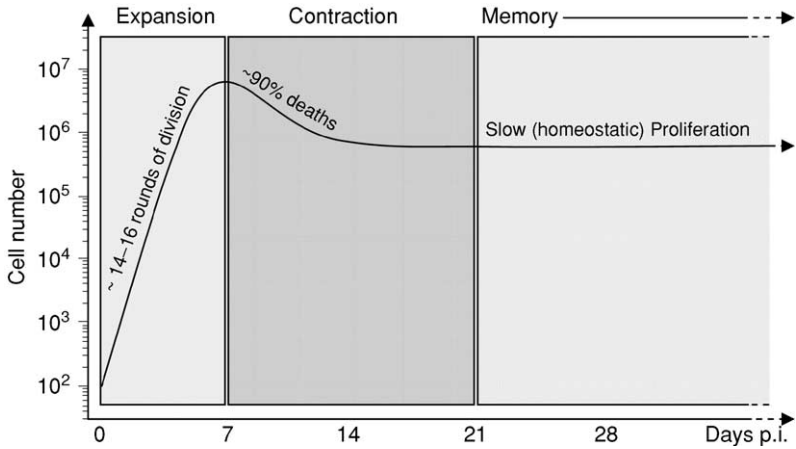

FIG 1. A quantitative overview of the $CD8^+$ T cell response to a viral epitope. This graph represents the changes that occur in response to LCMV infection in a population of naive $CD8^+$ T cells identical viral epitope specificity. Prior to infection, the size of this pool of identical precursor cells is assumed to be about 100 cells (in accordance with the data of Blattman *et al.*, 2002). The expansion, contraction, and memory phases are indicated by shading. As noted in Section IV.A, the transitions between the post infection (p.i.) phases are not abrupt, and it is likely that there is substantial temporal overlap between successive phases. This graph should be considered a representative graph only; the kinetics of the $CD8^+$ T cell response will differ depending on the nature of the infection (e.g., virus; acute vs persistent infection).

an activated APC expressing its cognate antigen may be rather brief; recent studies from several laboratories indicate that only a few hours of antigen exposure are required to cause a $CD8^+$ T cell and its progeny to proceed through the expansion, contraction, and memory phases and to express appropriate effector functions (Kaech and Ahmed, 2001; Mercado *et al.*, 2000; van Stipdonk *et al.*, 2001). Following antigen triggering, $CD8^+$ T cells divide and continue to do so rather rapidly. The T cell responses shown in Fig. 1 are intended to demonstrate the quantitative changes that may take place in a single population of about 100 naive cells, all of which are specific for the same epitope. In the first 7 days of a viral infection, a naive cell may undergo approximately 14 to 16 rounds of division, permitting a single cell to generate between 16,000 to 65,000 progeny (Blattman *et al.*, 2002). The graph in Fig. 1 assumes that all of the naive cells receive the appropriate antigenic signal, but this may not occur *in vivo*. For example, under some circumstances, increasing the antigen (epitope) expression increases the overall epitope-specific $CD8^+$ T cell response (Wherry *et al.*,

1999), consistent with the possibility that the lower expression may have recruited only a proportion of the epitope-specific naive cells into the responding population. It is also possible that all naive cells were activated at both levels of epitope expression, but that the higher level led to increased proliferation (e.g., more rapid, or more prolonged, cell division). Regardless of the mechanism, it is likely that the quantity of antigen available during the early phases of an infection can play a key role in determining the intensity of the $CD8^+$ T cell response. Does the duration of antigen availability have a marked effect? The effect of persistent antigen is unclear; it has been suggested that persistent antigen may cause the related T cells to undergo more rounds of cell division (Kaech *et al*., 2002), but others have noted that the duration of antigen exposure has a minimal effect on the $CD8^+$ T cell response (Badovinac *et al*., 2002).

In summary, naive $CD8^+$ T cells are preprogrammed and, after brief antigen contact, they expand, express their effector functions, contract, and enter the memory phase. This reliance on an easily triggered program may have advantages and disadvantages. Viruses have developed many strategies to evade the immune system, including the rapid down-regulation of APC function. If $CD8^+$ T cells were to require prolonged or repeated contact with antigen-charged APCs, there would be a significant risk that viruses could undermine the developing $CD8^+$ T cell response by limiting APC function. Because only brief antigen contact is needed to initiate the program, the host has a better chance of being able to mount a meaningful response. An additional advantage of programming is that it may permit the activated cell to quickly exit the lymphoid tissues (where the initial triggering occurs) and, presumably, to continue its rapid division in peripheral sites, even in the absence of ongoing antigen contact. In this way, the antiviral functions of the expanding cell population are more rapidly deployed than they would be if the cells had to remain in the lymphoid tissues to receive repeated antigenic signals. A possible disadvantage of programming is that the cells may continue to expand long after the virus (and the antigen) has been eradicated; this is potentially harmful, because autoaggressive $CD8^+$ T cells may play a role in virus-induced autoimmune disease (von Herrath *et al*., 2003).

Upon activation, $CD8^+$ T cells up-regulate the expression of several "activation markers," such as CD11a, CD25 (IL-2 receptor-α), CD44, and CD69; they also down-regulate other molecules, including CD62L (L-selectin) and CC-chemokine receptor 7 (CCR7). The understanding of these proteins' functions is incomplete, but several of the proteins appear to play an important part in regulating the anatomical

distribution of the T cells. It has been known for many years that T lymphocyte recirculation is not random, with some cells being retained in lymph nodes and others preferring peripheral tissues (Cahill *et al.*, 1977). Naive $CD8^+$ T cells appear to remain in lymphoid tissues because they express on their cell surface high levels of proteins such as CD62L (Gallatin *et al.*, 1983) and CCR7, which can mediate adhesion to lymph node venules (Baekkevold *et al.*, 2001). T cell activation results in the rapid down-regulation of these proteins, and this (along with other factors) allows the cell to exit the node. Once released from the nodes, T cells patrol the peripheral tissues, and settle preferentially in tissues that express particular ligands and/or chemokines, which interact with proteins on the T cell membrane (for example, with CD11a and CD44). There is increasing evidence that T cells home to specific organs, although it remains uncertain whether this behavior is imprinted on the individual T cell by the APC during priming or whether the behavior results from positive selection of activated T cells carrying the appropriate cell-surface molecules. In any event, it appears that during viral infections and other inflammatory processes, T cell trafficking is tightly controlled (reviewed in Weninger *et al.*, 2002).

2. $CD8^+$ T Cells: The Contraction Phase

Over the past decade, the expansion and memory phases of the T cell response have been exhaustively studied, but the intervening contraction (death) phase has received much less attention. Although this situation is changing, the understanding of the contraction phase is, at best, elementary. This results in some terminological confusion in the literature, which will, no doubt, be resolved as a clear picture of the contraction phase emerges over the coming years.

As already noted, about 90% of T cells die during the contraction phase, which is relatively brief, and is complete by approximately 15 to 21 days postinfection (Badovinac and Harty, 2002; Badovinac *et al.*, 2002; Kaech *et al.*, 2002; Sprent and Surh, 2002). Although the expansion and contraction phases usually are considered as temporally distinct, this is probably an oversimplification; T cell death begins even as T cell numbers continue to rise. Thus, between approximately 6 to 10 days postinfection, the expansion and contraction phases overlap. Conceptually, one can propose at least four general mechanisms that might precipitate T cell contraction; these mechanisms are not necessarily mutually exclusive. First, activated T cells may be destined to die, regardless of the milieu in which they find themselves; for example, it has been proposed that when a naive T cell first encounters its cognate antigen, a program may be triggered that leads to the death

of progeny cells after a certain number of divisions. This is an intriguing hypothesis, and there is experimental evidence consistent with an early programming event (Badovinac *et al.*, 2002). However, if such early programming takes place, it is unlikely to be sole regulator of contraction, because the fate of activated T cells can be altered by their environment; some of these environmental factors contribute to the remaining three mechanisms. Second, T cell numbers appear to decline in parallel with viral clearance; perhaps the cells die because they can no longer find their cognate antigen. Originally popular, this idea has fallen from favor. Third, T cells may reach a stage where antigen contact becomes lethal, rather than stimulatory [activation-induced cell death (AICD)]. Fourth, as the infection is resolved, there is a general reduction in the proinflammatory cytokine status, and activated T cells could die because of cytokine withdrawal [activated T cell autonomous death (ACAD)].

In addition to identifying the factor(s) that precipitate T cell death, it is important also to ask a related question: by what molecular mechanism do the cells die? Are they killed, or do they commit suicide? If the latter is true, are the cells intrinsically suicidal, or do they act upon instructions? Most studies suggest that the reduction in T cell numbers relies largely on programmed cell death (PCD), a process of cell suicide that is central to many aspects of cell regulation and tissue development. During PCD, the cell dismantles itself in an ordered manner; this contrasts with necrosis, a process of passive cellular disintegration. PCD is important for various aspects of immune regulation. It plays crucial roles in thymic T cell selection, in the killing of virus-infected cells by T cells, and, most relevant to this chapter, in the regulation of virus-specific T cell numbers following infection. Many papers on T cell contraction state that death occurs through apoptosis, and the terms PCD and apoptosis often are used interchangeably. However, the two are not synonymous; in several cell types, including T cells, PCD also can be mediated by triggering of nonapoptotic pathways, and some studies suggest that T cell contraction may be largely nonapoptotic (Holler *et al.*, 2000a). Three types of PCD have been proposed (reviewed in Jaattela and Tschopp, 2003), which can be categorized depending on the morphology of the dying cell, and the part played by caspases (aspartate-specific cysteine proteases that activate the effector phase of cell suicide; Cohen, 1997; Thornberry and Lazebnik, 1998).

1. Classic apoptosis, in which the chromatin of the dying cell condenses at the nuclear margins, and which is caspase-dependent

2. Apoptosis-like PCD, which also shows chromatin condensation, but may be caspase-independent
3. Necrosis-like PCD, in which chromatin condensation is absent, and which can be distinguished from regular necrosis because the former is driven by active cellular processes (Denecker *et al.*, 2001; Vercammen *et al.*, 1998)

We shall discuss the two main mechanisms thought to be responsible for PCD of virus-specific T cells: (i) activation-induced cell death (AICD), also called antigen-driven apoptosis, and (ii) activated T cell autonomous death (ACAD), also called growth factor deprivation-induced apoptosis (Hildeman *et al.*, 2002a; Janssen *et al.*, 2000; Lenardo *et al.*, 1999; Welsh and McNally, 1999).

a. AICD Activated T cells express the FasL molecule, permitting them to induce apoptosis of virus-infected cells that express the death receptor Fas. The Fas–FasL interaction can initiate a caspase cascade, beginning with the cleavage of procaspase-8, and culminating in apoptotic T cell death (reviewed in Budd, 2001). A role for the Fas pathway in T cell homeostasis *in vivo* is strongly supported by observations in mice lacking Fas (*lpr* mice) or FasL (*gld* mice), both of which develop uncontrolled lymphoproliferation (Nagata and Suda, 1995). However, inhibition of the caspase pathway, using either drugs (Hildeman *et al.*, 1999; Holler *et al.*, 2000a) or genetic manipulation (Smith *et al.*, 1996) usually does not result in lymphoproliferation, suggesting the existence of an alternative, caspase-independent pathway that may mediate T cell death. What is the evidence that the Fas pathway and/or the caspase cascade may play a role in AICD during the immune response to virus infection? The Fas pathway is thought to be central to AICD; antigen-driven overstimulation of the T cell receptor induces Fas expression, rendering the T cell susceptible to FasL-driven apoptosis (Brunner *et al.*, 1995). Since AICD is antigen-driven, it is thought to play its part relatively early in infection when the antigen load is high. Furthermore, T cells are rendered more sensitive to Fas-triggered apoptosis when they are actively dividing, and when IL-2 levels are high (Refaeli *et al.*, 1998); these are precisely the conditions present during the later part of the expansion phase. Thus, AICD may drive much of the T cell death that occurs toward the end of the expansion phase. Furthermore, AICD is thought to be crucial for the down-regulation of antiviral T cell responses in persistent virus infection (i.e., when antigen remains in the organism for an extended period of time) (Zhou *et al.*, 2002). However, the fact that the *in vitro* induction of AICD often requires repetitive stimulation through the TCR has led

to some doubt about its contribution to the contraction phase of an antiviral immune response *in vivo* (Hildeman *et al.*, 2002a), and this, together with the observations of caspase-independent death pathways, has led to the search for alternative mechanisms of T cell death.

b. ACAD ACAD is thought to be responsible for the bulk of virus-specific $CD8^+$ T cell death that occurs after the virus has been eradicated (i.e., later in the contraction phase). Unlike AICD, ACAD does not depend on the ligation of death receptors (i.e., it is Fas-independent), and it is instead controlled by molecular regulators within the T cell, with the bcl-2 protein family playing a key role (Strasser *et al.*, 1995; van Parijs *et al.*, 1998). The bcl-2 family comprises at least three subgroups of proteins, arrayed in opposing factions. The first subgroup contains antiapoptotic proteins, such as Bcl-2 and Bcl-x_L, and the second is populated by proapoptotic proteins, such as Bax and Bak. Members of the third subgroup, termed BH3-only proteins, favor apoptosis by inhibiting their antiapoptotic relatives or enhancing the activity of the proapoptotic molecules. One BH3-only protein, Bim, has been proposed as the key molecular regulator of ACAD (Hildeman *et al.*, 2002a,b). ACAD can be inhibited by the expression of high levels of Bcl-2, and IL-2 selectively induces the antiapoptotic members of the bcl-2 family, thereby preventing ACAD (Akbar *et al.*, 1996). Furthermore, elevated levels of Bcl-2 protein have been reported in memory T cells (Grayson *et al.*, 2001). The bcl-2 protein family controls mitochondrial outer membrane permeability, and the proapoptotic family members act by disregulating the membrane potential of these vital organelles; this lethal effect appears to be a final common pathway employed by various inducers of caspase-independent cell death. One consequence of the disregulation is the intracellular release of cytochrome c, which in turn activates procaspase-9 and triggers the caspase cascade. Thus, caspase activation occurs in both AICD and ACAD, but its significance differs greatly between the two pathways. In AICD, caspase activation is required for cell death. In contrast, caspase activation is a secondary feature of ACAD, being required for the DNA fragmentation characteristic of apoptosis, but cell death occurs even in the absence of caspases (Ferraro-Peyret *et al.*, 2002).

In summary, there are at least two independent but partially overlapping pathways that may induce T cell death. The likelihood that one or both pathways will be activated in any one cell depends on a variety of factors, which may include the history of antigen contact, the number of cell divisions, and the extracellular microenvironment. At the

peak of the infection, the rapid cell division and high IL-2 levels will tend to favor AICD but, as the inflammatory milieu dissipates, the decline in IL-2 will cause the balance of power within the warring factions of the bcl-2 family to shift, leading to changes in mitochondrial membrane potential, and caspase-independent cell death (ACAD). A recent study in which IL-2 was delivered *in vivo* at various times over the course of an antiviral immune response has confirmed that the timing of IL-2 administration is critical; IL-2 reduced T cell numbers when administered relatively soon after infection, during the expansion phase (consistent with IL-2 inducing AICD), but increased T cell survival when administered during the contraction phase (consistent with IL-2 preventing ACAD) (Blattman *et al.*, 2003).

3. CD8$^+$ T Cells: The Memory Phase

CD8$^+$ memory T cells play a critical role in protecting against many viral infections, and there is ample evidence that vaccines which induce only CD8$^+$ memory T cells can confer good protection against subsequent viral challenge (del Val *et al.*, 1991; Klavinskis *et al.*, 1989; Whitton *et al.*, 1993). The induction of virus-specific CD8$^+$ memory T cells is, therefore, a central goal of antiviral vaccine design. The number of CD8$^+$ T cells that enter the memory phase is related to the extent of the primary response (Hou *et al.*, 1994; Marshall *et al.*, 2001), but the ontogeny of CD8$^+$ memory T cells remains controversial. Some studies suggest that they arise directly from effector cells that escape the contraction phase (Jacob and Baltimore, 1999; Opferman *et al.*, 1999), and that passage into the memory phase may be a stochastic process (Sourdive *et al.*, 1998). Other data indicate that memory cells may represent a separate lineage that can be generated without expressing their effector functions (Lauvau *et al.*, 2001; Manjunath *et al.*, 2001). Regardless of precisely how these cells are generated, it is clear that, in immunocompetent animals, the resting level of memory cells remains relatively stable for a prolonged period (months or years) after infection or vaccination. The establishment of this stable CD8$^+$ T cell memory population requires CD4$^+$ T cells; in mice lacking CD4$^+$ T cells, where the primary CD8$^+$ T cell response (i.e., the expansion phase) can be relatively normal, the number of memory cells, and the extent of antiviral protection, decrease with each passing month (von Herrath *et al.*, 1996). The activity of CD4$^+$ T cells that stabilizes CD8$^+$ T cell memory may be exerted very early, perhaps when the naive CD8$^+$ T cells are being programmed (Janssen *et al.*, 2003; Shedlock and Shen, 2003; Sun and Bevan, 2003).

In normal mice, the maintenance of a stable level of $CD8^+$ memory T cells requires that the cells continue to divide in a homeostatic manner. This homeostatic division is regulated by cytokines, in particular by IL-7 and IL-15 (Becker *et al.*, 2002; Schluns *et al.*, 2000, 2002; Tan *et al.*, 2002), and occurs in the absence of cognate antigen (Lau *et al.*, 1994; Murali-Krishna *et al.*, 1999). It has been suggested (Sallusto *et al.*, 1999) that there may be two types of memory T cell, termed *effector memory* (cells that are cytolytic and produce cytokines immediately upon antigen encounter) and *central memory* ("effectorless" cells that do not express IFN-γ or perforin immediately upon antigen contact); evaluation of CCR7 expression suggested that $CCR7^+$ cells were central memory and $CCR7^-$ cells were effector memory. As proposed, this central/memory hypothesis had three distinguishing tenets: (i) effector memory cells are both constitutively lytic and cytokine competent, (ii) central memory cells express neither cytokines nor perforin upon antigen contact; and (iii) the effector and central memory populations can be distinguished on the basis of CCR7 expression. Emerging data are challenging all tenets of the hypothesis. First, several labs have shown that long after virus clearance, virus-specific memory cells can quickly produce IFN-γ in response to antigen contact, but most of them are nonlytic and thus would be excluded from the Sallusto/Lanzavecchia definition of effector memory cells. Second, in our own laboratory, we have found that almost all virus-specific $CD8^+$ memory T cells (identified using a tetramer) are also cytokine competent cells (data not shown), suggesting that virus-specific "effectorless" (i.e., central memory) cells, if they exist, are a very minor component of the response; similar findings have been reported in other models of infection (Masopust *et al.*, 2001). Third, with regard to CCR7 expression, Pircher and colleagues (Unsoeld *et al.*, 2002) used a new reagent to detect mouse CCR7 in LCMV TcR transgenic mice and found that, a few days after LCMV infection, virus-specific TcR transgenic $CD8^+$ T cells that were lytic and cytokine competent showed no clear pattern in their level of CCR7 expression (Unsoeld *et al.*, 2002). To determine the relationship (if any) between CCR7 expression and effector function in normal (not TcR transgenic) T cells, we infected mice with LCMV, and, several months later, the effector functions of their virus-specific $CD8^+$ memory T cells were evaluated directly *ex vivo* by intracellular cytokine staining (ICCS) using the dominant $NP_{118–126}$ peptide as stimulator. Cells were stained to detect CD8 and CCR7, and we included CD62L for comparative purposes. In addition, to determine the effects of antigen re-exposure,

some long-term LCMV-immune mice were reinfected with LCMV and sacrificed 4 days later; their splenocytes were analyzed as already described. Representative results are shown in Fig. 2; all cells shown are $CD8^+$ T cells and the axes represent IFN-γ/CD62L (Fig. 2A) or IFN-γ/CCR7 (Fig. 2B). Prior to secondary infection (left columns in Figs. 2A and 2B), peptide-responsive (i.e., IFN-γ^+) $CD8^+$ memory T cells in the spleen were almost all $CD62L^-$ and $CCR7^+$; this identification of $CCR7^+$ cells that respond immediately to antigen contact is not consistent with the central/effector memory hypothesis. Four days after virus infection (post-2°; right column in Fig. 2A and 2B), the cytokine competent cells had expanded and were still $CD62L^-$, but their CCR7 status had markedly changed, with the majority of the responding cells being $CCR7^-$. Thus, our data show that, in normal $CD8^+$ memory T cells, CCR7 expression does not correlate with the absence of immediate effector function. Rather, we suggest that it may correlate with the infection status, because CCR7 expression decreases markedly in the 4 days following secondary virus infection. CCR7 is thought to mediate attachment to endothelial cells (Campbell *et al*., 1998; Gunn *et al*., 1998) and alters the distribution of cells within the spleen (Potsch *et al*., 1999); presumably (like CD62L) CCR7 is down-regulated during infection to permit efficient extravasation of effector T cells. Similar findings have recently been reported by others (Ravkov *et al*., 2003). Furthermore, *in vivo* analyses have shown that both of these proposed classes of $CD8^+$ memory cell can confer protective immunity, and they might be better considered as parts of a continuum in which "effector memory" cells serve as the origin for "central memory" cells, which are distinguished more by their anatomical locations than by their effector functions (Wherry *et al*., 2003). In summary, although there is no doubt that $CD8^+$ memory T cells may be somewhat heterogenous in their function and distribution, there is little to support the original classification into two discrete populations based on marker expression and effector activity.

The great majority of published work on memory cells has focused on tissues in which T cells are abundant, usually in the spleen and lymph nodes. The information derived is interesting and relevant, but it represents an incomplete snapshot of antiviral $CD8^+$ T cell responses *in vivo* because many of their biologic effects must be exerted in nonlymphoid tissues. Although it has been known for many years that memory T cells can be found in nonlymphoid tissues (Mackay *et al*., 1992; Sprent, 1976), their detailed analysis is a new area of research, and the emerging data are rather inconclusive. One study showed that $CD8^+$ memory T cells in lung and liver were immediately cytolytic

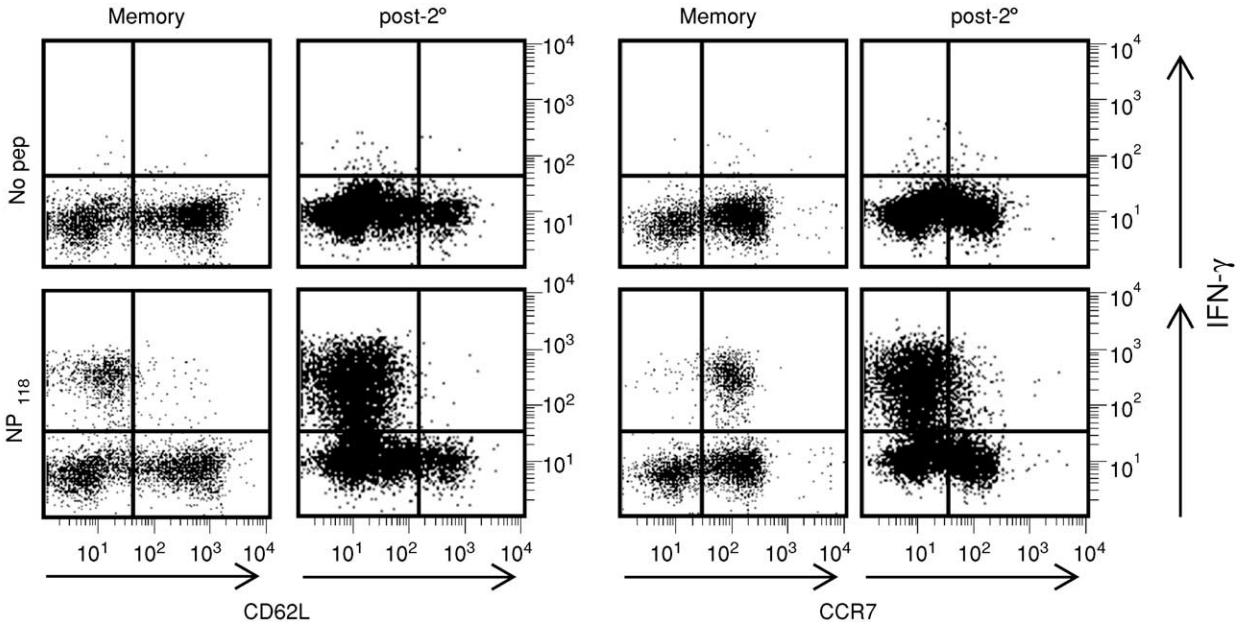

FIG 2. CCR7$^+$ cells express effector functions. Mice were infected with LCMV (Armstrong strain) and allowed to clear the virus. Some of these long-term immune mice were reinfected with LCMV 6 months later and they were sacrificed 4 days after reinfection (post-2°). The remaining mice were sacrificed without having been reinfected (memory). For both the memory and post-2° populations, the cell-surface phenotype (CD62L, left panel; CCR7, right panel) and antigen-responsiveness of splenic CD8$^+$ T cells were analyzed as described in Section V.A.3.

(Masopust *et al.*, 2001), but others have investigated the effector functions of $CD8^+$ memory T cells in the lung parenchyma and airways and have found that virus-specific cells are not immediately cytolytic (Hogan *et al.*, 2001a; Ostler *et al.*, 2001). Furthermore, even in an exhaustively studied organ, the spleen, controversy remains. Selin and Welsh (1997) showed that a small proportion of LCMV-specific memory cells in the spleen were cytolytic Oehen and Brduscha-Riem (1998) extended this observation in an LCMV transgenic TcR model, demonstrating that some of the transgenic LCMV-specific $CD8^+$ memory T cells in the spleens were cytolytic in a short-term (6 h) assay. However, the Lefrancois laboratory showed that, in contrast to Oehen's findings, $CD8^+$ memory cells in the spleen were not cytolytic (Masopust *et al.*, 2001). Our own observations suggest that, soon after the virus is cleared, lytic activity is rapidly lost by the great majority of LCMV-specific cells in the spleen (Rodriguez *et al.*, 2001). The regulation and expression of T cell effector functions in peripheral tissues will be the focus of much study in the coming years, as will the quantity of T cells that are resident at these sites. It is known that the number of virus-specific $CD8^+$ memory T cells in peripheral tissues, such as lung tissue, remains relatively stable for many months, but it remains uncertain whether this outcome is achieved by homeostatic division of lung-resident cells or whether the population is continually replenished by the immigration of new memory cells from lymphoid tissues; the available data favor the second mechanism (Ely *et al.*, 2003; Hogan *et al.*, 2002).

B. The Antiviral Functions of $CD8^+$ T Cells

Virus-specific $CD8^+$ T lymphocytes control microbial infections in two general ways: by secreting cytokines, such as IFN-γ and TNF, and by lysing infected cells. It has long been assumed that for controlling viral infections, the cytolytic function of $CD8^+$ T cells far outweighs the contribution made by their release of cytokines. There are at least two mechanisms by which $CD8^+$ T cells can cause lysis of infected target cells: first, by the insertion of a pore-forming protein, perforin, into the target cell membrane, thus facilitating the entry of toxic molecules such as granzymes and, second, by triggering "death pathways," as exemplified by the Fas/FasL pathway. In this pathway an interaction between the Fas molecule (on the target cell) and its ligand, FasL (on the T cell), results in apoptotic death of the infected cell. Many (probably most) $CD8^+$ T cells develop cytolytic capability within hours of antigen contact, but epitope-specific $CD8^+$ T cells can

differ in their cytolytic capacities, and virus-specific $CD8^+$ T cells very rapidly lose their lytic activity after virus clearance, although they remain cytokine competent—that is, capable of producing cytokines immediately upon antigen contact (Rodriguez *et al*., 2001). The role of the Fas/FasL pathway in direct antiviral defense is unclear; Fas/FasL interactions have been implicated in regulating virus infection of hepatocytes (Kafrouni *et al*., 2001) and neurons (Medana *et al*., 2000), but, in the absence of perforin, this pathway appears incapable of controlling LCMV infection (Walsh *et al*., 1994). There is no doubt that perforin is important in the clearance of several virus infections, such as LCMV (Kagi *et al*., 1994; Walsh *et al*., 1994), but cytolytic activity, long considered the crown jewel in the $CD8^+$ T cell armamentarium, is in some cases dispensable; perforin plays little role in controlling infections caused by vaccinia, Semliki Forest virus, vesicular stomatitis virus (Kagi *et al*., 1995), rotaviruses (Franco *et al*., 1997b), and coxsackie viruses (Gebhard *et al*., 1998). Furthermore, exposure to cytokines can directly reduce viral replication, and cytokines alone are able to "cure" some infected cells by inactivating viral replication in the absence of cell death (Estcourt *et al*., 1998; Guidotti and Chisari, 1996; Levy *et al*., 1996; Walker *et al*., 1991). Thus, both cytokines and cytotoxicity contribute to the antiviral activity of $CD8^+$ T cells.

C. $CD8^+$ T Cell Effector Functions Maturing Over the Course of Infection

Over the past decade, the majority of analyses of $CD8^+$ T cell responses to infection have been quantitative rather than qualitative. However, as indicated by the "central memory/effector memory" controversy, the focus is beginning to change. As already described, antibody responses mature over the course of infection and upon reexposure to cognate antigen, and it is reasonable to propose that the host might benefit if virus-specific $CD8^+$ T cells also were to improve with time. Our laboratory has investigated this possibility, and has shown that changes in antigen responsiveness do, indeed, take place. We have identified three distinct changes, at least two of which may enhance the ability of $CD8^+$ T cells to control virus infection. Each of these changes occurs at different times over the course of infection: the first is complete by about 8 days postinfection, the second is complete by about 21 days, and the third is complete after the cells have entered the memory phase. This chronology is reflected by the order of presentation in the following subsections.

1. Early in Infection, CD8$^+$ T Cells Improve Their Ability to be Triggered by Very Low Levels of Antigen

One way in which T cells could enhance their biological efficacy would be by optimizing their sensitivity to antigen contact; this could, in principle, be achieved by increasing the affinity of the TcR for the relevant epitope/MHC complex. Others have reported that, between 8 days postinfection and the memory phase, T cell populations carrying TcR of higher affinity are selectively expanded (Busch and Pamer, 1999; Savage *et al.*, 1999); however, this leads to only a very small (about twofold to fourfold) increase in the antigen-responsiveness of the T cell population and, for several reasons, it appears that TcR affinity can contribute nothing more to the maturing CD8$^+$ T cell response. Most importantly, and in contrast to antibodies (whose affinities for cognate antigen range from 10^{-7} to 10^{-12} M), the affinities of TcRs for peptide–MHC are very low *in vivo*, ranging from 10^{-4} to 10^{-7} M (Eisen *et al.*, 1996; Valitutti and Lanzavecchia, 1997). This low affinity is unlikely to result from structural constraints because *in vitro* mutagenesis of a TcR can generate a receptor with very high affinity for cognate antigen (Holler *et al.*, 2000b). Thus, the low affinity of TcR *in vivo* appears to result from selective pressures that favor cells bearing low-affinity receptors and/or oppose cells expressing high-affinity molecules. Consistent with the idea that high-affinity TcRs are not evolutionarily desirable, these receptors appear incapable of somatic hypermutation; the sequence of a TcR in a naive cell remains unaltered following activation and expansion. Several proposals have been advanced to explain this *in vivo* "affinity ceiling" for TcR–MHC interactions: (i) high-affinity TcR may be deleted during thymic selection; (ii) T cells bearing high-affinity TcR that escape thymic deletion may become dysfunctional, or be actively eliminated, by prolonged TcR contact with cognate antigen in the host periphery (Valitutti and Lanzavecchia, 1997); and (iii) cells carrying TcR with affinities of approximately 10^{-7} M can be triggered by very low levels of cognate antigen, so the host has no need to produce cells with higher affinity receptors (Salzmann and Bachmann, 1998; Sykulev *et al.*, 1995).

Does this mean that CD8$^+$ T cells improve their antigen responsiveness only about twofold to fourfold during viral infection? Prior studies compared cells at the peak of the immune response with cells in the memory phase; these time points were selected because cells were sufficiently numerous to be readily detectable by then-current methods. However, technological advances allowed us to investigate the antigen-responsiveness of virus-specific T cells from much earlier

times postinfection, when cells are few in number. We found that between approximately 4 and 8 days postinfection, the quantity of peptide antigen needed to trigger cytokine production by virus-specific $CD8^+$ T cells diminished by about 70-fold, and remained stable thereafter, essentially for the lifetime of the animal (Slifka and Whitton, 2001). By optimizing their ability to be triggered by very low levels of antigen, $CD8^+$ T cells ensure that they can recognize cells very early (minutes/hours) after they have become infected, thus maximizing the chance that the T cells' effector functions (e.g., cytokine production, cytolytic activity) will be exerted before the virus has had the opportunity to complete its cycle of replication, maturation, and egress. This occurs without a demonstrable selection of cells bearing high-affinity TcR. We proposed that this optimization is mediated by "hard-wiring" of the signal transduction apparatus, a suggestion confirmed by another study (Kersh *et al.*, 2003). In this light, we proposed an additional explanation for the *in vivo* affinity ceiling of TcRs. T cells are serial killers, and their biological function relies on their being able to rapidly disengage from one target cell and move to another; this antiviral activity might be fatally compromised if T cells were irrevocably linked to a target cell by high-affinity TcR. This explanation is consistent with an elegant study that showed there is an optimal "dwell time" in the interaction between an epitope/class I complex and the TcR of a $CD8^+$ T cell. If this interaction is too weak, or too strong, the T cell does not proliferate; only those $CD8^+$ T cells bearing TcR that are "just right" are rapidly expanded, and thus are included in the antiviral immune response (Kalergis *et al.*, 2001).

2. The Speed with Which $CD8^+$ T Cells Initiate IFN-γ Production Increases Until $\sim$ 21 Days Post-Infection

We have explained that T cells increase their antigen-responsiveness *in vivo* by becoming able to respond to lower levels of antigen. Recent studies suggest that as few as 10 peptide/MHC complexes are sufficient to stimulate coordinated signaling via the TcR (Irvine *et al.*, 2002), in which case the antigen-sensitivity of activated T cells approaches the lowest possible limit of antigen concentration on the cell surface. How else might the cells improve their effector response? We reasoned that they might do so by increasing the speed with which they begin cytokine production after being triggered by antigen contact. To determine how quickly an epitope-specific population of $CD8^+$ T cells could initiate IFN-γ synthesis in response to antigen contact (their "on-rate"), the proportion of cells synthesizing IFN-γ was evaluated after 1, 2, 3, 4, and 6 hr of peptide exposure. The response at 6 hr

was defined as 100%, the prior responses were plotted as a fraction of that maximum response, and the time taken for 50% of cells to respond to antigen (the half-maximal on-rate; OR1/2) was identified for each population. Representative results are shown in Fig. 3. The OR1/2 of cells harvested at 8 days postinfection was approximately 3.75 h, but the OR1/2 decreased markedly between day 8 and day 15 and, by 21 days postinfection, the cell populations had become maximally responsive to antigen contact (OR1/2 $\sim$ 1 h); this rapid response was retained in long-term immune animals (day 30+). Reinfection of long-term immune mice did not appreciably accelerate the response (day 4 post-2°), indicating that an OR1/2 of approximately 1 h may represent the fastest possible response by a $CD8^+$ T cell population (data not shown). It is important to ask whether this acceleration in response—from about 4 to 1 h—is likely to be biologically significant. Although this improvement may, at first blush, appear modest, one must remember that for most viruses, a single round of propagation (from infection, through replication, to release of infectious progeny) takes place over a short time period (usually about 6–24 h); consequently, even a small increase in the rapidity with which a triggered $CD8^+$ T cell can express an antiviral function might substantially decrease the ability of a virus to complete its replication cycle in an infected cell.

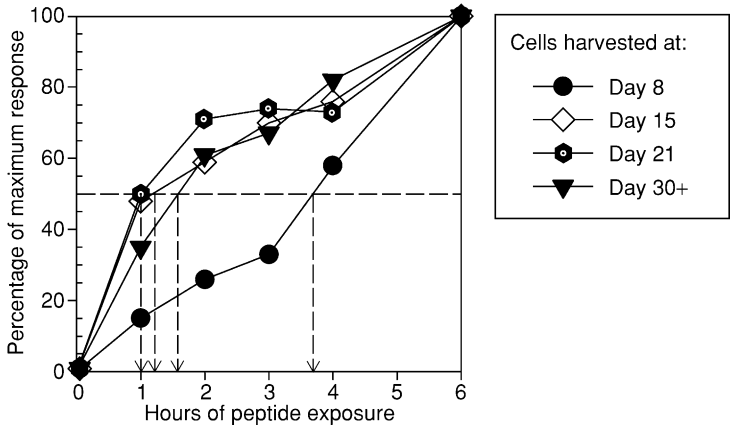

FIG 3. Onset of IFN-γ synthesis becomes more rapid as $CD8^+$ T cells mature. The C57BL/6 mice were infected with LCMV, and the maturation of effector function was followed by determining the OR1/2 of cells harvested over the course of infection. The OR1/2 values for one epitope-specific $CD8^+$ T cell population at 8, 15, 21, and 30+ days after primary virus infection are shown by drop-arrows.

3. CD8$^+$ Memory T Cells Produce Both IFN$^-$ and TNF Immediately Following In Vitro *Antigen Contact*

We have shown that cytokine production by CD8$^+$ T cells is very tightly regulated; IFN-γ and tumor necrosis factor (TNF) are produced only when the T cell is in contact with cognate antigen (Slifka *et al.*, 1999). However, as shown in Fig. 4, the pattern of cytokines produced by virus-specific cells changes as the immune response to infection proceeds (Slifka and Whitton, 2000a). When cells are harvested from LCMV-infected BALB/c mice during the expansion phase of the primary response (up to 7 to 8 days postinfection, for many viruses) and are exposed to antigen *in vitro*, two broad populations can be distinguished: one produces only IFN-γ, while the other produces both IFN-γ and TNF. As the response contracts (day 15), the ratio of these two populations changes, and double-positive cells outnumber single-positive cells by approximately 5:1; this process continues into the memory phase (day 60), at which time almost all cells respond to antigen contact by immediately producing IFN-γ and TNF. This maturational shift in cytokine profiles also was observed following LCMV infection of C57BL/6 mice, and during recombinant vaccinia virus infection (not shown). A similar change in phenotype occurs during the response to secondary infection (not shown); the cells are initially double-positive (i.e., they are memory phenotype), but, soon after infection, single-positive cells appear. After infection is cleared, the population reverts to the double-positive memory phenotype. These observations have been confirmed in both the influenza model (Belz *et al.*, 2001) and the murine gamma herpesvirus model (Liu *et al.*, 2002). The physiologic significance of this change has not been determined.

V. CD4$^+$ T Lymphocytes and Their Role in Antiviral Immune Responses

CD4$^+$ T cell responses can be detected after many infections in humans and in mice and, in many situations, are indispensable for effective immunity These cells display pleiotropic functions in the immune response to microbial infections. They are at the center of events, and orchestrate actions and movements by other subsets of cells including B cells, CD8$^+$ T cells, dendritic cells, and macrophages. In some cases, they also may combat infection directly, as described next.

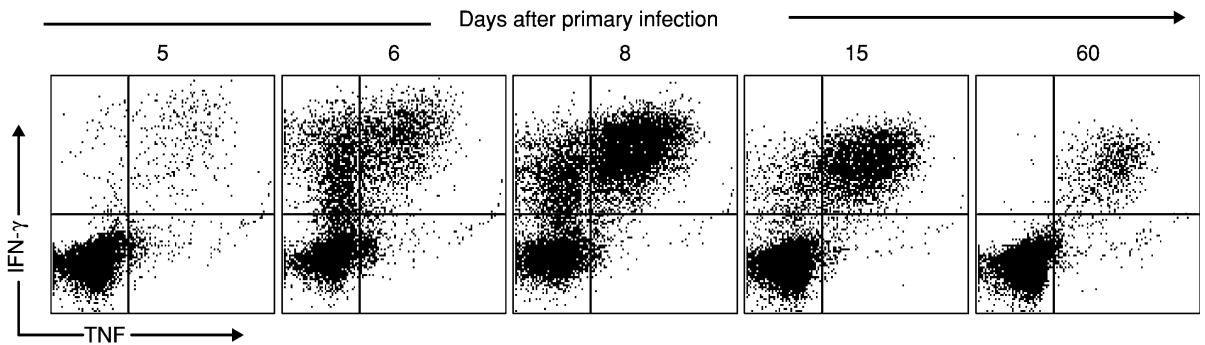

FIG 4. The cytokines produced upon antigen contact changes as the $CD8^+$ T cell response matures. Mice were infected with LCMV and, at the indicated time points postinfection, were sacrificed, and their spleens were harvested to determine the pattern of cytokine production by $CD8^+$ T cells over the course of a primary virus infection. Splenic $CD8^+$ T cell responses were determined using the intracellular cytokine staining procedure, and the acquired data were analyzed using CellQuest software. All cells shown are $CD8^+$ T cells and, as indicated, the *x*- and *y*-axes represent TNF and IFN-, respectively.

A. The Kinetics of the Antiviral CD4$^+$ T Cell Response

Technological developments have permitted accurate quantitation of virus-specific CD4$^+$ T cell responses without *in vitro* expansion. Using flow-cytometry-based assays, including intracellular staining for IFN-γ or TNF, CD4 responses have been followed in a number of infections. Like the CD8 response, the CD4 response shows three phases: expansion, contraction, and long-term memory. Differences between the two cell types, however, have been reported for all three phases.

1. CD4$^+$ T Cells: The Expansion Phase

Like the CD8 response, transient exposure to antigen induces a program of CD4 proliferation (Lee *et al.*, 2002), and the great majority of CD4$^+$ T cells activated during infection are antigen-specific (i.e., few cells are driven by nonspecific bystander activation) (Homann *et al.*, 2001; Whitmire *et al.*, 1998). However, CD4$^+$ T cell responses are typically much lower in magnitude than the concurrent CD8$^+$ T cell responses (Maini *et al.*, 1998, 2000; Whitmire *et al.*, 1998, 2000). This difference correlates with a lower proliferation rate, as revealed by BrdU incorporation or CFSE labeling; it has been estimated that, in the week following LCMV infection, a virus-specific CD4$^+$ T cell undergoes only about nine cell divisions (Homann *et al.*, 2001). The survival of the proliferating cells is improved with prolonged antigen stimulation (Lee *et al.*, 2002). The CD4 expansion phase also is regulated by several costimulatory interactions, including CD40L–CD40 (Whitmire *et al.*, 1999), CD28–B7 (Suresh *et al.*, 2001), OX40–OX40L (Kopf *et al.*, 1999), and ICOS (Kopf *et al.*, 2000) although these interactions are not required for T-help-independent CD8$^+$ T cell responses. CD4$^+$ T cells may be regulated more tightly than CD8$^+$ T cells via expression of CTLA4 or DR6 or other molecules that inhibit proliferation (Bird *et al.*, 1998; Doyle *et al.*, 2001). There is also evidence that there are intrinsic differences in the proliferative potential of CD4$^+$ T cells and CD8$^+$ T cells (Foulds *et al.*, 2002). Perhaps CD4$^+$ T cells differentiation requires prolonged/repeated antigen contact, whereas CD8$^+$ T cells commit to full differentiation after only brief stimulation (Kaech and Ahmed, 2001; van Stipdonk *et al.*, 2001).

2. CD4$^+$ T Cells: The Contraction Phase

Contraction of the CD4$^+$ T cell response is quantitatively similar to that of CD8$^+$ T cells, in that about 90% of the cells die; however, in contrast to the abrupt contraction of CD8$^+$ T cells, which is usually

complete within 1 week of the peak response, $CD4^+$ T cell contraction lingers over the course of several weeks (Homann *et al.*, 2001; Kamperschroer and Quinn, 1999). IL-2 treatment can increase both proliferation and survival of $CD4^+$ T cells (Blattman *et al.*, 2003), suggesting that cytokine withdrawal may play an important part in $CD4^+$ T cell contraction.

3. $CD4^+$ T Cells: The Memory Phase

A number of reports have shown that $CD4^+$ T cell responses to LCMV, Sendai virus, and influenza virus in mice can be readily detected long after the infection has been cleared (Topham and Doherty, 1998; Topham *et al.*, 1996a; Varga and Welsh, 1998; Whitmire *et al.*, 1998), and this $CD4^+$ T cell memory is maintained in the absence of antigen and MHC class II (Swain *et al.*, 1999). Some studies suggest that CD4 memory is even more stable than CD8 memory (Chang *et al.*, 2001; Varga *et al.*, 2001), but this is somewhat controversial; one study reported a decline in CD4 memory cell number over a prolonged period, in contrast to $CD8^+$ memory T cells, which remained stable in the same mice (Homann *et al.*, 2001). The changes correlated with levels of the antiapoptotic molecule Bcl2, which were lower in $CD4^+$ memory T cells than in $CD8^+$ memory T cells. Despite the conflicting conclusions from these studies, it is clear that $CD4^+$ T cell memory is regulated differently from $CD8^+$ T cell memory. For example, it appears that the survival of CD4 memory cells is not dependent on IL-15 or IL-7 (Tan *et al.*, 2002), and cells lacking a common cytokine receptor chain survive, indicating that IL-2, -4, -7, -9, and -15 may not be required (Lantz *et al.*, 2000). However—as evidence that the understanding of this process is far from complete—a recent paper reported that the homeostasis of $CD4^+$ memory T cells was regulated by IL-7 signaling (Seddon *et al.*, 2003). $CD4^+$ memory T cells show improved responsiveness to antigen, and can respond very quickly to antigen reencounter by producing cytokines (Homann *et al.*, 2001; Rogers *et al.*, 2000).

B. Two Subsets of $CD4^+$ T Cells

For the most part, $CD8^+$ T cells are at the front line; their biological effects are exerted directly upon infected cells. The functions of $CD4^+$ T cells are more disparate and usually serve to assist, or otherwise regulate, the responses of B cells and $CD8^+$ T cells. Because their main function is to provide help to other lymphocytes, $CD4^+$ T cells often are termed "T helper" (T_h) cells, and (at least) two different subsets of T_h can be defined according to their pattern of cytokine production. T_h1

cells produce IL-2, IFN-γ, lymphotoxin, and TNF, whereas T_h2 cells produce IL-4, IL-5, and IL-10. T_h1 cells are associated with the induction of the IgG2a subclass of IgG antibody, and T_h2 cells direct the production of IgG1. Both T_h1 and T_h2 cells can be induced following many viral infections, including CMV (Kallas *et al.*, 1998; Tsai *et al.*, 1997), HIV (Imami *et al.*, 2002), EBV (Steigerwald-Mullen *et al.*, 2000; Wilson *et al.*, 2001), RSV (Bendelja *et al.*, 2000; Tripp *et al.*, 2002), HBV (Diepolder *et al.*, 2001), HCV (Godkin *et al.*, 2002; Rosen *et al.*, 2002; Tsai *et al.*, 1997), and measles virus (Ovsyannikova *et al.*, 2003; Ward and Griffin, 1993) in humans. The cells can also be induced following LCMV (Su *et al.*, 1998; Whitmire *et al.*, 1998), influenza (Graham *et al.*, 1994), Sendai (Mo *et al.*, 1995), coxsackievirus B3 (Huber and Pfaeffle, 1994), RSV (Srikiatkhachorn *et al.*, 1999; Tripp *et al.*, 2001), and others in mice. The ratio of T_h1 to T_h2 cells induced by infection can vary markedly, depending on the infectious agent and host genetic background. Both cell types can be specific for the same epitopes (Varga *et al.*, 2000; Whitmire *et al.*, 1998), and there is evidence arguing that antigen dose alone does not dictate the type of T_h cell induced. There are some correlations between the T_h1/T_h2 ratio induced by virus infection and the clinical outcome (Imami *et al.*, 2002; Tsai *et al.*, 1997; Wang *et al.*, 2003), although this is a controversial issue (Bergamini *et al.*, 2001). For example, clearance of acute HCV infection is associated with a strong T_h1 cell response, whereas individuals who developed chronic HCV infection had predominantly T_h2 responses (Tsai *et al.*, 1997).

Some viruses, such as measles virus, HCMV, and MCMV, are found to induce a generalized T_h1 to T_h2 shift, and cause immune suppression. Studies of chronic LCMV infection have indicated that primary T_h1 T cell responses are reduced in magnitude (Ciurea *et al.*, 2001) compared to acute LCMV infection, with IL-2^+ cells being most affected (Fuller and Zajac, 2003), and the virus-specific $CD4^+$ T cells that are initially induced disappear over time (Fuller and Zajac, 2003; Oxenius *et al.*, 1998). In contrast, in mice infected with gamma-herpesvirus, which persists at low levels, virus-specific IL-2^+ CD4 T cell responses can be detected long after initial infection (Flano *et al.*, 2001). In terms of activation requirements, fewer T_h1 and T_h2 $CD4^+$ T cells are induced by virus infection in the absence of intact CD40–CD40L or B7–CD28 costimulation pathways (Whitmire *et al.*, 1999); however, other model systems have provided evidence that T_h2 cells have a lower reliance on costimulation. While the definition of the T_h1 and T_h2 subsets is clear—and there is evidence in cell lines for chromosomal restructuring, suggesting irrevocable differentiation—is it possible

that, during an *in vivo* infection, some cells at times make cytokines of both classes? There is a precedent for this in humans infected with CMV (Kallas *et al.*, 1998), but because IL-4 is best detected by ELISA or ELISPOT assays, proof of this in mice must await improved intracellular staining techniques that allow costaining for both IFN-γ and IL-4.

C. The Antiviral Functions of CD4$^+$ T Cells

1. CD4$^+$ T Cells Helping B Cells

A long recognized function of CD4$^+$ T cells is their ability to induce B cell differentiation; they are involved in class switching, and in the transition of virus-specific memory B cells into antibody-secreting plasma cells. Because preexisting antibody is a first line of defense against reinfection, these CD4$^+$ T cell functions are crucial for protection. CD4$^+$ T cells drive B cell differentiation and proliferation by acting through the CD40L–CD40 pathway, and they modify antibody class-switching by stimulating B cells with IFN-γ or IL-4. These differentiation events occur primarily in germinal centers where activated CD4$^+$ T cells associate with antigen-reactive B cells (Garside *et al.*, 1998; Pape *et al.*, 2003). CD4$^+$ T cells deliver their help to B cells in an antigen-specific manner. Memory B cells can internalize viral antigen via their surface-bound antibodies; these antigens are processed within the B cell, and epitope peptides are presented at the cell surface in association with MHC class II molecules (Section II.B). Only those CD4$^+$ T cells that are specific for the epitopes will, therefore, be triggered, ensuring that during a virus infection, only virus-specific CD4$^+$ T cells will be triggered to respond, and their signals will be delivered only to the virus-specific memory B cells. Recent studies have identified a protein named SAP that plays a key role in the differentiation of CD4$^+$ T cells (Wu *et al.*, 2001). SAP-deficient mice mount antigen-specific CD4$^+$ T cell responses to infection, but these cells cannot support the development of long-lived plasma cells (Crotty *et al.*, 2003). The reason that SAP$^{-/-}$ CD4$^+$ T cells are unable to provide this particular type of help remains unknown; the cells make normal amounts of IFN-γ, IL-2, and IL-4, and they express CD40L.

2. CD4$^+$ T Cell Importance in the Induction and/or Maintenance of CD8$^+$ T Cell Responses

Many viruses (e.g., LCMV, Sendai, vaccinia, influenza, ectromelia, gamma-herpesvirus-68, and Theiler's virus) induce strong primary CD8$^+$ T cell responses even in the absence of CD4$^+$ T cells (Ahmed

et al., 1988; Belz *et al*., 2002, 2003; Buller *et al*., 1987; Hou *et al*., 1995; Johnson *et al*., 1999; Mo *et al*., 1997). These infections often are systemic, and may directly activate innate defenses and APC costimulatory molecule expression by infecting APCs (Olson *et al*., 2001; Wu and Liu, 1994); these infections may instead express peptides of high avidity for MHC class I and TcR (Franco *et al*., 2000; Heath *et al*., 1993), thus triggering the program of $CD8^+$ T cell differentiation without the need for accessory molecule expression. In contrast, viruses that replicate to a lower extent or are localized to peripheral sites, often induce a detectable primary $CD8^+$ T cell response only when $CD4^+$ T cells are present. Examples of such T-help-dependent antiviral $CD8^+$ T cell responses in mice are those induced by Rauscher leukemia virus, Japanese encephalitis virus, herpes simplex virus, and mouse hepatitis virus (Edelmann and Wilson, 2001; Hom *et al*., 1991; Jennings *et al*., 1991; Murali-Krishna *et al*., 1996; Stohlman *et al*., 1998).

$CD4^+$ T cells can provide help to $CD8^+$ T cells in at least two ways. The first, described previously, involves APC licensing via the CD40-pathway; this enhances the costimulatory signals that are delivered to naive $CD8^+$ T cells, and triggers their program of proliferation and differentiation. A second means by which $CD4^+$ T cells can enhance primary CD8 responses is by secreting IL-2. The effects of IL-2 on $CD8^+$ T cells are manifold: this cytokine induces FasL expression on $CD8^+$ T cells, thus increasing those cells' cytotoxic potential (Esser *et al*., 1997), and IL-2 also may increase IFN-γ production by those cells (Cousens *et al*., 1995). IL-2 thus augments the proliferation of $CD8^+$ T cells (Cousens *et al*., 1995) and prolongs their survival (Akbar *et al*., 1996; Blattman *et al*., 2003; Kelly *et al*., 2002; Krummel *et al*., 1999) so that effector cells can pursue virus-infected cells for longer periods of time. For example, during coronavirus infection of the CNS, high numbers of $CD4^+$ T cells and $CD8^+$ T cells can be found in the brain parenchyma (Haring *et al*., 2001). If $CD4^+$ T cells are absent, coronavirus-specific $CD8^+$ T cells still migrate to sites of infection in the CNS, but they are much more likely to undergo apoptosis (Stohlman *et al*., 1998); this is consistent with their having an *in vivo* requirement for $CD4^+$-produced IL-2. Finally, there is strong evidence suggesting that $CD8^+$ memory T cell numbers are influenced by IL-2 (Blattman *et al*., 2003). However, IL-2 can also have deleterious effects on $CD8^+$ T cells, including increasing apoptosis of activated cells, depending on when and how much it is produced and to what extent the $CD8^+$ T cells are stimulated (Van Parijs *et al*., 1999). Given the possible opposing effects of IL-2, it will be interesting to learn how $CD4^+$ T cell production of this cytokine is regulated. It is conceivable that different

amounts of the cytokine are produced at different times depending on what effect is needed.

In addition to possible effects on the primary $CD8^+$ T cell response, $CD4^+$ T cells also may regulate the quality and quantity of $CD8^+$ T cell memory; primary CD8 responses to LCMV and influenza virus are relatively normal in $CD4^{-/-}$ mice, but memory responses measured by limiting dilution assay, or after secondary infection, are much reduced (Belz *et al.*, 2002; von Herrath *et al.*, 1996). Recent publications suggest that $CD4^+$ T cells may deliver a signal during the early programming of naive $CD8^+$ T cells, which facilitates their survival and ensures that they can respond appropriately to secondary antigen challenge (Section IV.A.3) $CD4^+$ T cells also may play a key role in sustaining $CD8^+$ T cells during persistent virus infections. During persistent infection, in the absence of $CD4^+$ T cells, $CD8^+$ T cells are quickly deleted or are rendered nonresponsive (Battegay *et al.*, 1994; Hunziker *et al.*, 2002; Matloubian *et al.*, 1994; Zajac *et al.*, 1998).

3. Direct and/or Bystander Antiviral Functions of $CD4^+$ T Cells

$CD4^+$ T cells orchestrate many aspects of the antiviral immune response, and these cells can, therefore, be said to have many distinct antiviral functions. For example, as already described, their provision of help to B cells can be considered an antiviral function because, in its absence, the antiviral antibody response is compromised, and the virus thereby gains an advantage. These effects—which result from the well-established helper activities of $CD4^+$ T cells—have been extensively catalogued and appear to constitute the great majority of antiviral effects of $CD4^+$ T cells (Doherty *et al.*, 1997). However, these effects are indirect, being mediated through other effector cells (e.g., $CD8^+$ T cells or B cells, described previously, or macrophages, not discussed further herein) or molecules produced by other cells (e.g., antibodies). In addition to these indirect effects, one can conceive of at least two other ways in which $CD4^+$ T cells might exert antiviral effects. First, the cells might act directly on virus-infected target cells that express viral epitopes in association with MHC class II; this would be analogous to the front line activities of $CD8^+$ T cells. Second, one could envision that a $CD4^+$ T cell might encounter an antigen-expressing (but uninfected) APC and be triggered to produce cytokines that directly inhibited the replication of viruses in adjacent infected (and probably MHC class II negative) cells; herein, we shall term this a "bystander" antiviral effect. Thus, we suggest that $CD4^+$ T cells may exert their antiviral effects in three ways: in a direct manner, in an indirect manner, or as bystanders. The indirect effects

were discussed previously. What is the evidence for the remaining two mechanisms?

a. Possible Direct Antiviral Effects of CD4$^+$ T Cells CD4$^+$ T cells may mediate direct purging of MHC class II-expressing cells, such as B cells (which can be infected by, for example, EBV or murine gamma herpesvirus), macrophages, and dendritic cells (which can be infected by several viruses), or microglia (targets of several CNS infections, including Theiler's virus or mouse hepatitis virus). However, the restricted anatomical distribution of MHC class II molecules means that CD4$^+$ T cells may be unable to recognize or act directly upon the great majority of cells that become infected following virus challenge, and this presents an obvious obstacle to the concept that CD4$^+$ T cells commonly exert direct antiviral effects (Section III). Nevertheless, it is clear that several cell types (e.g., epithelial cells) can up-regulate MHC class II expression during viral infection, rendering them potentially recognizable to CD4$^+$ T cells. Furthermore, the existence of cytolytic CD4$^+$ T cells is not in doubt, and one might infer that such cell—whose cytolytic effects rely on direct contact with an antigen-expressing target cell—are unlikely to exist without good reason. Cytolytic CD4$^+$ T cells were first identified *in vivo* (as distinct from CD4$^+$ T cell lines or clones) in LCMV-infected β2-microglobulin-deficient mice (Muller *et al.*, 1992), and subsequent careful analyses suggested that these virus-specific CD4$^+$ T cells could exert profound effects in the absence of CD8$^+$ T cells (Quinn *et al.*, 1993). Indeed, some β2-microglobulin-deficient mice cleared LCMV infection despite the lack of CD8$^+$ T cells (Muller *et al.*, 1992), suggesting (but not proving) that the CD4$^+$ T cells might have a direct antiviral effect. This interpretation is strengthened by the observations (Zajac *et al.*, 1996) that (i) the cytotoxic effect of the CD4$^+$ T cells is Fas-mediated, and (ii) the *in vivo* effects are Fas-dependent; Fas-dependence strongly suggests that the effects require a direct cell/cell interaction between a FasL$^+$ CD4$^+$ T cell and a Fas-expressing target cell. However, the model of β2-microglobulin-deficient mice is fraught with difficulties (reviewed in Frelinger and Serody, 1998), and direct cytolytic effects of CD4$^+$ T cells on infected cells *in vivo* remain to be demonstrated. Cytolytic CD4$^+$ T cells also have been identified following Epstein-Barr virus infection (Khanolkar *et al.*, 2001), and perforin$^+$ CD4$^+$ T cells have been reported in HIV infected individuals (Appay *et al.*, 2002). The mere existence of such cells, however, cannot be considered proof of their having a direct (or indeed, any) antiviral effect.

Perhaps the best evidence for direct CD4$^+$ T cell-mediated control of infection is in the murine gamma-herpesvirus model, in which it

appears likely (although not certain) that the $CD4^+$ T cells exert their effects, via IFN-γ production, directly on virus-infected MHC class II-positive target cells (Christensen *et al.*, 1999). There is other circumstantial evidence consistent with the idea that $CD4^+$ T cells may exert some direct effector functions. $CD4^+$ T cells are generally thought of as functioning in the spleen or lymph nodes, but there is increasing evidence that $CD4^+$ memory T cells can reside in peripheral, nonlymphoid sites (Hogan *et al.*, 2001b; Marzo *et al.*, 2002; McSorley *et al.*, 2002; Reinhardt *et al.*, 2001). These cells show a highly activated phenotype with elevated levels of CD25 and CD44, and decreased levels of CD45RB and CD11a, making them distinct from splenic memory $CD4^+$ T cells (Cauley *et al.*, 2002; Hogan *et al.*, 2001b). $CD4^+$ T cells in the periphery tend to make IFN-γ, (arguably, consistent with a direct effector function) while those that reside preferentially in lymphoid organs make more IL-2 (consistent with an immunoregulatory activity). However, these arguments are very much conjectural, and exceptions exist; as noted, $CD4^+$ T cells in the coronavirus-infected brain produce survival factors—possibly IL-2—to rescue $CD8^+$ T cells.

b. Possible Bystander Antiviral Effects of $CD4^+$T Cells Many situations exist in which transfer of virus-specific $CD4^+$ T cells has a profound effect on the outcome of virus infection and/or disease. For example, transfer of poliovirus-specific $CD4^+$ T cells into human-poliovirus-receptor transgenic mice protects the recipients from lethal poliovirus infection (Mahon *et al.*, 1995). Infection of neurons is required for a lethal outcome, and these cells do not usually express MHC class II molecules; therefore, this effect is unlikely to be direct. Similarly, transfer of HBV-specific effector $CD4^+$ T cells reduces viral load in mice transgenic for HBV (Franco *et al.*, 1997a), and $CD4^+$ T cells can mediate protection against Sendai infection independently of antibody or $CD8^+$ T cells (Zhong *et al.*, 2001). To evaluate possible bystander effects mediated by $CD4^+$ T cells, chimeric mice have been produced that express MHC class II molecules on some cells but not on others. Using this approach, it has been shown that influenza virus-specific $CD4^+$ T cells can clear infection from MHC class II-negative cells, consistent with bystander effects; however, indirect (antibody-mediated) effects were not excluded (Topham *et al.*, 1996b).

D. Maturation of the $CD4^+$T Cell Response

The cytokine profiles of $CD4^+$ T cells change over time. At the peak of the response to LCMV, most of the $CD4^+$ T cell response is T_h1 in phenotype, and there is a mixture of virus-specific $CD4^+$ T cells that

make IFN-γ; IFN-γ and IL-2; IFN-γ and TNF; or all three cytokines. The majority of these $CD4^+$ T cells make only IFN-γ in response to antigen contact although approximately 30% also make IL-2 (Harrington *et al*., 2002; Varga and Welsh, 2000), and a few synthesize TNF (Harrington *et al*., 2002; Homann *et al*., 2001; Varga and Welsh, 2000). In contrast, during the memory phase, very few single-positive cells can be identified, and essentially all of the cells are double-positive (IFN-γ^+ TNF^+ or IFN-γ^+ IL-2^+; Harrington *et al*., 2002; Homann *et al*., 2001). These patterns are reminiscent of the changes reported for $CD8^+$ T cells (Slifka and Whitton, 2000a) (Fig. 4). The significance of these changes is unclear. Could the cytokine double-positive cells have differentiated further than the single-positive cells? Are the single-positive cells a distinct lineage that terminally-differentiate into short-lived effector cells? Like $CD8^+$ T cells, $CD4^+$ T cells have been categorized into central memory cells (defined as $CD62L^{hi}$, $CCR7^+$) and effector memory cells ($CD62L^{lo}$, $CCR7^-$). The expression of CD62L and CCR7 affects the localization of T cells, with the central memory cells preferentially localized in the secondary lymphoid organs and the effector memory cells traveling through peripheral sites; however, as for $CD8^+$ T cells, there is no clear relationship between $CD4^+$ T cell effector function, and the expression of these marker proteins.

VI. Immunopathology

Immune responses are not invariably successful in controlling infection, and infections remain a major (and increasing) cause of human morbidity and mortality. It is easy to forgive the occasional failure on the part of the immune system, especially when one considers the innumerable strategies developed by microbes to evade its unwelcome attentions. However; it is important to realize that successful immune responses themselves are delivered at a cost because, in eradicating infection, the immune system also can damage the host. This phenomenon is termed *immunopathology*, and it is an extremely common feature of virus infection. Many of the symptoms of common viral infections—for example, the chills, muscle aches, and fever of influenza as well as the characteristic rash of measles—are not caused directly by the virus but instead result from the immune response to the virus. A detailed review of this topic would far exceed the scope of this chapter. Suffice it to say that the very nature of the $CD8^+$ T cell

response—which often involves lysis of infected cells or the release of highly toxic cytokines—means that immunopathology is an almost inevitable consequence of viral infection. We have argued that the need to minimize immunopathology has provided a strong selective pressure that ensures that the effector functions of $CD8^+$ T cells are held in an extraordinarily tight rein (Slifka and Whitton, 2000b; Slifka *et al.*, 1999). Moreover, antibodies should *not* be considered innocent parties. Antibody–antigen complexes may be deposited at various anatomical interfaces (e.g., in the basement membrane of the kidney) and can lead to complement activation and severe inflammatory damage. One key question, which has been approached only infrequently, is to ask if we can uncouple the harmful and the beneficial effects of the immune response. Coxsackie virus infection of humans or mice often results in severe myocarditis, and survivors have extensive myocardial scarring (often causing dilated cardiomyopathy, which is treatable only by transplantation). There is no approved treatment for coxsackie virus myocarditis. Using the mouse model, we have found that myocarditis and subsequent scarring are much reduced in perforin-deficient mice, but these animals clear the virus infection with kinetics indistinguishable from those observed in normal mice (Gebhard *et al.*, 1998). Thus, we suggest that the development of a reagent capable of specifically blocking perforin activity might permit the treatment of coxsackie virus myocarditis, without compromising the host's ability to recover from the infection.

VII. Summary

Evolutionary pressures imposed by an unremitting onslaught of infectious agents have shaped the mammalian immune system, and our very existence stands as proof of the potency of the antimicrobial immune response. The cooperative nature of the antibody and T cell arms of the adaptive immune system has long been recognized, but our understanding of the immune response to infection remains far from complete. Recent studies have begun to reveal previously unappreciated subtleties in the T cell response, which are tailored to most effectively detect a viral challenge and to provide a rapid and effective reply. If safer and more effective vaccines are to be designed, and if the harmful effects of the immune response are to be diminished while retaining beneficial components, we must not rest on our laurels; many important questions remain to be answered.

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REFERENCES

Anonymous (2003). Postexposure prophylaxis of hepatitis B. *Morb. Mortal. Wkly. Rep.* **33:**285–290.

Abney, E. R., Cooper, M. D., Kearney, J. F., Lawton, A. R., and Parkhouse, R. M. (1978). Sequential expression of immunoglobulin on developing mouse B lymphocytes: A systematic survey that suggests a model for the generation of immunoglobulin isotype diversity. *J. Immunol.* **120:**2041–2049.

Ahmed, R., Butler, L. D., and Bhatti, L. (1988). T4+ T helper cell function *in vivo*: Differential requirement for induction of antiviral cytotoxic T cell and antibody responses. *J. Virol.* **62:**2102–2106.

Akbar, A. N., Borthwick, N. J., Wickremasinghe, R. G., Panayoitidis, P., Pilling, D., Bofill, M., Krajewski, S., Reed, J. C., and Salmon, M. (1996). Interleukin-2 receptor common gamma-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: Selective induction of anti-apoptotic (bcl-2, bcl-xL) but not proapoptotic (bax, bcl-xS) gene expression. *Eur. J. Immunol.* **26:**294–299.

Appay, V., Zaunders, J. J., Papagno, L., Sutton, J., Jaramillo, A., Waters, A., Easterbrook, P., Grey, P., Smith, D., McMichael, A. J., Cooper, D. A., Rowland-Jones, S. L., and Kelleher, A. D. (2002). Characterization of $CD4^+$ CTLs *ex vivo*. *J. Immunol.* **168:**5954–5958.

Bachmann, M. F., Kundig, T. M., Odermatt, B., Hengartner, H., and Zinkernagel, R. M. (1994). Free recirculation of memory B cells versus antigen-dependent differentiation to antibody-forming cells. *J. Immunol.* **153:**3386–3397.

Badovinac, V. P., and Harty, J. T. (2002). $CD8^+$ T cell homeostasis after infection: Setting the "curve" *Microbes. Infect.* **4:**441–447.

Badovinac, V. P., Porter, B. B., and Harty, J. T. (2002). Programmed contraction of $CD8^+$ T cells after infection. *Nat. Immunol.* **3:**619–626.

Baekkevold, E. S., Yamanaka, T., Palframan, R. T., Carlsen, H. S., Reinholt, F. P., von Andrian, U. H., Brandtzaeg, P., and Haraldsen, G. (2001). The CCR7 ligand *elc* (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. *J. Exp. Med.* **193:**1105–1112.

Balfour, H. H., Jr., Groth, K. E., McCullough, J., Kalis, J. M., Marker, S. C., Nesbit, M. E., Simmons, R. L., and Najarian, J. S. (1977). Prevention or modification of varicella using zoster immune plasma. *Am. J. Dis. Child* **131:**693–696.

Battegay, M., Moskophidis, D., Rahemtulla, A., Hengartner, H., Mak, T. W., and Zinkernagel, R. M. (1994). Enhanced establishment of a virus carrier state in adult $CD4^+$ T cell-deficient mice. *J. Virol.* **68:**4700–4704.

Becker, T. C., Wherry, E. J., Boone, D., Murali-Krishna, K., Antia, R., Ma, A., and Ahmed, R. (2002). Interleukin 15 is required for proliferative renewal of virus-specific memory $CD8^+$ T cells. *J. Exp. Med.* **195:**1541–1548.

Belz, G. T., Liu, H., Andreansky, S., Doherty, P. C., and Stevenson, P. G. (2003). Absence of a functional defect in $CD8^+$ T cells during primary murine gamma-herpesvirus-68 infection of I-$A^{b-/-}$ mice. *J. Gen. Virol.* **84:**337–341.

Belz, G. T., Wodarz, D., Diaz, G., Nowak, M. A., and Doherty, P. C. (2002). Compromised influenza virus-specific $CD8^+$ T cell memory in $CD4^+$ T cell-deficient mice. *J. Virol.* **76:**12388–12393.

Belz, G. T., Xie, W., and Doherty, P. C. (2001). Diversity of epitope and cytokine profiles for primary and secondary influenza a virus-specific $CD8^+$ T cell responses. *J. Immunol.* **166:**4627–4633.

Ben-Nathan, D., Lustig, S., Tam, G., Robinzon, S., Segal, S., and Rager-Zisman, B. (2003). Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. *J. Infect. Dis.* **188:**5–12.

Bendelja, K., Gagro, A., Bace, A., Lokar-Kolbas, R., Krsulovic-Hresic, V., Drazenovic, V., Mlinaric-Galinovic, G., and Rabatic, S. (2000). Predominant type-2 response in infants with respiratory syncytial virus (RSV) infection demonstrated by cytokine flow cytometry. *Clin. Exp. Immunol.* **121:**332–338.

Bennett, S. R., Carbone, F. R., Karamalis, F., Flavell, R. A., Miller, J. F., and Heath, W. R. (1998). Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* **393:**478–480.

Bergamini, A., Bolacchi, F., Cerasari, G., Carvelli, C., Faggioli, E., Cepparulo, M., Demin, F., Uccella, I., Bongiovanni, B., Niutta, P., Capozzi, M., Lupi, M., Piscitelli, E., Rocchi, G., and Angelico, M. (2001). Lack of evidence for the Th2 predominance in patients with chronic hepatitis C. *Clin. Exp. Immunol.* **123:**451–458.

Bird, J. J., Brown, D. R., Mullen, A. C., Moskowitz, N. H., Mahowald, M. A., Sider, J. R., Gajewski, T. F., Wang, C. R., and Reiner, S. L. (1998). Helper T cell differentiation is controlled by the cell cycle. *Immunity* **9:**229–237.

Blattman, J. N., Antia, R., Sourdive, D. J., Wang, X., Kaech, S. M., Murali-Krishna, K., Altman, J. D., and Ahmed, R. (2002). Estimating the precursor frequency of naive antigen-specific $CD8^+$ T cells. *J. Exp. Med.* **195:**657–664.

Blattman, J. N., Grayson, J. M., Wherry, E. J., Kaech, S. M., Smith, K. A., and Ahmed, R. (2003). Therapeutic use of IL-2 to enhance antiviral T cell responses *in vivo*. *Nat. Med.* **9:**540–547.

Brunner, T., Mogil, R. J., LaFace, D., Yoo, N. J., Mahboubi, A., Echeverri, F., Martin, S. J., Force, W. R., Lynch, D. H., and Ware, C. F. (1995). Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T cell hybridomas. *Nature* **373:**441–444.

Budd, R. C. (2001). Activation-induced cell death. *Curr. Opin. Immunol.* **13:**356–362.

Buller, R. M. L., Holmes, K. L., Hugin, A., Frederickson, T. N., and Morse, H. C., III. (1987). Induction of cytotoxic T cell responses *in vivo* in the absence of CD4 helper cells. *Nature* **328:**77–79.

Burioni, R., Williamson, R. A., Sanna, P. P., Bloom, F. E., and Burton, D. R. (1994). Recombinant human Fab to glycoprotein D neutralizes infectivity and prevents cell-to-cell transmission of herpes simplex viruses 1 and 2 *in vitro*. *Proc. Natl. Acad. Sci. USA* **91:**355–359.

Burton, D. R. (2002). Antibodies, viruses, and vaccines. *Nat. Rev. Immunol.* **2:**706–713.

Busch, D. H., and Pamer, E. G. (1999). T cell affinity maturation by selective expansion during infection. *J. Exp. Med.* **189:**701–710.

Cahill, R. N., Poskitt, D. C., Frost, D. C., and Trnka, Z. (1977). Two distinct pools of recirculating T lymphocytes: Migratory characteristics of nodal and intestinal T lymphocytes. *J. Exp. Med.* **145:**420–428.

Campbell, J. J., Bowman, E. P., Murphy, K., Youngman, K. R., Siani, M. A., Thompson, D. A., Wu, L., Zlotnik, A., and Butcher, E. C. (1998). 6-C-kine (SLC), a lymphocyte adhesion-triggering chemokine expressed by high endothelium, is an agonist for the MIP-3beta receptor CCR7. *J. Cell Biol.* **141:**1053–1059.

Cauley, L. S., Cookenham, T., Miller, T. B., Adams, P. S., Vignali, K. M., Vignali, D. A., and Woodland, D. L. (2002). Cutting edge: Virus-specific $CD4^+$ memory T cells in nonlymphoid tissues express a highly activated phenotype. *J. Immunol.* **169:**6655–6658.

Chang, K. M., Thimme, R., Melpolder, J. J., Oldach, D., Pemberton, J., Moorhead-Loudis, J., McHutchison, J. G., Alter, H. J., and Chisari, F. V. (2001). Differential $CD4^+$ and $CD8^+$ T cell responsiveness in hepatitis C virus infection. *Hepatology* **33:**267–276.

Christensen, J. P., Cardin, R. D., Branum, K. C., and Doherty, P. C. (1999). $CD4^+$ T cell-mediated control of a gamma-herpesvirus in B cell-deficient mice is mediated by IFN-γ. *Proc. Natl. Acad. Sci. USA* **96:**5135–5140.

Ciurea, A., Hunziker, L., Klenerman, P., Hengartner, H., and Zinkernagel, R. M. (2001). Impairment of $CD4^+$ T cell responses during chronic virus infection prevents neutralizing antibody responses against virus escape mutants. *J. Exp. Med.* **193:**297–305.

Clayton, A. J. (1977). Lassa immune serum. *Bull. WHO* **55:**435–439.

Cohen, G. M. (1997). Caspases: The executioners of apoptosis. *Biochem. J.* **326:**1–16.

Cousens, L. P., Orange, J. S., and Biron, C. A. (1995). Endogenous IL-2 contributes to T cell expansion and IFN-γ production during lymphocytic choriomeningitis virus infection. *J. Immunol.* **155:**5690–5699.

Crotty, S., Kersh, E. N., Cannons, J., Schwartzberg, P. L., and Ahmed, R. (2003). SAP is required for generating long-term humoral immunity. *Nature* **421:**282–287.

del Val, M., Schlicht, H. J., Volkmer, H., Messerle, M., Reddehase, M. J., and Koszinowski, U. H. (1991). Protection against lethal cytomegalovirus infection by a recombinant vaccine containing a single nonameric T cell epitope. *J. Virol.* **65:**3641–3646.

Denecker, G., Vercammen, D., Declercq, W., and Vandenabeele, P. (2001). Apoptotic and necrotic cell death induced by death domain receptors. *Cell Mol. Life Sci.* **58:**356–370.

di Simone, C., and Buchmeier, M. J. (1995). Protection of mice from lethal LCMV infection by a reconstituted glycoprotein vaccine. *In* "Vaccines 95" (R. M. Chanock, F. Brown, H. S. Ginsberg, and E. Norrby, eds.). Cold Spring Harbor Laboratory, Plainview, NY.

Diepolder, H. M., Gruener, N. H., Gerlach, J. T., Jung, M. C., Wierenga, E. A., and Pape, G. R. (2001). Different levels of T cell receptor triggering induce distinct functions in hepatitis B and hepatitis C virus-specific human $CD4^+$ T cell clones. *J. Virol.* **75:**7803–7810.

Doherty, P. C., Topham, D. J., Tripp, R. A., Cardin, R. D., Brooks, J. W., and Stevenson, P. G. (1997). Effector $CD4^+$ and $CD8^+$ T cell mechanisms in the control of respiratory virus infections. *Immunol. Rev.* **159:**105–117.

Doyle, A. M., Mullen, A. C., Villarino, A. V., Hutchins, A. S., High, F. A., Lee, H. W., Thompson, C. B., and Reiner, S. L. (2001). Induction of cytotoxic T lymphocyte antigen 4 (CTLA-4) restricts clonal expansion of helper T cells. *J. Exp. Med.* **194:**893–902.

Edelmann, K. H., and Wilson, C. B. (2001). Role of CD28/CD80-86 and CD40/CD154 costimulatory interactions in host defense to primary herpes simplex virus infection. *J. Virol.* **75:**612–621.

Eisen, H. N., Sykulev, Y., and Tsomides, T. J. (1996). Antigen-specific T cell receptors and their reactions with complexes formed by peptides with major histocompatibility complex proteins. *Adv. Protein Chem.* **49:**1–56.

Ely, K. H., Roberts, A. D., and Woodland, D. L. (2003). Cutting edge: Effector memory $CD8^+$ T cells in the lung airways retain the potential to mediate recall responses. *J. Immunol.* **171:**3338–3342.

Enria, D. A., and Barrera Oro, J. G. (2002). Junin virus vaccines. *Curr. Top. Microbiol. Immunol.* **263:**239–261.

Esser, M. T., Dinglasan, R. D., Krishnamurthy, B., Gullo, C. A., Graham, M. B., and Braciale, V. L. (1997). IL-2 induces Fas ligand/Fas (CD95L/CD95) cytotoxicity in $CD8^+$ and $CD4^+$ T lymphocyte clones. *J. Immunol.* **158:**5612–5618.

Estcourt, M. J., Ramshaw, I. A., and Ramsay, A. J. (1998). Cytokine responses in virus infections: Effects on pathogenesis, recovery, and persistence. *Curr. Opin. Microbiol.* **1:**411–418.

Ferraro-Peyret, C., Quemeneur, L., Flacher, M., Revillard, J. P., and Genestier, L. (2002). Caspase-independent phosphatidylserine exposure during apoptosis of primary T lymphocytes. *J. Immunol.* **169:**4805–4810.

Fisher, R. G., and Edwards, K. M. (1998). Varicella-zoster. *Pediatr. Rev.* **19:**62–66.

Flano, E., Woodland, D. L., Blackman, M. A., and Doherty, P. C. (2001). Analysis of virus-specific $CD4^+$ T cells during long-term gamma-herpesvirus infection. *J. Virol.* **75:**7744–7748.

Fouchier, R. A., Kuiken, T., Schutten, M., van Amerongen, G., van Doornum, G. J., van den Hoogen, B. G., Peiris, M., Lim, W., Stohr, K., and Osterhaus, A. D. (2003). Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* **423:**240.

Foulds, K. E., Zenewicz, L. A., Shedlock, D. J., Jiang, J., Troy, A. E., and Shen, H. (2002). Cutting edge: $CD4^+$ and $CD8^+$ T cells are intrinsically different in their proliferative responses. *J. Immunol.* **168:**1528–1532.

Frame, J. D. (1989). Clinical features of Lassa fever in Liberia. *Rev. Infect. Dis.* **11:**S783–S789.

Franco, A., Guidotti, L. G., Hobbs, M. V., Pasquetto, V., and Chisari, F. V. (1997a). Pathogenetic effector function of CD4-positive T helper 1 cells in hepatitis B virus transgenic mice. *J. Immunol.* **159:**2001–2008.

Franco, A., Tilly, D. A., Gramaglia, I., Croft, M., Cipolla, L., Meldal, M., and Grey, H. M. (2000). Epitope affinity for MHC class I determines helper requirement for CTL priming. *Nat. Immunol.* **1:**145–150.

Franco, M. A., Tin, C., Rott, L. S., van Cott, J. L., McGhee, J. R., and Greenberg, H. B. (1997b). Evidence for $CD8^+$ T cell immunity to murine rotavirus in the absence of perforin, fas, and γ interferon. *J. Virol.* **71:**479–486.

Frelinger, J. A., and Serody, J. (1998). Immune response of β2-microglobulin-deficient mice to pathogens. *Curr. Top. Microbiol. Immunol.* **232:**99–114.

Fujinami, R. S., and Oldstone, M. B. A. (1979). Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell. *Nature* **279:**529–530.

Fuller, M. J., and Zajac, A. J. (2003). Ablation of $CD8^+$ and $CD4^+$ T cell responses by high viral loads. *J. Immunol.* **170:**477–486.

Gallatin, W. M., Weissman, I. L., and Butcher, E. C. (1983). A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* **304:**30–34.

Garside, P., Ingulli, E., Merica, R. R., Johnson, J. G., Noelle, R. J., and Jenkins, M. K. (1998). Visualization of specific B and T lymphocyte interactions in the lymph node. *Science* **281:**96–99.

Gebhard, J. R., Perry, C. M., Harkins, S., Lane, T., Mena, I., Asensio, V. C., Campbell, I. L., and Whitton, J. L. (1998). Coxsackie virus B3-induced myocarditis: Perforin exacerbates disease but plays no detectable role in virus clearance. *Am. J. Pathol.* **153:**417–428.

Gerhard, W. (2001). The role of the antibody response in influenza virus infection. *Curr. Top. Microbiol. Immunol.* **260:**171–190.

Godkin, A. J., Thomas, H. C., and Openshaw, P. J. (2002). Evolution of epitope-specific memory $CD4^+$ T cells after clearance of hepatitis C virus. *J. Immunol.* **169:**2210–2214.

Graham, M. B., Braciale, V. L., and Braciale, T. J. (1994). Influenza virus-specific $CD4^+$ T helper type 2 T lymphocytes do not promote recovery from experimental virus infection. *J. Exp. Med.* **180:**1273–1282.

Grayson, J. M., Murali-Krishna, K., Altman, J. D., and Ahmed, R. (2001). Gene expression in antigen-specific $CD8^+$ T cells during viral infection. *J. Immunol.* **166:**795–799.

Gregson, S., Terceira, N., Kakowa, M., Mason, P. R., Anderson, R. M., Chandiwana, S. K., and Carael, M. (2002). Study of bias in antenatal clinic HIV-1 surveillance data in a high contraceptive prevalence population in sub-Saharan Africa. *AIDS* **16:**643–652.

Guidotti, L. G., and Chisari, F. V. (1996). To kill or to cure: Options in host defense against viral infection. *Curr. Opin. Immunol.* **8:**478–483.

Gunn, M. D., Tangemann, K., Tam, C., Cyster, J. G., Rosen, S. D., and Williams, L. T. (1998). A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc. Natl. Acad. Sci. USA* **95:**258–263.

Gupta, M., Mahanty, S., Bray, M., Ahmed, R., and Rollin, P. E. (2001). Passive transfer of antibodies protects immunocompetent and immunodeficient mice against lethal Ebola virus infection without complete inhibition of viral replication. *J. Virol.* **75:**4649–4654.

Halper, J., Fu, S. M., Wang, C. Y., Winchester, R., and Kunkel, H. G. (1978). Patterns of expression of human "Ia-like" antigens during the terminal stages of B cell development *J. Immunol.* **120:**1480–1484.

Hammon, W. M., Coriell, L. L., Wehrle, P. F., and Stokes, J. (1953). Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis 4. Final report of results based on clinical diagnoses. *JAMA* **151:**1272–1285.

Haring, J. S., Pewe, L. L., and Perlman, S. (2001). High-magnitude, virus-specific $CD4^+$ T cell response in the central nervous system of coronavirus-infected mice. *J. Virol.* **75:**3043–3047.

Harrington, L. E., van der Most, R. G., Whitton, J. L., and Ahmed, R. (2002). Recombinant vaccinia virus induced T cell immunity: Quantitation of the response to the virus vector and the foreign epitope. *J. Virol.* **76:**3329–3337.

Heath, W. R., Kjer-Nielsen, L., and Hoffmann, M. W. (1993). Avidity for antigen can influence the helper dependence of $CD8^+$ T lymphocytes. *J. Immunol.* **151:**5993–6001.

Helmreich, E., Kern, M., and Eisen, H. N. (2003). The secretion of antibody by isolated lymph node cells. *J. Biol. Chem.* **236:**464–473.

Hibi, T., and Dosch, H. M. (1986). Limiting dilution analysis of the B cell compartment in human bone marrow. *Eur. J. Immunol.* **16:**139–145.

Hildeman, D. A., Mitchell, T., Teague, T. K., Henson, P., Day, B. J., Kappler, J., and Marrack, P. C. (1999). Reactive oxygen species regulate activation-induced T cell apoptosis. *Immunity* **10:**735–744.

Hildeman, D. A., Zhu, Y., Mitchell, T., Kappler, J., and Marrack, P. (2002a). Molecular mechanisms of activated T cell death *in vivo*. *Curr. Opin. Immunol.* **14:**354–359.

Hildeman, D. A., Zhu, Y., Mitchell, T. C., Bouillet, P., Strasser, A., Kappler, J., and Marrack, P. (2002b). Activated T cell death *in vivo* mediated by proapoptotic bcl-2 family member bim. *Immunity* **16:**759–769.

Hogan, R. J., Cauley, L. S., Ely, K. H., Cookenham, T., Roberts, A. D., Brennan, J. W., Monard, S., and Woodland, D. L. (2002). Long-term maintenance of virus-specific

effector memory $CD8^+$ T cells in the lung airways depends on proliferation. *J. Immunol.* **169:**4976–4981.

Hogan, R. J., Usherwood, E. J., Zhong, W., Roberts, A. A., Dutton, R. W., Harmsen, A. G., and Woodland, D. L. (2001a). Activated antigen-specific $CD8^+$ T cells persist in the lungs following recovery from respiratory virus infections. *J. Immunol.* **166:**1813–1822.

Hogan, R. J., Zhong, W., Usherwood, E. J., Cookenham, T., Roberts, A. D., and Woodland, D. L. (2001b). Protection from respiratory virus infections can be mediated by antigen-specific $CD4^+$ T cells that persist in the lungs. *J. Exp. Med.* **193:**981–986.

Holler, N., Zaru, R., Micheau, O., Thome, M., Attinger, A., Valitutti, S., Bodmer, J. L., Schneider, P., Seed, B., and Tschopp, J. (2000a). Fas triggers an alternative caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nat. Immunol.* **1:**489–495.

Holler, P. D., Holman, P. O., Shusta, E. V., O'Herrin, S., Wittrup, K. D., and Kranz, D. M. (2000b). *In vitro* evolution of a T cell receptor with high affinity for peptide/MHC. *Proc. Natl. Acad. Sci. USA* **97:**5387–5392.

Hom, R. C., Finberg, R. W., Mullaney, S., and Ruprecht, R. M. (1991). Protective cellular retroviral immunity requires both $CD4^+$ and $CD8^+$ immune T cells. *J. Virol.* **65:**220–224.

Homann, D., Teyton, L., and Oldstone, M. B. A. (2001). Differential regulation of antiviral T cell immunity results in stable $CD8^+$ but declining $CD4^+$ T cell memory. *Nat. Med.* **7:**913–919.

Hou, S., Hyland, L., Ryan, K. W., Portner, A., and Doherty, P. C. (1994). Virus-specific $CD8^+$ T cell memory determined by clonal burst size. *Nature* **369:**652–654.

Hou, S., Mo, X. Y., Hyland, L., and Doherty, P. C. (1995). Host response to Sendai virus in mice lacking class II major histocompatibility complex glycoproteins. *J. Virol.* **69:**1429–1434.

Huber, S. A., and Pfaeffle, B. (1994). Differential T_h1 and T_h2 cell responses in male and female BALB/c mice infected with coxsackie virus group B type 3. *J. Virol.* **68:**5126–5132.

Hunziker, L., Klenerman, P., Zinkernagel, R. M., and Ehl, S. (2002). Exhaustion of cytotoxic T cells during adoptive immunotherapy of virus carrier mice can be prevented by B cells or $CD4^+$ T cells. *Eur. J. Immunol.* **32:**374–382.

Hyland, L., Sangster, M., Sealy, R., and Coleclough, C. (1994). Respiratory virus infection of mice provokes a permanent humoral immune response. *J. Virol.* **68:**6083–6086.

Igarashi, A., Fukuoka, T., and Fukai, K. (1971). Passive immunization of mice with rabbit antisera against Chikungunya virus and its components. *Biken. J.* **14:**353–355.

Imami, N., Pires, A., Hardy, G., Wilson, J., Gazzard, B., and Gotch, F. (2002). A balanced type 1/type 2 response is associated with long-term nonprogressive human immunodeficiency virus type 1 infection. *J. Virol.* **76:**9011–9023.

Irvine, D. J., Purbhoo, M. A., Krogsgaard, M., and Davis, M. M. (2002). Direct observation of ligand recognition by T cells. *Nature* **419:**845–849.

Jaattela, M., and Tschopp, J. (2003). Caspase-independent cell death in T lymphocytes. *Nat. Immunol.* **4:**416–423.

Jacob, J., and Baltimore, D. (1999). Modelling T cell memory by genetic marking of memory T cells *in vivo*. *Nature* **399:**593–597.

Jansen, V. A. A., Stollenwerk, N., Jensen, H. J., Ramsay, M. E., Edmunds, W. J., and Rhodes, C. J. (2003). Measles outbreaks in a population with declining vaccine uptake. *Science* **301:**804.

Janssen, E. M., Lemmens, E. E., Wolfe, T., Christen, U., von Herrath, M. G., and Schoenberger, S. P. (2003). $CD4^+$ T cells are required for secondary expansion and memory in $CD8^+$ T lymphocytes. *Nature* **421:**852–856.

Janssen, O., Sanzenbacher, R., and Kabelitz, D. (2000). Regulation of activation-induced cell death of mature T-lymphocyte populations. *Cell Tissue Res.* **301:**85–99.

Jennings, S. R., Bonneau, R. H., Smith, P. M., Wolcott, R. M., and Chervenak, R. (1991). $CD4^+$ T lymphocytes are required for the generation of the primary but not the secondary $CD8^+$ cytolytic T lymphocyte response to herpes simplex virus in C57BL/6 mice. *Cell Immunol.* **133:**234–252.

Johnson, A. J., Njenga, M. K., Hansen, M. J., Kuhns, S. T., Chen, L., Rodriguez, M., and Pease, L. R. (1999). Prevalent class I-restricted T-cell response to the Theiler's virus epitope Db:VP2121-130 in the absence of endogenous CD4 help, tumor necrosis factor alpha, gamma interferon, perforin, or costimulation through CD28. *J. Virol.* **73:**3702–3708.

Kaech, S. M., and Ahmed, R. (2001). Memory $CD8^+$ T cell differentiation: Initial antigen encounter triggers a developmental program in naive cells. *Nat. Immunol.* **2:**415–422.

Kaech, S. M., Wherry, E. J., and Ahmed, R. (2002). Effector and memory T cell differentiation: Implications for vaccine development. *Nat. Rev. Immunol.* **2:**251–262.

Kafrouni, M. I., Brown, G. R., and Thiele, D. L. (2001). Virally infected hepatocytes are resistant to perforin-dependent CTL effector mechanisms. *J. Immunol.* **167:**1566–1574.

Kagi, D., and Hengartner, H. (1996). Different roles for cytotoxic T cells in the control of infections with cytopathic versus noncytopathic viruses. *Curr. Opin. Immunol.* **8:**472–477.

Kagi, D., Ledermann, B., Burki, K., Seiler, P., Odermatt, B., Olsen, K. J., Podack, E. R., Zinkernagel, R. M., and Hengartner, H. (1994). Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. *Nature* **369:**31–37.

Kagi, D., Seiler, P., Pavlovic, J., Ledermann, B., Burki, K., Zinkernagel, R. M., and Hengartner, H. (1995). The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses. *Eur. J. Immunol.* **25:**3256–3262.

Kalergis, A. M., Boucheron, N., Doucey, M. A., Palmieri, E., Goyarts, E. C., Vegh, Z., Luescher, I. F., and Nathenson, S. G. (2001). Efficient T cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex. *Nat. Immunol.* **2:**229–234.

Kallas, E. G., Reynolds, K., Andrews, J., Fitzgerald, T., Kasper, M., Menegus, M., and Evans, T. G. (1998). Cytomegalovirus-specific IFN-γ and IL-4 are produced by antigen expanded human blood lymphocytes from seropositive volunteers. *Immunol. Lett.* **64:**63–69.

Kamperschroer, C., and Quinn, D. G. (1999). Quantification of epitope-specific MHC class II-restricted T cells following lymphocytic choriomeningitis virus infection. *Cell Immunol.* **193:**134–146.

Keller, M. A., and Stiehm, E. R. (2000). Passive immunity in prevention and treatment of infectious diseases. *Clin. Microbiol. Rev.* **13:**602–614.

Kelly, E., Won, A., Refaeli, Y., and van Parijs, L. (2002). IL-2 and related cytokines can promote T cell survival by activating AKT. *J. Immunol.* **168:**597–603.

Kempe, C. H. (1960). Studies on smallpox and complications of smallpox vaccination. *Pediatrics* **25:**176–189.

Kempe, C. H., Bowles, C., Meiklejohn, G., Berge, T. O., St. Vincent, L., Babu, B. V. S., Govindarajan, S., Ratnakannan, N. R., Downie, A. W., and Murthy, V. R. (1961). The

use of vaccinia hyperimmune gammaglobulin in the prophylaxis of smallpox. *Bull. WHO* **25:**41–48.

Kersh, E. N., Kaech, S. M., Onami, T. M., Moran, M., Wherry, E. J., Miceli, M. C., and Ahmed, R. (2003). TCR signal transduction in antigen-specific memory $CD8^+$ T cells. *J. Immunol.* **170:**5455–5463.

Khanolkar, A., Yagita, H., and Cannon, M. J. (2001). Preferential utilization of the perforin/granzyme pathway for lysis of Epstein-Barr virus-transformed lymphoblastoid cells by virus-specific $CD4^+$ T cells. *Virol.* **287:**79–88.

Klavinskis, L. S., Oldstone, M. B. A., and Whitton, J. L. (1989). Designing vaccines to induce cytotoxic T lymphocytes: Protection from lethal viral infection. *In* "Vaccines 89. Modern approaches to new vaccines including prevention of AIDS" (F. Brown, R. Chanock, H. Ginsberg, and R. Lerner, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor.

Konig, R., Huang, L. Y., and Germain, R. N. (1992). MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. *Nature* **356:**796–798.

Kopf, M., Coyle, A. J., Schmitz, N., Barner, M., Oxenius, A., Gallimore, A., Gutierrez-Ramos, J. C., and Bachmann, M. F. (2000). Inducible costimulator protein (ICOS) controls T helper cell subset polarization after virus and parasite infection. *J. Exp. Med.* **192:**53–61.

Kopf, M., Ruedl, C., Schmitz, N., Gallimore, A., Lefrang, K., Ecabert, B., Odermatt, B., and Bachmann, M. F. (1999). OX40-deficient mice are defective in Th cell proliferation but are competent in generating B cell and CTL responses after virus infection. *Immunity* **11:**699–708.

Kreil, T. R., and Eibl, M. M. (1997). Pre- and postexposure protection by passive immunoglobulin but no enhancement of infection with a *Flavivirus* in a mouse model. *J. Virol.* **71:**2921–2927.

Krummel, M. F., Heath, W. R., and Allison, J. (1999). Differential coupling of second signals for cytotoxicity and proliferation in $CD8^+$ T cell effectors: Amplification of the lytic potential by B7. *J. Immunol.* **163:**2999–3006.

Kuiken, T., Fouchier, R. A., Schutten, M., Rimmelzwaan, G. F., van Amerongen, G., van Riel, D., Laman, J. D., de Jong, T., van Doornum, G., Lim, W., Ling, A. E., Chan, P. K., Tam, J. S., Zambon, M. C., Gopal, R., Drosten, C., van der, W. S., Escriou, N., Manuguerra, J. C., Stohr, K., Peiris, J. S., and Osterhaus, A. D. (2003). Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* **362:**263–270.

Lantz, O., Grandjean, I., Matzinger, P., and di Santo, J. P. (2000). Gamma chain required for naive $CD4^+$ T cell survival but not for antigen proliferation. *Nat. Immunol.* **1:**54–58.

Lanzavecchia, A. (1985). Antigen-specific interaction between T and B cells. *Nature* **314:**537–539.

Lau, L. L., Jamieson, B. D., Somasundaram, T., and Ahmed, R. (1994). Cytotoxic T cell memory without antigen. *Nature* **369:**648–652.

Lauvau, G., Vijh, S., Kong, P., Horng, T., Kerksiek, K., Serbina, N., Tuma, R. A., and Pamer, E. G. (2001). Priming of memory but not effector $CD8^+$ T cells by a killed bacterial vaccine. *Science* **294:**1735–1739.

Lee, W. T., Pasos, G., Cecchini, L., and Mittler, J. N. (2002). Continued antigen stimulation is not required during $CD4^+$ T cell clonal expansion. *J. Immunol.* **168:**1682–1689.

Lenardo, M., Chan, K. M., Hornung, F., McFarland, H., Siegel, R., Wang, J., and Zheng, L. (1999). Mature T lymphocyte apoptosis—Immune regulation in a dynamic and unpredictable antigenic environment. *Annu. Rev. Immunol.* **17:**221–253.

Levine, B., Hardwick, J. M., Trapp, B. D., Crawford, T. O., Bollinger, R. C., and Griffin, D. E. (1991). Antibody-mediated clearance of alphavirus infection from neurons. *Science* **254:**856–860.

Levy, J. A., Mackewicz, C. E., and Barker, E. (1996). Controlling HIV pathogenesis: The role of the noncytotoxic anti-HIV response of $CD8^+$ T cells. *Immunol. Today* **17:**217–224.

Liu, H., Andreansky, S., Diaz, G., Hogg, T., and Doherty, P. C. (2002). Reduced functional capacity of $CD8^+$ T cells expanded by postexposure vaccination of gamma-herpesvirus-infected CD4-deficient mice. *J. Immunol.* **168:**3477–3483.

Lutz, C., Ledermann, B., Kosco-Vilbois, M. H., Ochsenbein, A. F., Zinkernagel, R. M., Kohler, G., and Brombacher, F. (1998). IgD can largely substitute for loss of IgM function in B cells. *Nature* **393:**797–801.

Mackay, C. R., Marston, W. L., Dudler, L., Spertini, O., Tedder, T. F., and Hein, W. R. (1992). Tissue-specific migration pathways by phenotypically distinct subpopulations of memory T cells. *Eur. J. Immunol.* **22:**887–895.

Mahon, B. P., Katrak, K., Nomoto, A., Macadam, A. J., Minor, P. D., and Mills, K. H. (1995). Poliovirus-specific $CD4^+$ Th1 clones with both cytotoxic and helper activity mediate protective humoral immunity against a lethal poliovirus infection in transgenic mice expressing the human poliovirus receptor. *J. Exp. Med.* **181:**1285–1292.

Maini, M. K., Gudgeon, N., Wedderburn, L. R., Rickinson, A. B., and Beverley, P. C. (2000). Clonal expansions in acute EBV infection are detectable in the CD8 and not the CD4 subset and persist with a variable CD45 phenotype. *J. Immunol.* **165:**5729–5737.

Maini, M. K., Wedderburn, L. R., Hall, F. C., Wack, A., Casorati, G., and Beverley, P. C. (1998). A comparison of two techniques for the molecular tracking of specific T cell responses: $CD4^+$ human T cell clones persist in a stable hierarchy but at a lower frequency than clones in the $CD8^+$ population. *Immunology* **94:**529–535.

Manjunath, N., Shankar, P., Wan, J., Weninger, W., Crowley, M. A., Hieshima, K., Springer, T. A., Fan, X., Shen, H., Lieberman, J., and von Andrian, U. H. (2001). Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes. *J. Clin. Invest.* **108:**871–878.

Manz, R. A., Thiel, A., and Radbruch, A. (1997). Lifetime of plasma cells in the bone marrow. *Nature* **388:**133–134.

Marshall, D. R., Turner, S. J., Belz, G. T., Wingo, S., Andreansky, S., Sangster, M. Y., Riberdy, J. M., Liu, T., Tan, M., and Doherty, P. C. (2001). Measuring the diaspora for virus-specific $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA* **98:**6313–6318.

Marzo, A. L., Vezys, V., Williams, K., Tough, D. F., and Lefrancois, L. (2002). Tissue-level regulation of Th1 and Th2 primary and memory $CD4^+$ T cells in response to *Listeria* infection. *J. Immunol.* **168:**4504–4510.

Masopust, D., Vezys, V., Marzo, A. L., and Lefrancois, L. (2001). Preferential localization of effector memory cells in nonlymphoid tissue. *Science* **291:**2413–2417.

Matloubian, M., Concepcion, R. J., and Ahmed, R. (1994). $CD4^+$ T cells are required to sustain $CD8^+$ cytotoxic T cell responses during chronic viral infection. *J. Virol.* **68:**8056–8063.

McCormick, J. B. (1986). Clinical epidemiologic and therapeutic aspects of Lassa fever. *Med. Microbiol. Immunol.* **175:**153–155.

McSorley, S. J., Asch, S., Costalonga, M., Reinhardt, R. L., and Jenkins, M. K. (2002). Tracking *Salmonella*-specific $CD4^+$ T cells *in vivo* reveals a local mucosal response to a disseminated infection. *Immunity* **16:**365–377.

Medana, I. M., Gallimore, A., Oxenius, A., Martinic, M. M., Wekerle, H., and Neumann, H. (2000). MHC class I-restricted killing of neurons by virus-specific $CD8^+$ T lymphocytes is effected through the Fas/FasL but not the perforin pathway. *Eur. J. Immunol.* **30:**3623–3633.

Mercado, R., Vijh, S., Allen, S. E., Kerksiek, K., Pilip, I. M., and Pamer, E. G. (2000). Early programming of T cell populations responding to bacterial infection. *J. Immunol.* **165:**6833–6839.

Mo, X. Y., Sarawar, S. R., and Doherty, P. C. (1995). Induction of cytokines in mice with parainfluenza pneumonia. *J. Virol.* **69:**1288–1291.

Mo, X. Y., Tripp, R. A., Sangster, M. Y., and Doherty, P. C. (1997). The cytotoxic T-lymphocyte response to Sendai virus is unimpaired in the absence of γ interferon. *J. Virol.* **71:**1906–1910.

Monath, T. P. (2003). Yellow fever. *In* "Vaccines" (S. A. Plotkin and W. A. Orenstein, eds.). 4th ed. W. B. Saunders, Philadelphia.

Monath, T. P., and Cetron, M. S. (2002). Prevention of yellow fever in persons traveling to the tropics. *Clin. Infect. Dis.* **34:**1369–1378.

Muller, D., Koller, B. H., Whitton, J. L., LaPan, K., Brigman, K. K., Frelinger, J. A., and LaPan, K. E. (1992). LCMV-specific, class II-restricted cytotoxic T cells in β2-microglobulin-deficient mice. *Science* **255:**1576–1578.

Mupapa, K., Massamba, M., Kibadi, K., Kuvula, K., Bwaka, A., Kipasa, M., Colebunders, R., and Muyembe-Tamfum, J. J. (1999). Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J. Infect. Dis.* **179**(Suppl. 1)**:**S18–S23.

Murali-Krishna, K., Lau, L. L., Sambhara, S., Lemonnier, F., Altman, J. D., and Ahmed, R. (1999). Persistence of memory $CD8^+$ T cells in MHC class I-deficient mice. *Science* **286:**1377–1381.

Murali-Krishna, K., Ravi, V., and Manjunath, R. (1996). Protection of adult but not newborn mice against lethal intracerebral challenge with Japanese encephalitis virus by adoptively transferred virus-specific cytotoxic T lymphocytes: Requirement for $L3T4^+$ T cells. *J. Gen. Virol.* **77:**705–714.

Nagata, S., and Suda, T. (1995). Fas and Fas ligand: lpr and gld mutations. *Immunol. Today* **16:**39–43.

Nakayama, T., Zhou, J., and Fujino, M. (2003). Current status of measles in Japan. *J. Infect. Chemother.* **9:**1–7.

Nishimura, Y., Igarashi, T., Haigwood, N., Sadjadpour, R., Plishka, R. J., Buckler-White, A., Shibata, R., and Martin, M. A. (2002). Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. *J. Virol.* **76:**2123–2130.

Oehen, S., and Brduscha-Riem, K. (1998). Differentiation of naive CTL to effector and memory CTL: Correlation of effector function with phenotype and cell division. *J. Immunol.* **161:**5338–5346.

Ogilvie, M. M. (1998). Antiviral prophylaxis and treatment in chickenpox. A review prepared for the UK Advisory Group on Chickenpox on behalf of the British Society for the Study of Infection. *J. Infect* **36**(Suppl. 1)**:**31–38.

Olson, J. K., Girvin, A. M., and Miller, S. D. (2001). Direct activation of innate and antigen-presenting functions of microglia following infection with Theiler's virus. *J. Virol.* **75:**9780–9789.

Opferman, J. T., Ober, B. T., and Ashton-Rickardt, P. G. (1999). Linear differentiation of cytotoxic effectors into memory T lymphocytes. *Science* **283:**1745–1748.

Ostler, T., Hussell, T., Surh, C. D., Openshaw, P., and Ehl, S. (2001). Long-term persistence and reactivation of T cell memory in the lung of mice infected with respiratory syncytial virus. *Eur. J. Immunol.* **31:**2574–2582.

Ovsyannikova, I. G., Dhiman, N., Jacobson, R. M., Vierkant, R. A., and Poland, G. A. (2003). Frequency of measles virus-specific $CD4^+$ and $CD8^+$ T cells in subjects seronegative or highly seropositive for measles vaccine. *Clin. Diagn. Lab Immunol.* **10:**411–416.

Oxenius, A., Zinkernagel, R. M., and Hengartner, H. (1998). Comparison of activation versus induction of unresponsiveness of virus-specific $CD4^+$ and $CD8^+$ T cells upon acute versus persistent viral infection. *Immunity* **9:**449–457.

Padlan, E. A. (1994). Anatomy of the antibody molecule. *Mol. Immunol.* **31:**169–217.

Pantaleo, G., Demarest, J. F., Vaccarezza, M., Graziosi, C., Bansal, G. P., Koenig, S., and Fauci, A. S. (1995). Effect of anti-V3 antibodies on cell-free and cell-to-cell human immunodeficiency virus transmission. *Eur. J. Immunol.* **25:**226–231.

Pape, K. A., Kouskoff, V., Nemazee, D., Tang, H. L., Cyster, J. G., Tze, L. E., Hippen, K. L., Behrens, T. W., and Jenkins, M. K. (2003). Visualization of the genesis and fate of isotype-switched B cells during a primary immune response. *J. Exp. Med.* **197:**1677–1687.

Parren, P. W., Geisbert, T. W., Maruyama, T., Jahrling, P. B., and Burton, D. R. (2002). Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. *J. Virol.* **76:**6408–6412.

Parren, P. W., Marx, P. A., Hessell, A. J., Luckay, A., Harouse, J., Cheng-Mayer, C., Moore, J. P., and Burton, D. R. (2001). Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization *in vitro*. *J. Virol.* **75:**8340–8347.

Peirce, E. R., Melville, F. S., Downie, A. W., and Duckworth, M. J. (1958). Antivaccinial gamma-globulin in smallpox prophylaxis. *Lancet* **2:**635–638.

Perrin, L. H., Zinkernagel, R. M., and Oldstone, M. B. (1977). Immune response in humans after vaccination with vaccinia virus: Generation of a virus-specific cytotoxic activity by human peripheral lymphocytes. *J. Exp. Med.* **146:**949–969.

Potsch, C., Vohringer, D., and Pircher, H. (1999). Distinct migration patterns of naive and effector $CD8^+$ T cells in the spleen: Correlation with CCR7 receptor expression and chemokine reactivity. *Eur. J. Immunol.* **29:**3562–3570.

Prosniak, M., Faber, M., Hanlon, C. A., Rupprecht, C. E., Hooper, D. C., and Dietzschold, B. (2003). Development of a cocktail of recombinant-expressed human rabies virus-neutralizing monoclonal antibodies for postexposure prophylaxis of rabies. *J. Infect. Dis.* **188:**53–56.

Quinn, D. G., Zajac, A. J., Frelinger, J. A., and Muller, D. (1993). Transfer of lymphocytic choriomeningitis disease in β2-microglobulin-deficient mice by $CD4^+$ T cells. *Int. Immunol.* **5:**1193–1198.

Ravkov, E. V., Myrick, C. M., and Altman, J. D. (2003). Immediate early effector functions of virus-specific $CD8^+CCR7^+$ memory cells in humans defined by HLA and CC chemokine ligand 19 tetramers. *J. Immunol.* **170:**2461–2468.

Refaeli, Y., van Paris, L., London, C. A., Tschopp, J., and Abbas, A. K. (1998). Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. *Immunity* **8:**615–623.

Reinhardt, R. L., Khoruts, A., Merica, R., Zell, T., and Jenkins, M. K. (2001). Visualizing the generation of memory $CD4^+$ T cells in the whole body. *Nature* **410:**101–105.

Ridge, J. P., di Rosa, F., and Matzinger, P. (1998). A conditioned dendritic cell can be a temporal bridge between a $CD4^+$ T-helper and a T-killer cell. *Nature* **393:**474–477.

Robbins, A. S. (1993). Controversies in measles immunization recommendations. *West. J. Med.* **158:**36–39.

Rodriguez, F., Slifka, M. K., Harkins, S., and Whitton, J. L. (2001). Two overlapping subdominant epitopes identified by DNA immunization induce protective $CD8^+$ T cell populations with differing cytolytic activities. *J. Virol.* **75:**7399–7409.

Rogers, P. R., Dubey, C., and Swain, S. L. (2000). Qualitative changes accompany memory T cell generation: Faster, more effective responses at lower doses of antigen. *J. Immunol.* **164:**2338–2346.

Romero, J. R. (2003). Palivizumab prophylaxis of respiratory syncytial virus disease from 1998 to 2002: Results from four years of Palivizumab usage. *Pediatr. Infect. Dis. J.* **22:**S46–S54.

Rosen, H. R., Miner, C., Sasaki, A. W., Lewinsohn, D. M., Conrad, A. J., Bakke, A., Bouwer, H. G., and Hinrichs, D. J. (2002). Frequencies of HCV-specific effector $CD4^+$ T cells by flow cytometry: Correlation with clinical disease stages. *Hepatology* **35:**190–198.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* **401:**708–712.

Salter, R. D., Benjamin, R. J., Wesley, P. K., Buxton, S. E., Garrett, T. P. J., Clayberger, C., Krensky, A. M., Norment, A. M., Littman, D. R., and Parham, P. (1990). A binding site for the T cell coreceptor CD8 on the $\alpha 3$ domain of HLA-A2. *Nature* **345:**41–46.

Salzmann, M., and Bachmann, M. F. (1998). Estimation of maximal affinities between T cell receptors and MHC/peptide complexes. *Mol. Immunol.* **35:**65–71.

Savage, P. A., Boniface, J. J., and Davis, M. M. (1999). A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity* **10:**485–492.

Sawyer, W. A. (1931). Persistence of yellow fever immunity. *J. Prev. Med.* **5:**413–428.

Schluns, K. S., Kieper, W. C., Jameson, S. C., and Lefrancois, L. (2000). Interleukin-7 mediates the homeostasis of naive and memory $CD8^+$ T cells *in vivo*. *Nat. Immunol.* **1:**426–432.

Schluns, K. S., Williams, K., Ma, A., Zheng, X. X., and Lefrancois, L. (2002). Cutting edge: Requirement for IL-15 in the generation of primary and memory antigen-specific $CD8^+$ T cells. *J. Immunol.* **168:**4827–4831.

Schoenberger, S. P., Toes, R. E., van der Voort, E. I., Offringa, R., and Melief, C. J. (1998). T cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions. *Nature* **393:**480–483.

Seddon, B., Tomlinson, P., and Zamoyska, R. (2003). Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nat. Immunol.* **4:**680–686.

Selin, L. K., and Welsh, R. M. (1997). Cytolytically active memory CTL present in lymphocytic choriomeningitis virus-immune mice after clearance of virus infection. *J. Immunol.* **158:**5366–5373.

Shedlock, D. J., and Shen, H. (2003). Requirement for $CD4^+$ T cell help in generating functional $CD8^+$ T cell memory. *Science* **300:**337–339.

Shortridge, K. F., Zhou, N. N., Guan, Y., Gao, P., Ito, T., Kawaoka, Y., Kodihalli, S., Krauss, S., Markwell, D., Murti, K. G., Norwood, M., Senne, D., Sims, L., Takada, A., and Webster, R. G. (1998). Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virol.* **252:**331–342.

Slifka, M. K., and Ahmed, R. (1996). Long-term humoral immunity against viruses: Revisiting the issue of plasma cell longevity. *Trends Microbiol.* **4:**394–400.

Slifka, M. K., Antia, R., Whitmire, J. K., and Ahmed, R. (1998). Humoral immunity due to long-lived plasma cells. *Immunity* **8:**363–372.

Slifka, M. K., Matloubian, M., and Ahmed, R. (1995). Bone marrow is a major site of long-term antibody production after acute viral infection. *J. Virol.* **69:**1895–1902.

Slifka, M. K., Rodriguez, F., and Whitton, J. L. (1999). Rapid on/off cycling of cytokine production by virus-specific $CD8^+$ T cells. *Nature* **401:**76–79.

Slifka, M. K., and Whitton, J. L. (2000a). Activated and memory $CD8^+$ T cells can be distinguished by their cytokine profiles and phenotypic markers. *J. Immunol.* **164:**208–216.

Slifka, M. K., and Whitton, J. L. (2000b). Clinical implications of dysregulated cytokine production. *J. Mol. Med.* **78:**74–80.

Slifka, M. K., and Whitton, J. L. (2001). Functional avidity maturation of $CD8^+$ T cells without selection of higher affinity TCR. *Nat. Immunol.* **2:**711–717.

Smith, K. G., Strasser, A., and Vaux, D. L. (1996). CrmA expression in T lymphocytes of transgenic mice inhibits CD95 (Fas/APO-1)-transduced apoptosis but does not cause lymphadenopathy or autoimmune disease. *EMBO J.* **15:**5167–5176.

Sourdive, D. J., Murali-Krishna, K., Altman, J. D., Zajac, A. J., Whitmire, J. K., Pannetier, C., Kourilsky, P., Evavold, B., Sette, A., and Ahmed, R. (1998). Conserved T cell receptor repertoire in primary and memory $CD8^+$ T cell responses to an acute viral infection. *J. Exp. Med.* **188:**71–82.

Spear, G. T., Hart, M., Olinger, G. G., Hashemi, F. B., and Saifuddin, M. (2001). The role of the complement system in virus infections. *Curr. Top. Microbiol. Immunol.* **260:**229–245.

Sprent, J. (1976). Fate of H2-activated T lymphocytes in syngeneic hosts. I. Fate in lymphoid tissues and intestines traced with 3H-thymidine, 125I-deoxyuridine, and 51 chromium. *Cell Immunol.* **21:**278–302.

Sprent, J., and Surh, C. D. (2002). T cell memory. *Annu. Rev. Immunol.* **20:**551–579.

Srikiatkhachorn, A., Chang, W., and Braciale, T. J. (1999). Induction of Th1 and Th2 responses by respiratory syncytial virus attachment glycoprotein is epitope and major histocompatibility complex independent. *J. Virol.* **73:**6590–6597.

Steigerwald-Mullen, P., Kurilla, M. G., and Braciale, T. J. (2000). Type 2 cytokines predominate in the human $CD4^+$ T-lymphocyte response to Epstein-Barr virus nuclear antigen 1. *J. Virol.* **74:**6748–6759.

Stiehm, E. R. (1979). Standard and special human immune serum globulins as therapeutic agents. *Pediatrics* **63:**301–319.

Stohlman, S. A., Bergmann, C. C., Lin, M. T., Cua, D. J., and Hinton, D. R. (1998). CTL effector function within the central nervous system requires $CD4^+$ T cells. *J. Immunol.* **160:**2896–2904.

Strasser, A., Harris, A. W., Huang, D. C., Krammer, P. H., and Cory, S. (1995). Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. *EMBO J.* **14:**6136–6147.

Su, H. C., Cousens, L. P., Fast, L. D., Slifka, M. K., Bungiro, R. D., Ahmed, R., and Biron, C. A. (1998). $CD4^+$ and $CD8^+$ T cell interactions in IFN-γ and IL-4 responses to viral infections: Requirements for IL-2. *J. Immunol.* **160:**5007–5017.

Sun, J. C., and Bevan, M. J. (2003). Defective $CD8^+$ T cell memory following acute infection without $CD4^+$ T cell help. *Science* **300:**339–342.

Suresh, M., Whitmire, J. K., Harrington, L. E., Larsen, C. P., Pearson, T. C., Altman, J. D., and Ahmed, R. (2001). Role of CD28–B7 interactions in generation and maintenance of $CD8^+$ T cell memory. *J. Immunol.* **167:**5565–5573.

Swain, S. L., Hu, H., and Huston, G. (1999). Class II independent generation of $CD4^+$ memory T cells from effectors. *Science* **286:**1381–1383.

Sykulev, Y., Cohen, R. J., and Eisen, H. N. (1995). The law of mass action governs antigen-stimulated cytolytic activity of $CD8^+$ cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA* **92:**11990–11992.

Tan, J. T., Ernst, B., Kieper, W. C., LeRoy, E., Sprent, J., and Surh, C. D. (2002). Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype $CD8^+$ cells but are not required for memory phenotype $CD4^+$ cells. *J. Exp. Med.* **195:**1523–1532.

Thornberry, N. A., and Lazebnik, Y. (1998). Caspases: Enemies within. *Science* **281:**1312–1316.

Topham, D. J., and Doherty, P. C. (1998). Longitudinal analysis of the acute Sendai virus-specific $CD4^+$ T cell response and memory. *J. Immunol.* **161:**4530–4535.

Topham, D. J., Tripp, R. A., Hamilton-Easton, A. M., Sarawar, S. R., and Doherty, P. C. (1996a). Quantitative analysis of the influenza virus-specific $CD4^+$ T cell memory in the absence of B cells and Ig. *J. Immunol.* **157:**2947–2952.

Topham, D. J., Tripp, R. A., Sarawar, S. R., Sangster, M. Y., and Doherty, P. C. (1996b). Immune $CD4^+$ T cells promote the clearance of influenza virus from major histocompatibility complex class $II^{-/-}$ respiratory epithelium. *J. Virol.* **70:**1288–1291.

Tripp, R. A., Hou, S., Etchart, N., Prinz, A., Moore, D., Winter, J., and Anderson, L. J. (2001). $CD4^+$ T cell frequencies and T_h1/T_h2 cytokine patterns expressed in the acute and memory response to respiratory syncytial virus I-E(d)-restricted peptides. *Cell Immunol.* **207:**59–71.

Tripp, R. A., Moore, D., Barskey, A. T., Jones, L., Moscatiello, C., Keyserling, H., and Anderson, L. J. (2002). Peripheral blood mononuclear cells from infants hospitalized because of respiratory syncytial virus infection express T helper-1 and T helper-2 cytokines and CC chemokine messenger RNA. *J. Infect. Dis.* **185:**1388–1394.

Tsai, S. L., Liaw, Y. F., Chen, M. H., Huang, C. Y., and Kuo, G. C. (1997). Detection of type 2-like T-helper cells in hepatitis C virus infection: Implications for hepatitis C virus chronicity. *Hepatology* **25:**449–458.

Unsoeld, H., Krautwald, S., Voehringer, D., Kunzendorf, U., and Pircher, H. (2002). Cutting edge: $CCR7^+$ and $CCR7^-$ memory T cells do not differ in immediate effector cell function. *J. Immunol.* **169:**638–641.

Valitutti, S., and Lanzavecchia, A. (1997). Serial triggering of TCRs: A basis for the sensitivity and specificity of antigen recognition. *Immunol. Today* **18:**299–304.

van Parijs, L., Peterson, D. A., and Abbas, A. K. (1998). The Fas/Fas ligand pathway and Bcl-2 regulate T cell responses to model self and foreign antigens. *Immunity* **8:**265–274.

van Parijs, L., Refaeli, Y., Lord, J. D., Nelson, B. H., Abbas, A. K., and Baltimore, D. (1999). Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. *Immunity* **11:**281–288.

van Stipdonk, M. J., Lemmens, E. E., and Schoenberger, S. P. (2001). Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat. Immunol.* **2:**423–429.

Vanderplasschen, A., Hollinshead, M., and Smith, G. L. (1997). Antibodies against vaccinia virus do not neutralize extracellular enveloped virus but prevent virus release from infected cells and comet formation. *J. Gen. Virol.* **78:**2041–2048(Pt 8).

Varga, S. M., Selin, L. K., and Welsh, R. M. (2001). Independent regulation of lymphocytic choriomeningitis virus-specific T cell memory pools: Relative stability of CD4 memory under conditions of CD8 memory T cell loss. *J. Immunol.* **166:**1554–1561.

Varga, S. M., and Welsh, R. M. (1998). Detection of a high frequency of virus-specific $CD4^+$ T cells during acute infection with lymphocytic choriomeningitis virus. *J. Immunol.* **161:**3215–3218.

Varga, S. M., and Welsh, R. M. (2000). High frequency of virus-specific interleukin-2-producing $CD4^+$ T cells and Th1 dominance during lymphocytic choriomeningitis virus infection. *J. Virol.* **74:**4429–4432.

Varga, S. M., Wissinger, E. L., and Braciale, T. J. (2000). The attachment (G) glycoprotein of respiratory syncytial virus contains a single immunodominant epitope that elicits both T_h1 and T_h2 $CD4^+$ T cell responses. *J. Immunol.* **165:**6487–6495.

Veazey, R. S., Shattock, R. J., Pope, M., Kirijan, J. C., Jones, J., Hu, Q., Ketas, T., Marx, P. A., Klasse, P. J., Burton, D. R., and Moore, J. P. (2003). Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat. Med.* **9:**343–346.

Vercammen, D., Brouckaert, G., Denecker, G., van de Craen, M., Declercq, W., Fiers, W., and Vandenabeele, P. (1998). Dual signaling of the Fas receptor: Initiation of both apoptotic and necrotic cell death pathways. *J. Exp. Med.* **188:**919–930.

von Herrath, M. G., Fujinami, R. S., and Whitton, J. L. (2003). Microorganisms and autoimmunity—Making the barren field fertile? *Nature Reviews Microbiology* **1:**151–157.

von Herrath, M. G., Yokoyama, M., Dockter, J., Oldstone, M. B. A., and Whitton, J. L. (1996). CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge. *J. Virol.* **70:**1072–1079.

Walker, C. M., Erickson, A. L., Hsueh, F. C., and Levy, J. A. (1991). Inhibition of human immunodeficiency virus replication in acutely infected $CD4^+$ cells by $CD8^+$ cells involves a noncytotoxic mechanism. *J. Virol.* **65:**5921–5927.

Walsh, C. M., Matloubian, M., Liu, C. C., Ueda, R., Kurahara, C. G., Christensen, J. L., Huang, M. T., Young, J. D., Ahmed, R., and Clark, W. R. (1994). Immune function in mice lacking the perforin gene. *Proc. Natl. Acad. Sci. USA* **91:**10854–10858.

Wang, J. H., Layden, T. J., and Eckels, D. D. (2003). Modulation of the peripheral T cell response by CD4 mutants of hepatitis C virus: Transition from a T_h1 to a T_h2 response. *Hum. Immunol.* **64:**662–673.

Ward, B. J., and Griffin, D. E. (1993). Changes in cytokine production after measles virus vaccination: Predominant production of IL-4 suggests induction of a T_h2 response. *Clin. Immunol. Immunopathol.* **67:**171–177.

Ward, R., and Krugman, S. (1962). Etiology, epidemiology, and prevention of viral hepatitis. *Prog. Med. Virol.* **4:**87–118.

Welsh, R. M., and McNally, J. M. (1999). Immune deficiency, immune silencing, and clonal exhaustion of T cell responses during viral infections. *Curr. Opin. Microbiol.* **2:**382–387.

Weninger, W., Manjunath, N., and von Andrian, U. H. (2002). Migration and differentiation of $CD8^+$ T cells. *Immunol. Rev.* **186:**221–233.

Wherry, E. J., Puorro, K. A., Porgador, A., and Eisenlohr, L. C. (1999). The induction of virus-specific CTL as a function of increasing epitope expression: Responses rise steadily until excessively high levels of epitope are attained. *J. Immunol.* **163:**3735–3745.

Wherry, E. J., Teichgraber, V., Becker, T. C., Masopust, D., Kaech, S. M., Antia, R., von Andrian, U. H., and Ahmed, R. (2003). Lineage relationship and protective immunity of memory $CD8^+$ T cell subsets. *Nat. Immunol.* **4:**225–234.

White, H. A. (1972). Lassa fever. A study of 23 hospital cases. *Trans. R. Soc. Trop. Med. Hyg.* **66:**390–401.

Whitmire, J. K., Asano, M. S., Murali-Krishna, K., Suresh, M., and Ahmed, R. (1998). Long-term CD4 Th1 and Th2 memory following acute lymphocytic choriomeningitis virus infection. *J. Virol.* **72:**8281–8288.

Whitmire, J. K., Flavell, R. A., Grewal, I. S., Larsen, C. P., Pearson, T. C., and Ahmed, R. (1999). CD40–CD40 ligand costimulation is required for generating antiviral $CD4^+$

T cell responses but is dispensable for $CD8^+$ T cell responses. *J. Immunol.* **163:**3194–3201.

Whitmire, J. K., Murali-Krishna, K., Altman, J. D., and Ahmed, R. (2000). Antiviral $CD4^+$ and $CD8^+$ T cell memory: Differences in the size of the response and activation requirements. *Philos. Trans. R. Soc. London B Biol. Sci.* **355:**373–379.

Whitton, J. L., Sheng, N., Oldstone, M. B. A., and McKee, T. A. (1993). A "string-of-beads" vaccine, comprising linked minigenes, confers protection from lethal-dose virus challenge *J. Virol.* **67:**348–352.

Wilson, A. D., Hopkins, J. C., and Morgan, A. J. (2001). *In vitro* cytokine production and growth inhibition of lymphoblastoid cell lines by $CD4^+$ T cells from Epstein-Barr virus (EBV) seropositive donors. *Clin. Exp. Immunol.* **126:**101–110.

Wittes, J. T., Kelly, A., and Plante, K. M. (1996). Meta-analysis of CMVIG studies for the prevention and treatment of CMV infection in transplant patients. *Transplant. Proc.* **28:**17–24.

Wright, K. E., and Buchmeier, M. J. (1991). Antiviral antibodies attenuate T cell-mediated immunopathology following acute lymphocytic choriomeningitis virus infection. *J. Virol.* **65:**3001–3006.

Wu, C., Nguyen, K. B., Pien, G. C., Wang, N., Gullo, C., Howie, D., Sosa, M. R., Edwards, M. J., Borrow, P., Satoskar, A. R., Sharpe, A. H., Biron, C. A., and Terhorst, C. (2001). SAP controls T cell responses to virus and terminal differentiation of Th2 cells. *Nat. Immunol.* **2:**410–414.

Wu, Y., and Liu, Y. (1994). Viral induction of costimulatory activity on antigen-presenting cells bypasses the need for $CD4^+$ T cell help in $CD8^+$ T cell responses. *Curr. Biol.* **4:**499–505.

Youngman, K. R., Franco, M. A., Kuklin, N. A., Rott, L. S., Butcher, E. C., and Greenberg, H. B. (2002). Correlation of tissue distribution, developmental phenotype, and intestinal homing receptor expression of antigen-specific B cells during the murine antirotavirus immune response. *J. Immunol.* **168:**2173–2181.

Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. D., Suresh, M., Altman, J.D., and Ahmed, R. (1998). Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188:**2205–2213.

Zajac, A. J., Quinn, D. G., Cohen, P.L., and Frelinger, J. A. (1996). Fas-dependent $CD4^+$ cytotoxic T-cell-mediated pathogenesis during virus infection. *Proc. Natl. Acad. Sci. USA* **93:**14730–14735.

Zhong, W., Roberts, A. D., and Woodland, D. L. (2001). Antibody-independent antiviral function of memory $CD4^+$ T cells *in vivo* requires regulatory signals from $CD8^+$ effector T cells. *J. Immunol.* **167:**1379–1386.

Zhou, S., Ou, R., Huang, L., and Moskophidis, D. (2002). Critical role for perforin-, Fas/FasL-, and TNFR1-mediated cytotoxic pathways in down-regulation of antigen-specific T cells during persistent viral infection. *J. Virol.* **76:**829–840.