Adaptive immune responses

J. Lindsay Whitton

Department of Neuropharmacology, CVN-9, The Scripps Research Institute,
10550 N Torrey Pines Rd, La Jolla, CA 92037, USA

INTRODUCTION

Human urbanization, and the associated domestication of wild animals, afforded many viruses the opportunity to colonize, and better adapt to, our species. In the ensuing millennia, they have taken full advantage, killing more than a billion humans, and establish persistent infections with such efficiency that essentially all adult humans carry lifelong virus infections. Widespread immunization has reduced the toll of several acute virus infections: it has permitted the eradication of smallpox virus and the approaching extermination of poliovirus, and many young physicians have never encountered a patient with acute measles, mumps or rubella. Furthermore, as exemplified by the results of hepatitis B virus (HBV) vaccination in Taiwan (Huang & Lin, 2000), protection against acute virus infection can lead to a reduction in the number of persistently infected individuals (and, for HBV, yields an additional bounty – a lower incidence of the related hepatocellular carcinoma). However, despite the success of antiviral vaccination, infectious diseases are responsible for >20 % of deaths worldwide, and remain a leading cause of death even in so-called 'first world' countries.

Public health measures can dramatically reduce exposure to infectious organisms, classically illustrated by Dr John Snow in 1854, when a cholera outbreak in London was interrupted by his removing the handle of the Broad Street water pump (Cameron & Jones, 1983). But, if exposure occurs, what protects the individual from infection and disease? First come the barrier defences (skin and other epithelia, saliva, gastric acid), which constitute a physico-chemical barricade against intrusion. These are vitally important: consider how much more easily one becomes infected when the skin is

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breached, either by accident (an existing wound is an open invitation to microbes) or by design (penetration by the proboscis of an infected mosquito). Next in line is the multi-faceted innate immune system, whose weaponry includes natural killer (NK) cells, macrophages, polymorphonuclear leukocytes, complement and a plethora of cytokines. The subtlety and complexity of the innate immune response is only now being appreciated, and its role in suppressing virus infection has been highlighted by a recent elegant study of mouse cytomegalovirus (MCMV) infection of SCID mice, which lack an adaptive immune system. It has been known for some time that MCMV infection of SCID mice usually is rapidly lethal, indicating the importance of the adaptive immune response in protecting against this virus. However, occasional mice survive the infection, and appear to clear the virus, indicating the potential effectiveness of innate immunity. Recent work focused on the ultimate fate of these rare survivors, and revealed an ongoing battle; several weeks after their apparent recovery, virus reappeared, and the animals succumbed. DNA sequencing, and other data, indicated that the recrudescent viruses were variants that carried mutations which prevented their recognition by NK cells (French *et al*., 2004). This is the first study to show that DNA viruses evolve in response to pressure from NK cells, and it further underlines the importance of innate immune functions for holding viruses in check. Nevertheless, the barrier and innate immune systems often provide only incomplete protection, and an additional key component protecting us against microbial onslaught is the adaptive immune system. The importance of adaptive immunity is best revealed by two simple facts. *First*, individuals in whom adaptive immunity is compromised (e.g. SCID mice; or humans with agammaglobulinaemia) are at greatly increased risk of both infection and severe disease. *Second*, the success of vaccination – arguably, the most important single medical advance in history – relies almost entirely on the stimulation of adaptive immune responses. In this chapter, I shall outline the nature of the adaptive immune response, then will describe recent studies from my laboratory, investigating the regulation and maturation of one aspect of the adaptive response over the course of an infection.

THE ADAPTIVE IMMUNE RESPONSE DEPENDS ON LYMPHOCYTES

The adaptive immune response is mediated by lymphocytes, which are driven to expand, and to express their effector functions, by contact with specific antigenic moieties that can be recognized by receptors on the lymphocyte membrane. Usually, on any one lymphocyte, all of the surface receptors are identical; therefore – at least broadly speaking – a lymphocyte can respond to only one antigen (i.e. lymphocytes are *antigen-specific*; and the moiety that a lymphocyte recognizes is termed its *cognate* antigen). However, the receptors vary subtly from one lymphocyte to the next, ensuring

that the host can recognize, and mount an immune response to, an enormous diversity of antigens. Any given virus contains several antigens and, therefore, the adaptive immune response to a virus comprises a collection of several distinct antigen-specific responses, each of which is reliant on a lymphocyte expressing appropriate receptor proteins. Upon first encounter with cognate antigen, the host mounts a *primary immune response*, in which antigen-specific effector functions will be somewhat slow to develop, usually becoming detectable only after several days; and this relatively slow response often gives the virus sufficient time to replicate, disseminate, cause illness and spread to the next susceptible host. The somewhat lethargic nature of the primary response results from (i) the host having only a few naïve precursor lymphocytes specific for any one antigen, which means that some time must pass before these antigen-specific lymphocytes can multiply to a biologically meaningful number and (ii) the slow expression of effector functions by the newly activated lymphocytes. In individuals who survive the infection, the primary response wanes (hopefully, after eradication of the microbe), but antigen-specific *memory* lymphocytes remain. These antigen-specific cells are much greater in number than were their naïve progenitors and (as described later for $CD8^+$ T cells) have optimized their effector functions, so, if the host is again exposed to the same virus, these memory cells allow the host to mount a greatly accelerated *secondary immune response*, which usually rapidly shuts down virus replication. The resulting rapid control of infection has at least two favourable consequences: not only does it protect the infected host against disease, it also diminishes the likelihood of virus transmission to susceptible individuals in the community (this latter benefit is termed herd immunity). Memory lymphocytes are induced not only by infection, but also by immunization, and *these cells serve as the cornerstone of all vaccines*.

Two classes of lymphocyte are involved in the adaptive immune response: B lymphocytes (so named because they were first identified in the avian organ, the bursa of Fabricius) and T lymphocytes (which are derived from the thymus). T lymphocytes can be further subdivided into two groups, characterized by the cell-surface expression of accessory proteins named CD4 and CD8; generally, mature T cells express only one of these proteins. B lymphocytes and their progeny (including plasma cells) are the source of antibodies, soluble effector molecules which act mainly to diminish the infectivity of cell-free virus. In contrast, $CD8^+$ T lymphocytes eradicate virus-infected cells (the role of $CD4^+$ T cells is reviewed briefly later). Thus antibodies and $CD8^+$ T lymphocytes act in a complementary manner (reviewed by Whitton & Oldstone, 2001): antibodies neutralize viruses in the fluid phase (e.g. blood, interstitial spaces), thereby reducing the number of cells that become infected; and $CD8^+$ T lymphocytes exert their antiviral effects upon infected cells, thereby reducing the production and release of virus into the extracellular milieu. Both antibodies (B cells) and T cells are key

components of the antiviral immune response, but in this chapter I shall concentrate on T cells, as they are a major research focus of my laboratory.

T CELLS RECOGNIZE FRAGMENTS OF VIRUS PROTEINS DISPLAYED ON THE CELL SURFACE BY MHC MOLECULES

As noted earlier, each lymphocyte expresses multiple copies of a unique receptor which dictates the antigen specificity of the cell. For both $CD4^+$ and $CD8^+$ T cells, this T-cell receptor (TcR) recognizes short (9–24 amino acid) peptide fragments (*epitopes*) generated inside the 'target' cell by degradation of viral proteins. These epitopes are displayed on the target cell surface, for T-cell perusal, by host glycoproteins encoded in the major histocompatibility complex (MHC); the epitope/MHC complex is, therefore, the antigen for which a TcR (and its lymphocyte) is specific. There are two types of MHC glycoprotein, termed MHC class I and MHC class II. These classes differ in several ways, of which three are particularly important.

(i) Interactions with CD4 or CD8 proteins

MHC molecules on target cells not only present epitopes for TcR recognition, but also are directly recognized by these T-cell accessory proteins. Conserved regions on MHC class I molecules interact directly with CD8 (Salter *et al*., 1990), and there is a similar interaction between MHC class II proteins and CD4 (Konig *et al*., 1992). Thus $CD8^+$ T cells can exert their activities only on target cells that express MHC class I; and, similarly, the effects of $CD4^+$ T cells are limited to target cells that express MHC class II.

(ii) Anatomical distribution

MHC class I molecules are expressed on the surface of almost all somatic cells, but expression of MHC class II molecules is restricted to specialized antigen presenting cells (APCs; these cells usually also express class I MHC).

(iii) Source of epitopes

MHC class I molecules present epitopes from proteins *synthesized inside* a target cell, which means that $CD8^+$ T cells can recognize virus-infected cells. In contrast, epitopes presented by MHC class II molecules are derived from proteins *that have been taken up from the extracellular milieu*. This protein uptake and degradation is the purview of APCs, and these cells, therefore, can be recognized by $CD4^+$ T cells even though they are not actively infected.

These three differences in MHC have profound biological implications for their T-cell partners. $CD8^+$ T cells can, in principle, recognize (and exert their antiviral effects

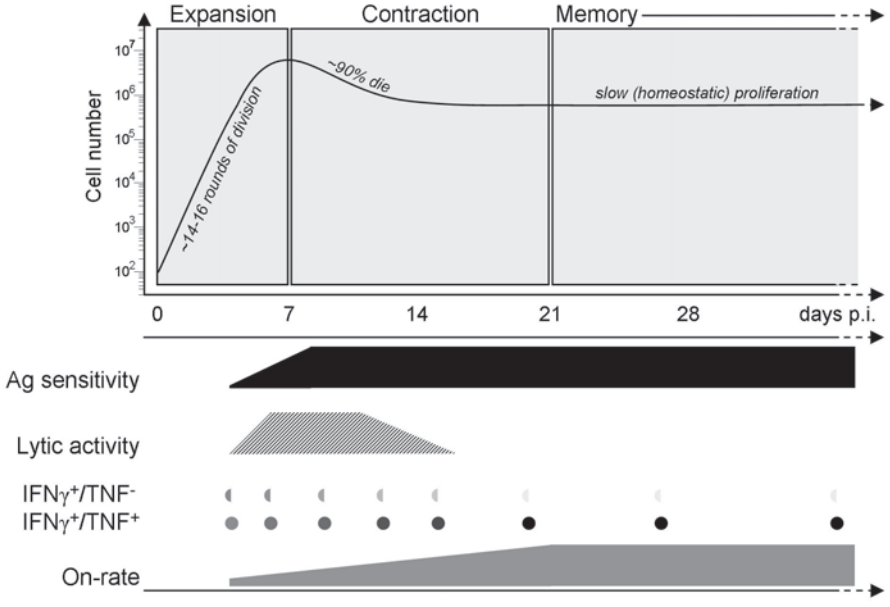

Fig. 1. Quantitative and qualitative summary of the antiviral CD8+ T cell response. The upper part of the figure shows a quantitative summary of a strong CD8+ T cell response against a single viral epitope, assuming a starting number of 100 naïve epitope-specific precursor cells. Note that the *y*-axis is a $\log_{10}$ scale. Qualitative changes in four aspects of CD8+ T cell effector function, discussed in the text, are shown in the lower part of the figure.

upon) almost any infected cell; thus CD8+ T cells are the foot soldiers of the immune response, engaging in hand-to-hand combat against viruses within cells. In contrast, CD4+ T cells are unable to recognize the majority of infected cells (which do not express MHC class II), and so they seem unlikely to play a direct role in eradicating the microbe; these cells instead orchestrate the antiviral campaign. The majority of studies in my laboratory are aimed at understanding CD8+ T cell responses; these are, therefore, the topic of the remainder of this chapter.

THE THREE PHASES OF THE ANTIVIRAL CD8+ T CELL RESPONSE

The quantitative and qualitative changes that take place in a single population of epitope-specific CD8+ T cells over the course of a virus infection, and beyond, are summarized in Fig. 1. In this figure, the number of precursor cells (i.e. in a naïve mouse) that are specific for a single epitope is assumed to be 10^2, consistent with published data (Blattman *et al.*, 2002). The antiviral CD8+ T cell response is traditionally considered as having three overlapping phases: expansion, contraction and memory.

The expansion phase

Naïve $CD8^+$ T cells express high levels of proteins such as CD62L (Gallatin *et al*., 1983) and CCR7, which mediate adhesion to lymph node venules, and cause the naïve cells to be retained within lymphoid tissues, which are rich in APCs (Baekkevold *et al*., 2001). When a naïve antigen-specific $CD8^+$ T cell encounters its cognate antigen presented by an APC, a TcR-dependent signal triggers a programme which leads to the cell's division and differentiation. T-cell activation results in the rapid down-regulation of CD62L and CCR7, and this (along with other factors) allows the newly activated cell to exit the node. Recent studies indicate that a few hours of antigen exposure can trigger a $CD8^+$ T cell to complete its entire developmental programme in the apparent absence of additional antigenic contact (Kaech & Ahmed, 2001; Mercado *et al*., 2000; van Stipdonk *et al*., 2001). This rapid loss of antigen dependence implies that a newly triggered $CD8^+$ T cell can very quickly be released from lymphoid tissues (where antigen is most abundant), to take up its duties in peripheral sites, while continuing to divide and differentiate. T cells require only ~6 h between each division, so each activated precursor generates many thousands of progeny cells. The virus-specific T-cell response usually peaks around 7–10 days after infection, after which the cell numbers decline.

The contraction phase (aka the death phase)

This phase – the least well understood of the three – begins as early as 6 days post-infection (and, therefore, overlaps with the end of the expansion phase). Approximately 90 % of T cells die during the contraction phase, a dramatic attrition that is complete by ~21 days post-infection (Badovinac *et al*., 2002; Badovinac & Harty, 2002; Kaech *et al*., 2002; Sprent & Surh, 2002). The mechanisms regulating T-cell death remain controversial, but the most widely accepted theory is that death is apoptotic, and results from one of two mechanisms: activation-induced cell death (AICD) or cytokine withdrawal [activated T cell autonomous death (ACAD)]. The probability that one or both of these apoptotic pathways will be activated in any one $CD8^+$ T cell may be determined by, for example, the cell's history (number of prior divisions, antigen contact) and the surrounding microenvironment. Timing appears to play a critical part, as revealed by a recent study in which IL-2 was administered at different times after virus infection. When administered during the expansion phase, this cytokine increased T-cell death (consistent with its inducing AICD), but the same treatment during the contraction phase led to increased T-cell survival (consistent with its preventing ACAD; Blattman *et al*., 2003).

The memory phase

As noted earlier, $CD8^+$ memory T cells are important in protecting against many viral infections, and experimental vaccines that induce only $CD8^+$ memory T cells (but no

antibodies) can confer good protection against viral challenge (del Val *et al*., 1991; Klavinskis *et al*., 1989; Whitton *et al*., 1993). The number of memory cells induced by an infection is related to the degree of $CD8^+$ T cell expansion during the primary response (Hou *et al*., 1994; Marshall *et al*., 2001) and, under normal circumstances, the resting level of memory cells remains relatively stable for months or years after infection or vaccination. This stable level of $CD8^+$ memory T cells requires ongoing homeostatic division that 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 does not require ongoing antigen contact (Lau *et al*., 1994; Murali-Krishna *et al*., 1999). Memory T cells are often relatively abundant in non-lymphoid tissues (Mackay *et al*., 1992; Sprent, 1976). Intuitively, this makes sense: most virus infections begin at mucosal surfaces, and the presence of virus-specific effector cells at or near these locations might improve the host's ability to rapidly eradicate the invaders. However, the ability of memory cells to immediately express certain effector functions is somewhat controversial. For example, some studies suggest that $CD8^+$ memory T cells in lung and liver might be immediately cytolytic (Masopust *et al*., 2001), while others have reported that virus-specific $CD8^+$ memory cells in the lung are not immediately cytolytic (Hogan *et al*., 2001; Ostler *et al*., 2001). The factors that regulate the numbers of primary and memory $CD8^+$ T cells resident in peripheral tissues, and the expression of their effector functions, are important topics; their identification may facilitate the development of better therapeutic and prophylactic approaches to controlling virus infection.

THE ANTIVIRAL EFFECTOR FUNCTIONS OF $CD8^+$ T CELLS

Perhaps the best-known antiviral effector function of virus-specific $CD8^+$ T lymphocytes is their cytolytic activity; these cells often can kill infected target cells, usually by secreting the pore-forming protein perforin. Indeed, for this reason, these cells often are termed 'cytotoxic T lymphocytes', and the resulting acronym (CTL) often is used as synonym for all $CD8^+$ T cells. Perforin-mediated cytotoxicity is required for the clearance of several virus infections [e.g. lymphocytic choriomeningitis virus (LCMV): Kagi *et al*., 1994; Walsh *et al*., 1994]. As shown in Fig. 1, CTL activity usually is first detected at ~4–5 days post-infection, peaks at around 7–10 days, and declines quite quickly thereafter; we have shown that, by ~15 days p.i., most of the remaining virus-specific $CD8^+$ T cells have lost their capacity to rapidly lyse infected target cells (Rodriguez *et al*., 2001). However, cytolytic activity is not always the primary means by which $CD8^+$ T cells control virus infections, and it plays little part in eradicating vaccinia, Semliki Forest and vesicular stomatitis viruses (Kagi *et al*., 1995), rotaviruses (Franco *et al*., 1997), coxsackieviruses (Gebhard *et al*., 1998) and HBV (Guidotti *et al*., 1996, 1999). In these, and other, cases, $CD8^+$ T cells can control virus infection by a non-lytic mechanism; they can secrete cytokines, such as interferon-gamma (IFN-γ) and tumour necrosis factor (TNF), which can directly reduce virus replication, and may

even 'cure' infected cells by inactivating virus replication in the absence of cell death (Estcourt *et al*., 1998; Guidotti & Chisari, 1996; Levy *et al*., 1996; reviewed by Slifka & Whitton, 2000a; Walker *et al*., 1991). Thus, in terms of the effector functions (lytic activity/cytokine synthesis) that are induced by antigen contact, $CD8^+$ T cells fall into four broad classes: (i) non-lytic, and unable to immediately make abundant cytokines (typical of naïve precursor cells); (ii) lytic, but unable to make cytokines (such cells are unusual); (iii) lytic and $cytokine^{competent}$ (fully fledged antiviral cells, found between ~7 and 15 days post-infection); and (iv) non-lytic, but $cytokine^{competent}$ (as shown diagrammatically in Fig. 1, after day 15 most virus-specific $CD8^+$ T cells appear to be in this category). The existence of class (iv) appears to be overlooked by some investigators, as shown by two misnomers that are rampant in the literature. *First*, many investigators reserve the term 'effector cell' for a $CD8^+$ T cell that is lytic, thereby implying that non-lytic cells are 'non-effectors'; however, cells in class (iv) produce antiviral cytokines and, even in the absence of immediate lytic activity, can be profoundly antiviral, and most certainly deserve the 'effector' title. *Second*, the existence of these cells indicates that the term CTL is an inappropriate synonym for virus-specific $CD8^+$ T cells; in my view, 'CTL' should be used only to describe cells for which lytic activity has been demonstrated.

$CD8^+$ T CELL EFFECTOR FUNCTIONS MATURE OVER THE COURSE OF INFECTION

Antibody responses mature over the course of infection; there is a change both in the type of antibody produced ('class switching') and in the affinity of the antigen-binding region, which can increase dramatically following re-encounter with the antigen. The mechanisms underlying these antibody maturation processes are reviewed elsewhere, and are mentioned here mainly as a counterpoint to $CD8^+$ T cells, which, until recently, were not known to undergo a marked maturation over the course of infection. However, my laboratory has identified three distinct ways in which $CD8^+$ T cell responses improve over time.

$CD8^+$ T cells become more sensitive to low levels of epitope/MHC on target cells

We investigated the antigen-responsiveness of virus-specific T cells at various times post-infection, and found that, between ~4 and ~8 days post-infection, the quantity of epitope/MHC needed to trigger cytokine production or cytolytic activity by virus-specific $CD8^+$ T cells diminished by ~70-fold, and remained stable thereafter, essentially for the lifetime of the animal (Slifka & Whitton, 2001). How might this benefit the host? We proposed that, by optimizing their ability to be triggered by very low levels of epitope/MHC, $CD8^+$ T cells ensure that they can recognize cells very soon (minutes/hours) after they have become infected, at a time when epitope levels on the infected cell

surface are still rising. Recent studies have shown that as few as 10 peptide/MHC complexes may be sufficient to stimulate highly activated T cells (Irvine *et al.*, 2002), and this extraordinary sensitivity should allow the T cells' effector functions (cytokine production, cytolytic activity) to be exerted prior to virus maturation, thereby preventing the production and release of infectious particles. However, if a T cell can see as few as 10 epitope/MHC complexes, this is close to the lowest possible limit of antigen concentration on the cell surface, and it is difficult to conceive of any significant further enhancement of T-cell sensitivity. How else might T cells improve their biological functions?

CD8$^+$ T cells accelerate the initiation of IFN-γ production

We reasoned that, having maximized their ability to be triggered by low levels of antigen, T cells might improve their biological efficacy by improving their cytokine production (for example, by producing more cytokine, and/or by increasing the speed with which they begin cytokine production). We have evaluated the speed with which various epitope-specific populations of CD8$^+$ T cells can initiate IFN-γ synthesis in response to antigen contact by measuring their 'on-rate' (Liu *et al.*, 2004). For some epitope-specific populations, the initiation of cytokine synthesis became progressively faster over the course of infection, reaching optimal performance by ~21 days p.i., and retaining it thereafter. Typically, the on-rate of IFN-γ synthesis at day 21 was ~two- to fourfold faster than at day 8, an improvement of ~1–3 h. This change may seem small, but we consider it likely to be biologically significant, because the life cycle of most viruses is very short (a few hours); consequently, even a small increase in the speed of cytokine production will increase the probability that virus replication in the target cell can be interrupted, thereby substantially benefiting the host.

Different patterns of cytokine production by CD8$^+$ primary and memory T cells

Some time ago, we showed that IFN-γ and TNF production by CD8$^+$ T cells takes place only when the cell is in contact with cognate antigen (Slifka *et al.*, 1999). However, the pattern of cytokines produced by virus-specific CD8$^+$ T cells changes over the course of virus infection (Slifka & Whitton, 2000b). During the primary immune response to LCMV infection, two broad populations of CD8$^+$ T cells can be distinguished: one produces only IFN-γ, while the other produces both IFN-γ and TNF. As the primary response declines, the ratio of these two populations changes, and double-positive cells outnumber single-positive cells by ~5 : 1; and this process continues into the memory phase, at which time almost all cells respond to antigen contact by immediately producing IFN-γ and TNF. These observations have been confirmed by others, using models of influenza virus infection (Belz *et al.*, 2001) and murine gamma herpesvirus infection (Liu *et al.*, 2002). If this difference in cytokine responses between primary and

memory cells is, indeed, a characteristic of most infections, then it seems likely to be biologically significant; however, this remains to be proven.

In conclusion, $CD8^+$ T cell responses to virus infection are important. Many laboratories have focused on quantitative analyses of cell numbers in primary or secondary lymphoid tissues and, although groundbreaking, these studies are only now being complemented by thorough investigations of $CD8^+$ cell numbers in peripheral sites. Furthermore, our understanding of the subtleties of T-cell effector functions, in both lymphoid and non-lymphoid tissues, remains rudimentary, and such qualitative evaluations will, doubtless, yield many fascinating results in the years to come.

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