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Review

Antibodies and B Cell Memory in Viral Immunity

Thomas Dörner[1] and Andreas Radbruch[1,*]
[1]Charite Universitätsmedizin Berlin & Deutsches Rheumaforschungszentrum Berlin, 10117 Berlin, Germany
*Correspondence: radbruch@drfz.de
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Humoral immunity, in particular secreted neutralizing antibodies, is of central importance to protect the body against acutely cytopathic viruses, whereas noncytopathic viruses have found ways of balanced coexistence with the immune system to avoid antibody-mediated elimination. There is evidence that polyspecific "natural" antibodies provide early protection, independent of T cell help. If that line of defense is crossed, T cell-dependent immune responses then generate a humoral memory provided by long-lived plasma cells secreting specific antibodies of adapted avidity and function, i.e., isotype, even in the absence of virus. Secreted protective antibodies of humoral memory provide an efficient line of defense against reinfection and are backed up by specific B and T memory cells of reactive memory. Whereas humoral memory has developed effective antiviral protection, some viruses (i.e., HIV) have managed to develop specific evasion strategies to escape it. Thus, coevolution provides us with some insight into just how substantial antiviral antibodies and memory B cell are in protecting the host from virus infection.

Introduction

Viruses are intracellular pathogens and depend on living hosts for their survival. Viruses have developed an impressive array of strategies to cope with the hosts' antiviral defense, in particular with the immune system. Often, the immune response against acutely cytopathic viruses (such as neurotropic poliovirus, rabies virus, and smallpox virus in humans) and noncytopathic viruses (such as hepatitis B and C viruses [HBV, HCV] and herpesvirsuses, a family that includes cytomegalo virus [CMV], herpes simplex virus 1 and 2 [HSV-1, 2], Epstein-Barr virus [EBV], and human herpes virus 6 [HHV6]) causes more damage than the virus itself. Acutely cytopathic viruses have to be eliminated by an effective immune response or they will kill their host. The role of the immune system in the dialog between viruses and their host can thus be both protective and pathogenic. With regard to the latter, a number of viruses, e.g., CMV, EBV, varizella zoster virus (VZV), human immunodeficiency virus (HIV), HBV, and HCV have developed strategies by coevolution to avoid elimination by the immune system that is of clinical relevance today.

Success of the immune system depends on multiple lines of defense, addressing the pathogen at several points of its attack. Recently, research has been directed to barrier mechanisms and mechanisms of the innate immune system. With respect to the adaptive immune system, interest mostly focuses on cytotoxic and helper T lymphocytes. The role of B cells and their antibodies seems to be discussed less, although it is clear from the early days of immunology that antibodies generated by provocation with pathogen can protect from reiterated challenge with pathogen, elegantly demonstrated by Kitasato and Behring more than 100 years ago (Behring, 1900).

How is "protective" humoral memory of the immune system defined? In epidemiological terms, survival after infection serves as the easiest outcome criterion, but its use is restricted to animal experiments with respect to intentional experimental infection. There is a very limited availability of biomarkers as "correlates of protection," in particular in humans, although such biomarkers would be very informative as to how protection is achieved. Antibodies specific for a pathogen and able to neutralize it in vitro are probably one of the best correlates of protection available so far. Although "neutralization," i.e., the ability to prevent infection of host cells by an infectious pathogen in vitro, is a very efficient way of protection, it is probably not the only one, and in vivo protection assays may reveal alternative ways of protection (reviewed in Hangartner et al., 2006).

Humoral memory, i.e., secretion of specific protective antibodies over long periods in the apparent absence of the antigen (Figure 1), provides the host with a first line of defense against reinfection, defines protection against a range of pathogens (Ahmed and Gray, 1996; Slifka and Ahmed, 1996), and also is an informative biomarker for previous exposure to a particular pathogen.

Pathogen-specific antibodies of the serum can be but are not necessarily protective. It is not even clear which proportion of serum immunglobulin (Ig) is pathogen specific and provided by the humoral memory, as compared to "natural" antibodies that are preformed polyspecific IgM antibodies not shaped by postrecombination processes (somatic hypermutation, class switching). Because the half life of secreted antibodies is rather short, in the range of days (Radbruch et al., 2006), their maintenance over long time periods requires the persistent presence of cells secreting them. These cells, plasmablasts and plasma cells, are generated from specific, activated B cells, a process that can be driven by persistent antigen (Zinkernagel and Hengartner, 2006) or that can reflect true immunological memory. Here, we consider memory as the maintenance of immune information in the absence of persistent instruction. B cell memory is bipartite, consisting of

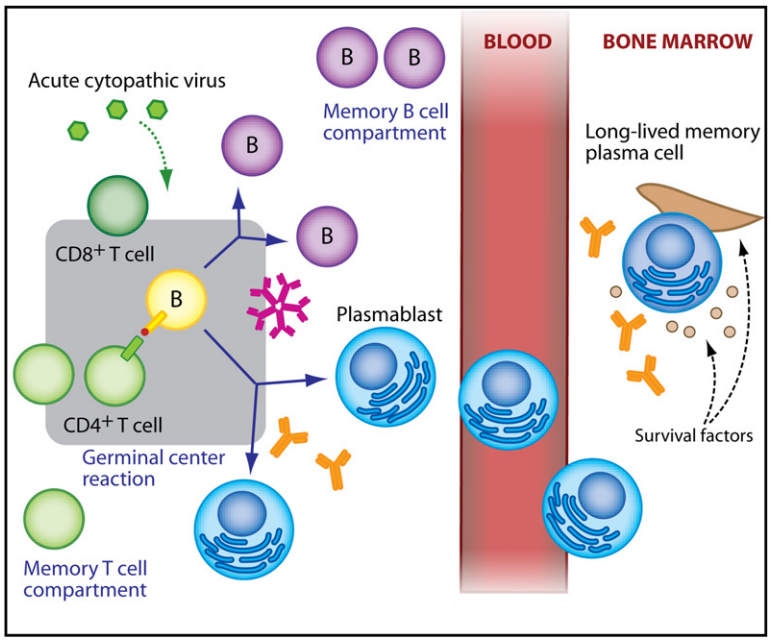

Figure 1. Long-Term Antiviral Memory Induction Induced by an Acutely Cytopathic Virus

After vaccination or infection, B1 cells (not shown) differentiate into plasmablasts producing polyspecific IgM antibodies at extrafollicular sites in a T cell-independent manner. Simultaneously, activation of antigen-specific $CD8^+$ and $CD4^+$ T cells occurs. As part of the germinal center (GC)-reaction, $CD4^+$ T cells provide help to naive B cells (yellow), which eventually become long-lived plasma cells producing high amounts of neutralizing IgG antibodies in the bone marrow. These naive B cells also give rise to long-term memory B cells. Survival niches for these cells are likely within secondary lymphoid organs. The GC reaction is dependent on antigen recognized by specific B cell receptors and the presence of specific T cells.

Survival of established long-lived plasma cells residing in the bone marrow and memory B cells is thought to be critically dependent on soluble and insoluble survival factors (as indicated for plasma cells in the bone marrow) but independent of the cognate antigen and T cell help. The hypothetical survival niche for long-lived plasma cells is thought to comprise adhesion molecules, cellular components, and soluble factors, whereas the site and conditions of long-lived memory B cells need to be deciphered.

long-lived antibody-secreting plasma cells (humoral memory) and long-lived memory B cells, able to react quickly to a recurrent antigenic challenge (reactive memory) (Ahmed and Gray, 1996). We will discuss the role of B cell (reactive) and plasma cell (protective) memory in the protection against acutely cytopathic viruses, a prototypic example of long-term protective memory (Figure 1).

B Cells Provide Several Lines of Antiviral Defense

Successful vaccination against certain viruses (measles, chicken pox, rubella, etc.) in childhood reflects the importance of protective antibodies, and so far, vaccines in clinical practice depend on the induction of neutralizing antibodies but not exclusively on T cell-mediated immunity (Zinkernagel and Hengartner, 2006). Most importantly, all current vaccines that are clinically protective are dependent on neutralizing antibody responses (Letvin, 2007, in this issue of *Immunity*). Whereas cytotoxic lymphocytes can eliminate infected cells, antibodies have the potential to both eliminate infected cells and prevent infectious virus from infecting a cell (neutralization).

Placental transfer of protective IgG antibodies from mother to offspring is an impressive "demonstration by nature" of the efficacy of humoral immunity. However, agammaglobulinemic or hypogammaglobulinemic children are less susceptible to severe viral infections than children lacking cellular immunity. From this observation it has often been argued that T cells are more important than B cells in antiviral immunity. This argument neglects the crucial importance of T helper cells not only for cellular but also for humoral immunity, in particular the generation of memory B cells and long-lived plasma cells. After orthopoxvirus infection, T cells are crucial for the induction of humoral memory in primary responses (Figure 1) and become of secondary importance in secondary responses, when neutralizing antibodies play an important role in protection against reinfection (Edghill-Smith et al., 2005).

A number of studies compared the induction and maintenance of specific T and B cell responses and resulting antiviral titers. In this regard, it was shown that antiviral antibody can protect mice efficiently, even if $CD4^+$ or $CD8^+$ T cells have been depleted prior to viral (re)challenge (Belyakov et al., 2003; Xu et al., 2004). Humans make strong $CD8^+$ and $CD4^+$ T cell immune responses after receiving smallpox vaccine (Edghill-Smith et al., 2005; Hammarlund et al., 2003). Quantitative studies (Figure 2) examining the magnitude and duration of vaccinia-specific T cell responses found that effector $CD8^+$ T cell responses peak at approximately 2 (Amara et al., 2004; Terajima et al., 2003) to 5 weeks after vaccination (Amara et al., 2004; Hammarlund et al., 2003; Rock et al., 2005). Interestingly, a decline of circulating vaccinia-specific $CD8^+$ T cells (Amara et al., 2004; Hammarlund et al., 2003; Terajima et al., 2003) and specific $CD4^+$ T cells (Amara et al., 2004) was noted. Antivaccine Ig was more stable than $CD8^+$ and $CD4^+$ T cells (Hammarlund et al., 2003). In this context, a cross-sectional analysis (Hammarlund et al., 2003) of 306 individuals who received smallpox vaccine demonstrated that vaccinia virus-specific memory $CD4^+$ T cells were long lived with an overall half-life time of 8–12 years (Amara et al., 2004; Crotty et al., 2003). Vaccinia-specific memory $CD8^+$ T cells were also long lived, but only half of the vaccinees had detetable

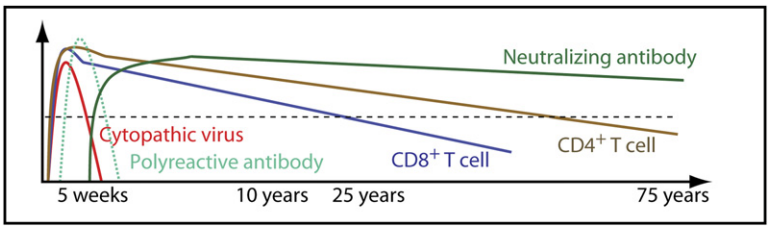

Figure 2. Timeline of a Specific Immune Defense against an Acutely Cytopathic Virus

At early stages of infection with an acutely cytopathic virus such as vaccinia (viral load in red line), specific $CD4^+$ and $CD8^+$ T cells are expanded. Polyreactive antiviral IgM antibodies are likely induced as a first defense that lacks clear identification in antiviral responses. As a result of successful GC reactions, long-lived plasma cells are induced, providing neutralizing antibodies. The half-life of long-lived plasma cells has been reported to be longer than that of $CD4^+$ or $CD8^+$ T cells, although tested by only a limited number of studies. It has been argued that there is no intrinsic half-life of long-lived plasma cells, but rather that their survival is conditional on being in a survival niche (Radbruch et al., 2006). The vertical dotted line indicates the assumed lower level of detection.

vaccine-specific memory $CD8^+$ T cells 20 years after vaccination. The longevity of B cell and $CD4^+$ and $CD8^+$ T cell memory and antiviral antibody responses observed in available human studies appears to be different and likely reflects the complex and different regulation of these memory compartments. How their induction and maintenance are managed and whether different vaccinations or viral infections lead to differences in their halflives remain to be elucidated.

The characteristic of long-standing antiviral antibodies to be dependent on previous T cell instruction has been analyzed by a number of studies. Mice infected with a cytopathic virus, which cannot persist in the host, e.g., inactivated vesicular stomatitis virus (VSV) Indiana, develop very high titers of neutralizing antibodies rapidly (Ochsenbein et al., 2000). By day 300 after infection, however, antibody titers decline below detectable amounts for neutralizing antibodies. These antibodies are likely thus secreted by short-lived plasma cells. Titers of non-neutralizing antibodies against the virus remain high beyond day 300. They are either derived from long-lived plasma cells or from short-lived plasma cells generated continously in a chronic immune reaction driven by persistent antigen. Thus a primary immune response against an acutely cytopathic virus, which is eliminated rather quickly and before the specific memory T and B cells can be reactivated by virus, is apparently not sufficient to generate humoral memory of protective antibodies. In transfer experiments, stable titers of neutralizing antibodies in naive recipients developed only after transfer of primed T and B cells plus antigen and not without antigen (Ochsenbein et al., 2000), in line with the concept that long-lived plasma cells can be generated only from memory B cells (Ochsenbein et al., 2000; reviewed in Radbruch et al., 2006), either by reinfection with acutely cytopathic viruses, like VSV, or in the prolonged initial immune response to replicating, persistent virus, like LCMV, allowing reactivation of memory B cells (Bachmann et al., 2004). Such secondary activations in initial immune responses have been described and analyzed in detail in mice immunized with 4-hydroxy-3-nitrophenyl)acetyl-KLH (NP-KLH) (Blink et al., 2005).

A drastic example of the maintenance of stable titers of neutralizing antibodies in the absence of pathogen is provided by poxvirus vaccination. Antibody responses after secondary vaccination are detected as early as day 4 after immunization (Amanna et al., 2006). Peak antibody responses decline during the first year after vaccination, but antibody titers then stabilize and remain nearly constant for up to 75 years after immunization (Hammarlund et al., 2003; Crotty and Ahmed, 2004; Slifka, 2004).

In humans, application of vaccinia immunoglobulin reduces the spreading of smallpox virus infection, when administered simulataneously with the vaccine to contacts (Amanna et al., 2006), and also reduces disease severity (Peirce et al., 1958). High neutralizing antibody titers are associated with protective immunity against acutely cytopathic smallpox infection, although there is no simple correlation between stable antibody titers and T cell memory (Crotty et al., 2003; Hammarlund et al., 2005). This argues against the idea that high antibody titers recognizing glycoprotein structures are simply reflecting pre-existing T cell memory with reactivity against peptide structures because of their distinct biochemical receptor recognition pattern. Rather, the competence to generate long-lived plasma cell memory might depend on the availability of precursor memory B cells.

Apart from humoral memory, the humoral immune system also provides a first line of defense against initial viral infection, namely the polyspecific (''natural'') antibodies secreted by plasma cells derived from B1 B cells (Haury et al., 1997; Baumgarth et al., 1999). These antibodies are considered a link between innate and adaptive immunity. They limit dissemination of the pathogen, form immune complexes for activating the adaptive immune reaction, recruit antigen into germinal centers, and activate complement. The antiviral repertoire of ''natural antibodies'' seems to be far from complete, and apparently they are not as efficient protectors as antibodies of humoral memory, which provide additional effector functions by switched isotype, i.e., constant region of the heavy chain, and improved affinity. The increase in affinity may be critical in maintaining avidity for the pathogen in the switch from decavalent IgM to bivalent IgG, IgE, or IgA.

It is remarkable that plasma cells secreting ''natural'' antibodies are rarely recruited into the pool of long-lived plasma cells. It has been argued that most of those antibodies probably are also autoreactive (Haynes et al., 2005) and thus are eliminated from memory by tolerance mechanisms yet to be defined. In this context, two human mAb with anti-HIV neutralizing capacity 2F5 and 4E10 have been reported that also showed reactivity against the autoantigen cardiolipin (Haynes et al., 2005). If not

eliminated, affinity maturation may render these antibodies oligo- or monospecific. In humoral protection against highly variable viruses, these antibodies may play a crucial role. Rare long-term nonprogressing HIV-infected persons (Deeks and Walker, 2007, in this issue of *Immunity*) have been described who are apparently also protected by their polyspecific antibodies, covering a wide range of HIV genotypes (Mascola and Montefiori, 2003; Braibant et al., 2006). Indeed, in view of our current concept of the stability of plasma cell memory (see below), HIV has managed to circumvent targeting by oligospecific antibodies by mutation as well as targeting by polyspecific antibodies. In addition, the lack of $CD4^+$ T cell help results in a substantial reduction of memory B cells, limiting reactive memory.

In antiviral defense, B cells play a central role by providing protective "natural" antibodies in the first line, humoral memory antibodies in the second line, and reactive memory B cells in the third line of protection.

Humoral Memory Establishment Is Dependent on T Cell Help

In systemic T cell-dependent immune responses to protein antigens and viruses, the early antibody response is provided by short-lived plasmablasts and plasma cells, whereas later on the humoral response is primarily provided by long-lived plasma cells, with specific memory B cells maintained as backup system (Odendahl et al., 2005). Recruitment of plasma cells generated late in the immune reaction to the pool of long-lived plasma cells ensures that the memory plasma cells secrete antibodies of the highest affinity (Blink et al., 2005). After acute viral infection, the virus-specific antibody-secreting cells of bone marrow differ from those in the spleen in terms of increased virus-specific ELISPOT formation, suggesting that affinity maturation and selection occurs before plasma cells accumulate in the bone marrow or when plasmablasts develop into plasma cells (Slifka and Ahmed, 1996). Convincing evidence supporting the hypothesis that selection occurs before plasma cell migration and/or accumulation in the bone marrow has been provided by characterization of affinity maturation in the spleen and bone-marrow compartments after a primary antibody response (Smith et al., 1997).

The precise mechanisms governing the recruitment of plasma cells to the memory plasma cell compartment are still not clear. As discussed in detail elsewhere (Höfer et al., 2006; Manz et al., 2005; Radbruch et al., 2006), long-lived plasma cells depend on a dedicated environment for their survival, the "plasma cell survival niche." Such niches are found predominantly in the bone marrow and to a lesser extent in secondary lymphoid organs, like the spleen, and in inflamed tissue. Whereas plasmablasts are migratory for about 1 week after their generation, plasma cells are not. Plasmablasts from systemic immune responses are attracted by the chemokine CXCL12 (SDF-1α), the ligand of CXCR4, and are dependent on instruction by IFN-γ and by CXCL9, 10, and 11, the ligands of CXCR3. Interestingly, although long-lived plasma cells are no longer migratory, their survival is supported by CXCL12, i.e., plasmablasts are attracted to and can migrate toward survival niches for long-lived plasma cells. We have postulated and provided indirect evidence for competition between newly generated plasmablasts and established resident long-lived plasma cells for habitation of a limited number of survival niches (Odendahl et al., 2005). Such a mechanism would combine a remarkable stability of established humoral memory responses with an efficient recruitment of new specificities to memory (Höfer et al., 2006), because for each newly recruited plasmablast, only one "old" plasma cell would be eliminated. At an estimated repertoire size of 1,000 to 10,000 specificities of humoral memory, this would imply the elimination of 0.1% to 0.01% of "old" plasma cells of each specificity to adapt one new specificity to the pool. It should be noted that the ability to compete with long-lived plasma cells for survival niches seems to be dependent on as-yet-undefined molecular competence of plasmablasts and that long- and short-lived plasma cells can coexist even in the same organ (Hoyer et al., 2004). Efficient generation of long-lived plasma cells so far has been observed mostly in secondary systemic T cell-dependent immune reactions.

Vaccinia virus infection and immunization is one of the classical examples of generation of long-term immunity against viral reinfection. The kinetics of the specific immune responses vary quantitatively and qualitatively for the various hosts analyzed, depending on the hosts' immunocompetence, the route of adminstration of antigen, adjuvants used, dose of antigen, and previous immunizations of the host (Earl et al., 2004), according to rules not defined so far.

In vaccinia virus-specific responses, the kinetics and duration of T cell responses (Harrington et al., 2002) showed strong $CD8^+$ T cell responses (about 30% of all $CD8^+$ T cells) at day 7 postinfection in mice, which then declined over time and stabilized by day 30 for up to 300 days after infection. In monkeys immunized with an attenuated modified vaccinia virus Ankara vaccine, a strong $CD8^+$ T cell response peaked at approximately 2 weeks after immunization (Earl et al., 2004) at a frequency of 0.1% and 1% specific cells of $CD8^+$ T cells. Vaccinia-specific $CD4^+$ T cell responses in mice also peak at approximately 1 week after infection, with 4%–5% of total splenic $CD4^+$ T cells (Harrington et al., 2002; Xu et al., 2004), and reach a plateau 1 to 7 months after infection (Harrington et al., 2002).

Vaccinia-specific memory B cells can make up up to 1% of circulating IgG^+ memory B cells 1–6 months after vaccination (Crotty et al., 2003). The number of vaccine-specific B cells declines by about 90% during the first year after vaccination and then stabilizes, with vaccinia-specific IgG^+ memory B cells being maintained for more than 50 years (Crotty et al., 2003). The kinetics of generation of vaccinia-specific serum antibodies has mainly been analyzed in mice (Tscharke et al., 2002; Wyatt et al., 2004). Antibody responses after vaccinia virus infection were detectable at low amounts at day 7 after

infection, followed by strong IgM and IgG responses after day 14 (Spriggs et al., 1992). Antibody titers reached a plateau 1 month after infection (Xu et al., 2004). Interestingly, these antivaccinia responses were almost completely $CD4^+$ T cell dependent, initially with a minor T cell-independent IgM component (Xu et al., 2004). Dissecting the relative contributions of $CD8^+$ and $CD4^+$ T cells and B cells by genetic or serological depletion has clarifyed the relative contributions of cellular and humoral immune components in immunity to vaccinia virus in mice. Transfer of serum or primed $CD8^+$ T cells both was protective, but $CD4^+$ T cell-dependent antiviral antibodies clearly played a more important role in clearing the virus than $CD8^+$ T cell-mediated protection, as was evident from the differential inactivation (Xu et al., 2004). Humans make antibody responses to several vaccinia virus proteins after immunization (Demkowicz et al., 1992; Davies et al., 2005a; Jones-Trower et al., 2005) with distinct immunodominant targets, like H3L, which have been shown to be protective in mice (Davies et al., 2005b). The H3L protein is a viral receptor involved in cell adhesion, and it is highly conserved among the orthopoxviruses. There is a correlation between pre-existing serum-neutralizing antibody titers and protection against smallpox (Mack et al., 1972). In case of vaccinia, H3L has been identified as the first target of neutralizing antibodies against a smallpox vaccine (Dryvax) (Davies et al., 2005b). Thus, protection and conservation are probably closely related in that long-term protection by antibodies secreted by long-lived plasma cells, which can no longer change the affinity of their antibodies, is most efficient if the target antigen cannot be modified by the virus.

Rapid anamnestic antibody responses after infection appear to be primarily driven by the size of the reactive memory B cell compartment and the quality of its antibodies. Individuals with an enlarged frequency of memory B cells specific for major antigenic targets would be expected to be more efficiently protected than individuals with a memory B cell pool dominated by specificities for nonprotective antigens. With regard to the T (memory) cells, the role of CD40/40L and ICOS/ICOS-L and cytokines regulating plasmablast differentiation, Ig class switching, and affinity maturation are relevant (Durandy et al., 2004).

So far, generation of long-lived plasma cells has been observed only in T cell-dependent immune reactions. Whether this reflects the requirement of a particular costimulatory signal making plasmablasts competent for longevity or whether such competent plasmablasts can be generated only from memory B cells, which in turn require T cell help for their generation, is currently not clear (Radbruch et al., 2006). By definition, induction of T-dependent (TD) humoral immune responses requires $CD4^+$ T cell help, although the precise molecular nature of this help may vary (Cobbold et al., 1984; Coulie et al., 1985; Wofsy et al., 1985). $CD4^+$ T cells appear to play different roles depending on the antigen and the phase of the immune response during induction, maintenance, and reactivation after secondary challenge.

$CD4^+$ T cell depletion studies have shown that the induction of strong antibody responses requires an intact $CD4^+$ T cell compartment (Vieira and Rajewsky, 1990). Although a very early stage of immune reaction seems to be T cell independent (Blink et al., 2005; Hangartner et al., 2006; Haury et al., 1997; Baumgarth et al., 1999), subsequent $CD4^+$ T cell help is strictly dependent on CD40L costimulation (Banchereau et al., 1994; Whitmire et al., 1996) and may also involve other CD28 family members, such as ICOS/ICOS-L (Löhning et al., 2003). The induction phase is rather short, and the later T cells are depleted after immunization in experimental setups, the less it matters for the magnitude of the humoral response, indicating the dispensability for $CD4^+$ T cell help after the induction phase (Coulie et al., 1985; Vieira and Rajewsky, 1990; Wofsy et al., 1985). If $CD4^+$ T cell depletion is delayed until approximately 3 weeks after immunization, then antigen-specific antibody responses are no longer affected (Vieira and Rajewsky, 1990).

The critical role of $CD4^+$ T cell help for the establishment of B and plasma cell memory is also evident from patients with HIV infection. In these patients with aquired immunodeficiency syndrome, i.e., impairment of the $CD4^+$ T helper cell compartment, immune responses to other viruses can be compromised. In this regard, AIDS patients with $CD4^+$ T cell counts below 50 cells/mm^3, when immunized with a recombinant strain of vaccinia virus, were reported with fatal viral complications (Zagury, 1991), consistent with a report on vaccination with replication-competent vaccinia virus (Bartlett, 2003) that resulted in life-threatening infection of a patient with $CD4^+$ T cell counts below 25 cells/mm^3.

In summary, interaction of antigen-specific T and B lymphocytes is required to generate humoral antiviral memory upon prolonged or repeated challenge with a viral pathogen. This memory is provided by long-lived plasma cells and is independent of B and T memory cells, once it is established.

Persistence of Humoral Immunity

Major advances have been made in identifying antigen-independent mechanisms of maintaining immunological memory (Becker et al., 2002; Blattman et al., 2002; Freitas and Rocha, 2000; Lanzavecchia and Sallusto, 2002). First of all, maintenance of B cell memory and long-term antibody production can occur in the absence of continued $CD4^+$ T cell help (Vieira and Rajewsky, 1990). $CD4^+$ T cells were continuously depleted for up to 6 weeks without any substantial effect on B cell memory (Vieira and Rajewsky, 1990). Memory B cells, when adoptively transferred into T cell-deficient hosts, survive for at least 90 days in the absence of a detectable $CD4^+$ T cell population (Hebeis et al., 2004).

Memory $CD4^+$ T cells and memory $CD8^+$ T cells can persist in the absence of antigen (reviewed in Amanna et al., 2006). Murine memory $CD8^+$ T cells undergo homeostatic proliferation, and this is independent of antigen or MHC I-dependent antigen presentation (Kassiotis et al., 2002; Murali-Krishna et al., 1999; Sprent and Surh, 2002, 2003;

Swain et al., 1999; Tanchot et al., 1997). In mice, memory $CD8^+$ T cells are stably maintained, whereas $CD4^+$ memory T cell numbers appear to decline slowly (Homann et al., 2001).

There is no statistical correlation between $CD4^+$ T cell memory and long-term serum antibody titers against vaccinia (Crotty and Ahmed, 2004; Hammarlund et al., 2003), in line with the concept that $CD4^+$ T cell help is not required for the maintenance of this specific humoral memory. Maintenance of humoral memory in face of HIV-induced depletion of T cell help has also been studied in AIDS patients. Pre-existing antibody titers to a variety of pathogens do not change in these patients (Naniche et al., 2004). A lack of quantitative correlation between T cell memory and humoral memory has also been described for memory to measles virus, after natural infection or vaccination 23 to 47 years after exposure to the virus or 1–34 years after vaccination (Naniche et al., 2004). A substantial decrease in $CD4^+$ T cell memory at time points more than 21 years after vaccination was observed, but a similar decrease was not seen in measle virus-specific antibody titers.

If neither T cells nor antigen maintain memory B and long-lived plasma cells, what does? Concepts regarding the mechanisms underlying the persistence of specific antibody production have focused primarily on the memory B cell component of humoral immunity (Schittek and Rajewsky, 1992; Gray et al., 1996; Lam et al., 1997; Maruyama et al., 2000) and plasma cell longevity (Manz et al., 1997; Slifka et al., 1998).

For long-lived plasma cells, it is still discussed whether they may have an intrinsic half-life or whether their survival is conditional on the specialized environment of the ''plasma cell survival niche'' (reviewed in Radbruch et al., 2006). For memory B cells, it remains controversial whether or not they undergo homeostatic proliferation and how this might be regulated (Schittek and Rajewsky, 1992). From in vivo labeling with heavy glucose (Makela and Nossal, 1962) or analysis of expression of the proliferation marker Ki67 (Gray et al., 1996), it has been concluded that approximately 2% of human memory B cells divide every day. This may or may not be driven by their antigen receptor (Lam et al., 1997), similar to T cells (Murali-Krishna et al., 1999). Independent of antigen receptor, human memory B cells could be maintained through activation via pathogen-associated molecular pattern receptors, e.g., toll-like receptors (TLRs), and cytokine receptors, although Bernasconi and collegues have shown that memory B cells, when bystander activated in vitro with TLRs ligands and/or cytokines, differentiate exclusively into plasmablasts (Bernasconi et al., 2002) and not also into memory B cells. Programmed homeostatic proliferation has been suggested to maintain human memory B cells, perhaps by a depot of antigen (Ochsenbein et al., 2000; Haberman and Shlomchik, 2003; Mandel et al., 1980), although it has been shown recently that secreted antibody, i.e., immune complexes and antigen on follicular dendritic cells, are not required to maintain B cell memory (Anderson et al., 2006). Persistent antigen may play a role in chronic immune reactions, with persistent antigen perpetuating memory B cells, and at the same time generating plasmablasts. In this context, preplasma cells proliferating and differentiating have been described (Kelsoe, 1996; Miller, 1964), as well as steady-state, low-frequency, peripheral antibody-secreting cells (Slifka and Ahmed, 1998). However, the continous generation of virus-specific plasma cells is difficult to explain for acutely cytopathic viruses, such as smallpox (Hammarlund et al., 2003) with no clinically apparent retention of the virus and a decline of $CD4^+$ and $CD8^+$ T cell memory over time (Hammarlund et al., 2003).

Long-lived plasma cells have been shown to survive independent of antigen (Manz et al., 1998; Slifka et al., 1998). Instead, they require distinct signals from their environment, the plasma cell survival niche (Cassese et al., 2003). Although the molecular code of the ''plasma cell survival niche'' is not deciphered so far, signals such as CXCL12, IL6, B cell-activating factor (BAFF), apoptosis-inducing ligand (APRIL), and CD44 are most likely involved, although not essential. Genetic blocking of the respective signaling pathways has in no instance eliminated the pool of long-lived plasma cells. Whereas the interaction of CXCR4 and CXCL12 is important for the recruitment of newly generated competent migratory and competent plasmablasts to the pool of long-lived plasma cells, the ligation of FcγRIIb (CD32) with immune complex and subsequent signaling has been discussed with respect to elimination of plasma cells from the this pool (Xiang et al., 2007). It should, however, be noted that so far it has not been shown that long-lived plasma cells can be killed by immune complexes in their survival niches.

Taken together, T cell, B cell, and plasma cell memories against viruses are apparently differentially regulated and can be maintained independent of each other. The distinct instructions and conditions of survival of these memory cells are of utmost interest.

Viral Evasion and Deception Strategies

There are increasing numbers of reports on viral evasion strategies (reviewed by Hangartner et al., 2006; Gruenberg and van der Goot, 2006; Mohamadzadeh et al., 2006). Viruses can deteriorate T-B cell and T-DC interaction within secondary lymphoid organs and impair their architecture. In this context, filovirus glycoproteins can bind to receptors of immune cells and interfere with their function as well as mask important antigenic sites (Mohamadzadeh et al., 2006). Another example of interference with B cell reactivity is direct binding of HIV-1 to CD21, the complement receptor 2 on B cells (Moir et al., 2000) promoting virus dissemination to T cells. For HSV and CMV, it has been shown that CD1 antigen presentation by DCs is a target of evasion (Raftery et al., 2006).

With regard to humoral memory, viruses have developed escape and deception strategies to escape natural antibodies, by infecting unprotected individuals and changing their antigenicity (serotypes or subtypes). A typical example is influenza A virus, which mutates antigenic

determinants to create "antigenic drift." Antigenic drift also includes modifications of O- and N-glycosylation and glycoprotein shedding of filoviruses (Mohamadzadeh et al., 2006) supporting the relevance of glycoprotein structures for recognition of antigens by B and T cell receptors. Continous mutation of the target epitopes to escape neutralizing antiviral antibodies is an immune evasion mechanism used by HIV. Sera from infected patients were unable to neutralize HIV from the same donor but could readily neutralize isolates obtained months ago (Richman et al., 2003; Wei et al., 2003). This indicates that HIV immune evasion is due to (1) escape of virus from the specificity of initially generated humoral memory and (2) the inability of the immune system to create protective humoral memory for the mutated virus, probably by impairment of T cell help. It has been hypothesized that the unique ability of B cells to vary their antigen receptors by somatic hypermutation may enable the human host to compete with the viral mutation rates (Hangartner et al., 2006), but by escape from protective memory and subversion of reactive memory, HIV may have found a way to win the race.

More successful in evolutionary terms are probably nonacutely cytopathic viruses, which have found numerous ways to coexist with their human hosts, thereby avoiding the generation of efficient protective humoral memory in the first place.

Conclusions

The humoral immune system of vertebrates plays an essential role in their protection against acutely cytopathic viruses. The two compartments of memory B cells and long-lived plasma cells provide separate lines of humoral immunity. Long-lived plasma cells secrete protective antibodies of high specificity and adapted function. Memory B cells provide the potential to react fast and with even more adapted antibodies against reinfection. Induction of long-term plasma cells and memory B cells apparently is dependent on $CD4^+$ T cell help and B cell receptor signaling. Their maintenance appears to be independent of viral antigen. Viral evasion and deception strategies reflect the essential role of protective long-lived plasma cells and reactive memory B cells in antiviral defense. Understanding the rules governing the differential generation of memory B cells versus long-lived plasma cells from the same precursors, and the mechanisms maintaining them, will provide exciting new options to develop novel, efficient antiviral vaccines.

REFERENCES

Ahmed, R., and Gray, D. (1996). Immunological memory and protective immunity: understanding their relation. Science *272*, 54–60.

Amanna, I.J., Slifka, M.K., and Crotty, S. (2006). Immunity and immunological memory following smallpox vaccination. Immunol. Rev. *211*, 320–337.

Amara, R.R., Nigam, P., Sharma, S., Liu, J., and Bostik, V. (2004). Long-lived poxvirus immunity, robust CD4 help, and better persistence of CD4 than CD8 T cells. J. Virol. *78*, 3811–3816.

Anderson, S.M., Hannum, L.G., and Shlomchik, M.J. (2006). Cutting edge: memory B cell survival and function in the absence of secreted antibody and immune complexes on follicular dendritic cells. J. Immunol. *176*, 4515–4519.

Bachmann, M.F., Hunziker, L., Zinkernagel, R.M., Storni, T., and Kopf, M. (2004). Maintenance of memory CTL responses by T helper cells and CD40-CD40 ligand: antibodies provide the key. Eur. J. Immunol. *34*, 317–326.

Banchereau, J., Bazan, F., Blanchard, D., Briere, F., Galizzi, J.P., Vankooten, C., Liu, Y.J., Rousset, F., and Saeland, S. (1994). The Cd40 antigen and its ligand. Annu. Rev. Immunol. *12*, 881–922.

Bartlett, J.G. (2003). Smallpox vaccination and patients with human immunodeficiency virus infection or acquired immunodeficiency syndrome. Clin. Infect. Dis. *36*, 468–471.

Baumgarth, N., Herman, O.C., Jager, G.C., Brown, L., Herzenberg, L.A., and Herzenberg, L.A. (1999). Innate and acquired humoral immunities to influenza virus are mediated by distinct arms of the immune system. Proc. Natl. Acad. Sci. USA *96*, 2250–2255.

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.

Behring, E. (1900). Determination of the value of tetanus antitoxin and its application in human and veterinaty. Dtsch. Med. Wochenschr. *26*, 29–32.

Belyakov, I.M., Earl, P., Dzutsev, A., Kuznetsov, V.A., Lemon, M., Wyatt, L.S., Snyder, J.T., Ahlers, J.D., Franchini, G., Moss, B., and Berzofsky, J.A. (2003). Shared modes of protection against poxvirus infection by attenuated and conventional smallpox vaccine viruses. Proc. Natl. Acad. Sci. USA *100*, 9458–9463.

Bernasconi, N.L., Traggiai, E., and Lanzavecchia, A. (2002). Maintenance of serological memory by polyclonal activation of human memory B cells. Science *298*, 2199–2202.

Blattman, J.N., Antia, R., Sourdive, D.J.D., Wang, X.C., 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.

Blink, E.J., Light, A., Kallies, A., Nutt, S.L., Hodgkin, P.D., and Tarlinton, D.M. (2005). Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. J. Exp. Med. *201*, 545–554.

Braibant, M., Brunet, S., Costagliola, D., Rouzioux, C., Agut, H., Katinger, H., Autran, B., and Barin, F. (2006). Antibodies to conserved epitopes of the HIV-1 envelope in sera from long-term non-progressors: prevalence and association with neutralizing activity. AIDS *20*, 1923–1930.

Cassese, G., Arce, S., Hauser, A.E., Lehnert, K., Moewes, B., Mostarac, M., Muehlinghaus, G., Szyska, M., Radbruch, A., and Manz, R.A. (2003). Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. J. Immunol. *171*, 1684–1690.

Cobbold, S.P., Jayasuriya, A., Nash, A., Prospero, T.D., and Waldmann, H. (1984). Therapy with monoclonal-antibodies by elimination of T-cell subsets in vivo. Nature *312*, 548–551.

Coulie, P.G., Coutelier, J.P., Uyttenhove, C., Lambotte, P., and Vansnick, J. (1985). In vivo suppression of T-dependent antibody-responses by treatment with a monoclonal anti-L3T4 antibody. Eur. J. Immunol. *15*, 638–640.

Crotty, S., and Ahmed, R. (2004). Immunological memory in humans. Semin. Immunol. *16*, 197–203.

Crotty, S., Felgner, P., Davies, H., Glidewell, J., Villarreal, L., and Ahmed, R. (2003). Cutting edge: long-term B cell memory in humans after smallpox vaccination. J. Immunol. *171*, 4969–4973.

Davies, D.H., Liang, X.W., Hernandez, J.E., Randall, A., Hirst, S., Mu, Y.X., Romero, K.M., Nguyen, T.T., Kalantari-Dehaghi, M., Crotty, S., et al. (2005a). Profiling the humoral immune response to infection by

using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. Proc. Natl. Acad. Sci. USA *102*, 547–552.

Davies, D.H., McCausland, M.M., Valdez, C., Huynh, D., Hernandez, J.E., Mu, Y.X., Hirst, S., Villarreal, L., Felgner, P.L., and Crotty, S. (2005b). Vaccinia virus H3L envelope protein is a major target of neutralizing antibodies in humans and elicits protection against lethal challenge in mice. J. Virol. *79*, 11724–11733.

Deeks, S.G., and Walker, B.D. (2007). Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. Immunity *27*, this issue, 406–416.

Demkowicz, W.E., Maa, J.S., and Esteban, M. (1992). Identification and characterization of vaccinia virus genes encoding poteins that are highly antigenic in animals and are immunodominant in vaccinated humans. J. Virol. *66*, 386–398.

Durandy, A., Revy, P., and Fischer, A. (2004). Human models of inherited immunoglobulin class switch recombination and somatic hypermutation defects (hyper-IgM syndromes). Adv. Immunol. *82*, 295–330.

Earl, P.L., Americo, J.L., Wyatt, L.S., Eller, L.A., Whitbeck, J.C., Cohen, G.H., Eisenberg, R.J., Hartmann, C.J., Jackson, D.L., Kulesh, D.A., et al. (2004). Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. Nature *428*, 182–185.

Edghill-Smith, Y., Golding, H., Manischewitz, J., King, L.R., Scott, D., Bray, M., Nalca, A., Hooper, J.W., Whitehouse, C.A., Schmitz, J.E., et al. (2005). Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. Nat. Med. *11*, 740–747.

Freitas, A.A., and Rocha, B. (2000). Population biology of lymphocytes: the flight for survival. Annu. Rev. Immunol. *18*, 83–111.

Gray, D., Siepmann, K., vanEssen, D., Poudrier, J., Wykes, M., Jainandunsing, S., Bergthorsdottir, S., and Dullforce, P. (1996). B-T lymphocyte interactions in the generation and survival of memory cells. Immunol. Rev. *150*, 45–61.

Gruenberg, J., and van der Goot, F.G. (2006). Mechanisms of pathogen entry through the endosomal compartments. Nat. Rev. Mol. Cell Biol. *7*, 495–504.

Haberman, A.M., and Shlomchik, M.J. (2003). Opinion—reassessing the function of immune-complex retention by follicular dendritic cells. Nat. Rev. Immunol. *3*, 757–764.

Hammarlund, E., Lewis, M.W., Hansen, S.G., Strelow, L.I., Nelson, J.A., Sexton, G.J., Hanifin, J.M., and Slifka, M.K. (2003). Duration of antiviral immunity after smallpox vaccination. Nat. Med. *9*, 1131–1137.

Hammarlund, E., Lewis, M.W., Carter, S.V., Amanna, I., Hansen, S.G., Strelow, L.I., Wong, S.W., Yoshihara, P., Hanifin, J.M., and Slifka, M.K. (2005). Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. Nat. Med. *11*, 1005–1011.

Hangartner, L., Zinkernagel, R.M., and Hengartner, H. (2006). Antiviral antibody responses: the two extremes of a wide spectrum. Nat. Rev. Immunol. *6*, 231–243.

Harrington, L.E., van der Most, R., 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.

Haury, M., Sundblad, A., Grandien, A., Barreau, C., Coutinho, A., and Nobrega, A. (1997). The repertoire of serum IgM in normal mice is largely independent of external antigenic contact. Eur. J. Immunol. *27*, 1557–1563.

Haynes, B.F., Fleming, J., St Clair, E.W., Katinger, H., Stiegler, G., Kunert, R., Robinson, J., Scearce, R.M., Plonk, K., Staats, H.F., et al. (2005). Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science *308*, 1906–1908.

Hebeis, B.J., Klenovsek, K., Rohwer, P., Ritter, U., Schneider, A., Mach, M., and Winkler, T.H. (2004). Activation of virus-specific memory B cells in the absence of T cell help. J. Exp. Med. *199*, 593–602.

Höfer, T., Muehlinghaus, G., Moser, K., Yoshida, T., Mei, H.E., Hebel, K., Hauser, A., Hoyer, B., Luger, E.O., Dorner, T., et al. (2006). Adaptation of humoral memory. Immunol. Rev. *211*, 295–302.

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.

Hoyer, B.F., Moser, K., Hauser, A.E., Peddinghaus, A., Voigt, C., Eilat, D., Radbruch, A., Hiepe, F., and Manz, R.A. (2004). Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. J. Exp. Med. *199*, 1577–1584.

Jones-Trower, A., Garcia, A., Meseda, C.A., He, Y., Weiss, C., Kumar, A., Weir, J.P., and Merchlinsky, M. (2005). Identification and preliminary characterization of vaccinia virus (Dryvax) antigens recognized by vaccinia immune globulin. Virology *343*, 128–140.

Kassiotis, G., Garcia, S., Simpson, E., and Stockinger, B. (2002). Impairment of immunological memory in the absence of MHC despite survival of memory T cells. Nat. Immunol. *3*, 244–250.

Kelsoe, G. (1996). Life and death in germinal centers (redux). Immunity *4*, 107–111.

Lam, K.P., Kuhn, R., and Rajewsky, K. (1997). In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell *90*, 1073–1083.

Lanzavecchia, A., and Sallusto, F. (2002). Progressive differentiation and selection of the fittest in the immune response. Nat. Rev. Immunol. *2*, 982–987.

Letvin, N.L. (2007). Correlates of immune protection and the development of a human immunodeficiency virus vaccine. Immunity *27*, this issue, 366–369.

Löhning, M., Hutloff, A., Kallinich, T., Mages, H.W., Bonhagen, K., Radbruch, A., Hamelmann, E., and Kroczek, R.A. (2003). Expression of ICOS in vivo defines CD4(+) effector T cells with high inflammatory potential and a strong bias for secretion of interleukin 10. J. Exp. Med. *197*, 181–193.

Mack, T.M., Thomas, D.B., and Noble, J. (1972). A prospective study of serum antibody and protection against smallpox. Am. J. Trop. Med. Hyg. *21*, 214–218.

Makela, O., and Nossal, G.J.V. (1962). Autoradiographic studies on immune response. 2. DNA synthesis amongst single antibody-producing cells. J. Exp. Med. *115*, 231–244.

Mandel, T.E., Phipps, R.P., Abbot, A., and Tew, J.G. (1980). The follicular dendritic cell—long-term antigen retention during immunity. Immunol. Rev. *53*, 29–59.

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

Manz, R.A., Lohning, M., Cassese, G., Thiel, A., and Radbruch, A. (1998). Survival of long-lived plasma cells is independent of antigen. Int. Immunol. *10*, 1703–1711.

Manz, R.A., Hauser, A.E., Hiepe, F., and Radbruch, A. (2005). Maintenance of serum antibody levels. Annu. Rev. Immunol. *23*, 367–386.

Maruyama, M., Lam, K.P., and Rajewsky, K. (2000). Memory B-cell persistence is independent of persisting immunizing antigen. Nature *407*, 636–642.

Mascola, J.R., and Montefiori, D.C. (2003). HIV-1: nature's master of disguise. Nat. Med. *9*, 393–394.

Miller, J.J. (1964). Autoradiographic study of plasma cell and lymphocyte survival in rat popliteal lymph nodes. J. Immunol. *92*, 673–681.

Mohamadzadeh, M., Chen, L.P., Olinger, G.G., Pratt, W.D., and Schmaljohn, A.L. (2006). Filoviruses and the balance of innate, adaptive, and inflammatory responses. Viral Immunol. *19*, 602–612.

Moir, S., Malaspina, A., Li, Y.X., Chun, T.W., Lowe, T., Adelsberger, J., Baseler, M., Ehler, L.A., Liu, S.Y., Davey, R.T., et al. (2000). B cells of HIV-1-infected patients bind virions through CD21-complement

interactions and transmit infectious virus to activated T cells. J. Exp. Med. *192*, 637–645.

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

Naniche, D., Garenne, M., Rae, C., Manchester, M., Buchta, R., Brodine, S.K., and Oldstone, M.B.A. (2004). Decrease in measles virus-specific CD4 T cell memory in vaccinated subjects. J. Infect. Dis. *190*, 1387–1395.

Ochsenbein, A.F., Pinschewer, D.D., Sierro, S., Horvath, E., Hengartner, H., and Zinkernagel, R.M. (2000). Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. Proc. Natl. Acad. Sci. USA *97*, 13263–13268.

Odendahl, M., Mei, H., Hoyer, B.F., Jacobi, A.M., Hansen, A., Muehlinghaus, G., Berek, C., Hiepe, F., Manz, R., Radbruch, A., and Dörner, T. (2005). Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response. Blood *105*, 1614–1621.

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

Radbruch, A., Muehlinghaus, G., Luger, E.O., Inamine, A., Smith, K.G.C., Dörner, T., and Hiepe, F. (2006). Competence and competition: the challenge of becoming a long-lived plasma cell. Nat. Rev. Immunol. *6*, 741–750.

Raftery, M.J., Winau, F., Kaufmann, S.H.E., Schaible, U.E., and Schönrich, G. (2006). CD1 antigen presentation by human dendritic cells as a target for herpes simplex virus immune evasion. J. Immunol. *177*, 6207–6214.

Richman, D.D., Wrin, T., Little, S.J., and Petropoulos, C.J. (2003). Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc. Natl. Acad. Sci. USA *100*, 4144–4149.

Rock, M.T., Yoder, S.M., Wright, P.F., Talbot, T.R., Edwards, K.M., and Crowe, J.E. (2005). Differential regulation of granzyme and perforin in effector and memory T cells following smallpox immunization. J. Immunol. *174*, 3757–3764.

Schittek, B., and Rajewsky, K. (1992). Natural occurrence and origin of somatically mutated memory B-cells in mice. J. Exp. Med. *176*, 427–438.

Slifka, M.K. (2004). Immunological memory to viral infection—commentary. Curr. Opin. Immunol. *16*, 443–450.

Slifka, M.K., and Ahmed, R. (1996). Long-term antibody production is sustained by antibody-secreting cells in the bone marrow following acute viral infection. Ann. N Y Acad. Sci. *797*, 166–176.

Slifka, M.K., and Ahmed, R. (1998). Long-lived plasma cells: a mechanism for maintaining persistent antibody production. Curr. Opin. Immunol. *10*, 252–258.

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

Smith, K.G.C., Light, A., Nossal, G.J.V., and Tarlinton, D.M. (1997). The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. EMBO J. *16*, 2996–3006.

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

Sprent, J., and Surh, C.D. (2003). Cytokines and T cell homeostasis. Immunol. Lett. *85*, 145–149.

Spriggs, M.K., Koller, B.H., Sato, T., Morrissey, P.J., Fanslow, W.C., Smithies, O., Voice, R.F., Widmer, M.B., and Maliszewski, C.R. (1992). Beta-2-microglobulin-, $CD8^+$ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered Igg responses. Proc. Natl. Acad. Sci. USA *89*, 6070–6074.

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

Tanchot, C., Lemonnier, F.A., Perarnau, B., Freitas, A.A., and Rocha, B. (1997). Differential requirements for survival and proliferation of CD8 naive or memory T cells. Science *276*, 2057–2062.

Terajima, M., Cruz, J., Raines, G., Kilpatrick, E.D., Kennedy, J.S., Rothman, A.L., and Ennis, F.A. (2003). Quantitation of CD8(+) T cell responses to newly identified HLA-A*0201-restricted T cell epitopes conserved among vaccinia and variola (smallpox) viruses. J. Exp. Med. *197*, 927–932.

Tscharke, D.C., Reading, P.C., and Smith, G.L. (2002). Dermal infection with vaccinia virus reveals roles for virus proteins not seen using other inoculation routes. J. Gen. Virol. *83*, 1977–1986.

Vieira, P., and Rajewsky, K. (1990). Persistence of memory B-cells in mice deprived of T-cell help. Int. Immunol. *2*, 487–494.

Wei, X.P., Decker, J.M., Wang, S.Y., Hui, H.X., Kappes, J.C., Wu, X.Y., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., et al. (2003). Antibody neutralization and escape by HIV-1. Nature *422*, 307–312.

Whitmire, J.K., Slifka, M.K., Grewal, J.S., Flavell, R.A., and Ahmed, R. (1996). CD40 ligand-deficient mice generate a normal primary cytotoxic T-lymphocyte response but a defective humoral response to a viral infection. J. Virol. *70*, 8375–8381.

Wofsy, D., Mayes, D.C., Woodcock, J., and Seaman, W.E. (1985). Inhibition of humoral immunity in vivo by monoclonal-antibody to L3T4—studies with soluble-antigens in intact mice. J. Immunol. *135*, 1698–1701.

Wyatt, L.S., Earl, P.L., Eller, L.A., and Moss, B. (2004). Highly attenuated smallpox vaccine protects mice with and without immune deficiencies against pathogenic vaccinia virus challenge. Proc. Natl. Acad. Sci. USA *101*, 4590–4595.

Xiang, Z., Cutler, A.J., Brownlie, R.J., Fairfax, K., Lawlor, K.E., Severinson, E., Walker, E.U., Manz, R.A., Tarlinton, D.M., and Smith, K.G.C. (2007). Fc gamma RIIb controls bone marrow plasma cell persistence and apoptosis. Nat. Immunol. *8*, 419–429.

Xu, R., Johnson, A.J., Liggitt, D., and Bevan, M.J. (2004). Cellular and humoral immunity against vaccinia virus infection of mice. J. Immunol. *172*, 6265–6271.

Zagury, D. (1991). Anti-HIV cellular immunotherapy in AIDS. Lancet *338*, 694–695.

Zinkernagel, R.M., and Hengartner, H. (2006). Protective 'immunity' by pre-existent neutralizing antibody titers and preactivated T cells but not by so-called 'immunological memory'. Immunol. Rev. *211*, 310–319.