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Long-term humoral immunity against viruses: revisiting the issue of plasma cell longevity

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Immunity to viral infections is achieved by a complex series of events composed primarily of humoral and cell-mediated immune responses. Often, the most important factor in preventing reinfection is pre-existing antibody, which stands guard against pathogens by persisting in the serum and along mucosal surfaces. Likewise, passive transfer of immunoglobulin that is specific for viruses, such as measles, polio, hepatitis A, rubella and smallpox, can modify, or even prevent, disease. As the half-life of serum immunoglobulin is less than 2–3 weeks, antibody levels can only be maintained by continuous antibody production. Although B cells can secrete antibody at different stages of differentiation, antibody responses are typically maintained by terminally differentiated plasma cells that have a high rate of antibody production. It has been estimated that plasma cells may secrete >5000 molecules of antibody per second. Plasma cells are considered to be the most important type of antibody-secreting cell (ASC) and both terms are used interchangeably in the context of this review.

Several characteristics distinguish plasma cells from memory B cells. Plasma cells are terminally differentiated, nondividing cells that spontaneously secrete antibody and have lost nearly all surface-bound immunoglobulin and major histocompatibility complex (MHC) class II molecules. Memory B cells do not spontaneously secrete antibody but, after antigenic stimulation, mount rapid secondary responses by proliferating and differentiating into ASC as well as generating more memory B cells. Memory B cells, equipped with high-affinity antigen receptors (i.e., surface-bound immunoglobulin), increased surface expression of MHC class II molecules and inducible co-stimulatory molecules, such as B7, are also extremely efficient antigen-presenting cells (APC) for stimulating T cell responses. As pre-existing antibody is the first line of defense against reinfection, ASC are directly required to maintain antibody production. Memory B cells, therefore, do not directly prevent reinfection but are thought to be involved in maintaining ASC numbers by interacting with immune complexes on follicular dendritic cells (FDC). If reinfection occurs, memory B cells may help prevent disease or decrease the severity of disease by limiting the spread of infection.

Lifelong antibody production against viruses

Many acute viral infections induce a protective humoral response that is characterized by pre-existing antibody. The duration of this antibody response varies among viral infections and can range from a few months to many years. Table 1 shows the longevity of humoral responses following several different acute human viral infections. Sustained antibody production at mucosal sites has been demonstrated in humans and in some animal models; however, mucosal infections confined to the respiratory or gastrointestinal tract often do not provide the same long-lived immunity induced by systemic viral infections. In general, mucosal antibody responses are shorter-lived than serum antibody responses and may, in part, indicate why recurrent respiratory or intestinal infections are common, whereas re-infection leading to clinical disease is rare in most acute systemic viral infections.

Whether long-term antibody responses are restricted to the structural proteins of the virion or whether the antibody responses to nonstructural proteins are also maintained is a question that has received little attention. Human subjects, after infection with wild-type (Oman) polio or live attenuated polio vaccines, maintain serum antibody responses against nonstructural proteins, as well as against the structural proteins of the virion, for many years. Likewise, antibody to nonstructural proteins of hepatitis A virus has been identified >25 years after infection. Antibody to structural proteins provides antiviral activity mechanisms including neutralization, agglutination and opsonization by phagocytic cells. Antibody responses to nonstructural proteins are unlikely to utilize these mechanisms and antibodies against internal or nonstructural viral proteins are generally not believed to play a major role in host defense. However, animal studies have shown that monoclonal antibodies against nonstructural proteins of yellow fever, dengue,
murine hepatitis virus and rabies are protective against viral challenge and reduce mortality rates. The mechanism(s) by which antibody to nonstructural proteins confer protection is not fully understood and deserves further investigation.

**Site of antibody production**

Initial T-cell-dependent antibody responses occur in the germinal centers of the spleen and other lymphoid organs. After primary vaccination with nonreplicating antigens, such as sheep red blood cells, the number of ASC peaks in the spleen and lymph nodes during the initial stages of the immune response but then declines to near baseline levels. Likewise, serum antibody titers are generally also short-lived after primary immunization. After secondary vaccination, the ASC response in the spleen and lymph nodes is higher and faster but again declines rapidly. However, as the splenic ASC numbers drop after secondary vaccination, a large number of ASC appear in the bone marrow and this usually corresponds with higher serum antibody titers of longer duration. By contrast, many acute viral infections induce long-term antibody responses without requiring repeated exposure. After resolving a single acute viral infection, the splenic ASC numbers rise and decline within a few weeks, while large numbers of virus-specific ASC accumulate in the bone marrow. Thus, the humoral response to viral infection is analogous to that induced by multiple vaccinations of a nonreplicating antigen. In animal models, the bone marrow has been identified as the predominant site of long-term antibody production after acute viral infections such as lymphocytic choriomeningitis virus (LCMV), Sendai virus, influenza and vesicular stomatitis virus (VSV). We have studied the anatomical site of virus-specific antibody production by infecting mice with LCMV, a natural murine pathogen. Acute LCMV infection of adult mice is cleared within 2 weeks by a potent cytotoxic T cell (CTL) response and virus-specific serum antibody is then maintained for the life span of the immune animal. The initial virus-specific ASC populations develop in the spleen and then decline rapidly after resolving the infection (Fig. 1). Despite extensive polyclonal B cell expansion in the spleen, appreciable numbers of antiviral ASC are not detected in the bone marrow until day 15. After viral clearance, and for the life span of the immune animal, the bone marrow is the major source of antiviral ASC. Upon reinfection with LCMV, the virus is cleared within 3–5 days and stimulates potent memory B cell and T cell responses. Again, the ASC response in the spleen rises transiently before returning to near baseline levels within 2 weeks. After clearance of the secondary infection and re-establishment of homeostasis, the bone marrow still remains the major site of antibody production. Intuitively, it makes sense to have anatomical segregation of B cell differentiation and long-term antibody production. Antigenic stimulation of naïve B cells, and their differentiation into memory B cells and plasma cells, occurs in the germinal centers of the spleen and lymph nodes. If plasma cells were to remain in the germinal centers, then in a case of yellow fever, for example, a human lymphoid follicle would need to allocate space for yellow-fever-specific plasma cells for the following 75 years, as well as the numerous
Mechanisms of long-term antibody production

It is interesting that common childhood diseases, such as polio, measles and mumps, induce serum antibody responses that are sustained into adulthood. The longevity of these immune responses may explain why these are considered as ‘childhood diseases’ because the first exposure, lifelong protection against reinfection and disease is afforded. To explain these long-term antibody responses, several mechanisms have been proposed (Table 2). The most simple explanation for the maintenance of humoral immunity may explain why common childhood diseases, such as polio, measles and mumps, induce serum antibody responses that are sustained into adulthood. The longevity of these immune responses may explain why these are considered as ‘childhood diseases’ because the first exposure, lifelong protection against reinfection and disease is afforded. To explain these long-term antibody responses, several mechanisms have been proposed (Table 2). The most simple explanation for the maintenance of humoral immunity may be that memory T cells facilitate memory B cell homing, ready to mount new antibody responses. Alternatively, a low-grade viral infection may persist, or viral antigen may remain, in the form of antigen-specific ASC. Moreover, plasma cells of other specificities that would accrue over this extended period. By targeting plasma cells to the bone marrow, peripheral lymphoid organs, such as the spleen and lymph node, are able to return to homeostasis, ready to mount new antibody responses. Evidence supporting this phenomenon includes studies showing that germinal centers recede by ~3 weeks post-antigenic stimulation, whereas ASC in the bone marrow are not only maintained but continue to accumulate over time.

Role of CD4+ T Cells

By definition, T-cell-dependent antibody responses require CD4+ T cells to initiate both primary and secondary antigen-specific antibody responses, but what role do they play in the maintenance of humoral immunity? The involvement of CD4+ T cells in antibody responses can be divided into four stages: (1) differentiation of naive B cells into ASC and memory B cells, (2) differentiation of memory B cells into ASC during secondary responses, (3) maintenance of memory B cells and (4) maintenance of ASC. It is well established that CD4+ T cells deplete abrogates the ability of naive B cells to produce T-cell-dependent antibody responses and prevents the generation of memory B cells. One study has shown that after initiation of a primary humoral response, depletion of CD4+ T cells in vivo appears to have minimal effect on the maintenance of memory B cells. However, this issue needs to be examined in more detail. The induction of secondary humoral responses again requires CD4+ T cell help, but whether the assistance of CD4+ T cells is required for the survival of plasma cells remains to be determined.

During anamnestic responses, antigen-specific ASC migrate to the bone marrow; a phenomenon that has been studied in the context of both naive and memory CD4+ T cell help. During a secondary antibody response to a T-cell-dependent antigen, memory B cells, but not necessarily memory T cells, appear to be required to initiate migration of ASC to the bone marrow compartment. If memory T cells are present, the ASC numbers in the spleen are greatly increased after antigenic stimulation, showing that memory T cells facilitate memory B cell proliferation and differentiation into ASC. However, this does not result in a substantially greater population of ASC accumulating in the bone marrow. T cell help may not be an absolute requirement for this migratory phenomenon because T-cell-independent antibody responses also induce antigen-specific ASC migration to the bone marrow.

In summary, T cell help may be necessary only to initiate the antibody response or to boost antibody production intermittently upon restimulation with antigen, however, additional studies are needed to determine the role of CD4+ T cells in the maintenance of memory B cells, as well as the longevity of antibody secreting cells and their migration to the bone marrow.
of polio and yellow fever outbreaks in isolated communities confirm Panum's results by demonstrating long-term humoral immunity in the absence of recurrent infection. In addition, immunization with vaccinia virus induces antibody production that is maintained for at least 15 years. Re-exposure is unlikely in each of these examples because there were either no reported cases of infection during the decades in between epidemics or, in the case of vaccinia, there is no longer any circulating virus because smallpox vaccination was discontinued in 1977 and none of the subjects in the study worked in laboratories where vaccinia was used.

Some viral infections that are normally classified as acute may actually persist after clinical symptoms have subsided. For example, measles and rubella virus have been found to persist for years after the initial infection. However, it is difficult to believe that low-grade chronic infection is the only mechanism behind long-term antibody production because a variety of acute infections induce life-long immunity (Table 1) and it is unlikely that they each develop into low-grade persisting infections. In addition, nonreplicating antigens, such as tetanus toxoid, induce antibody responses that last >20 years, and pre-existing antibody to diphtheria toxoid has been identified 25-30 years post-immunization. Overall, long-term serum antibody responses are a common theme among acute systemic viral infections and the longevity of such responses does not appear to be dependent upon repeated exposure or chronic viral infection.

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Box 1. Model of long-term antibody production

(a) During acute infection
- High antigen level (1–3 weeks)
- Continuous B-cell stimulation

(b) After acute infection
- Intermediate antigen level (weeks, months or longer?)
- Memory B-cell restimulation by immune complexes

(c) Long-term homeostasis
- Low to no antigen (>10 years)
- Memory B cells enter a resting state

• Antibody (80–90% of serum antibody produced by plasma cells in spleen, lymph nodes, etc.)
• Long-lived plasma cells in bone marrow

Antibody responses. Although cross-reactivity to self antigens or the development of idiotypic networks are plausible, current dogma suggests that antibody production is maintained by memory B cells that are stimulated to become ASC by antigen trapped on the surface of FDC. Most serum antibody is produced in the spleen (lymph nodes, etc.) and few plasma cells accumulate in the bone marrow. At this stage, most serum antibody is produced by long-lived plasma cells in the bone marrow, with only a few plasma cells remaining in the spleen. Which cell type migrates to the bone marrow has not been established but it is possible that terminal differentiation of plasma cells may occur after migration. Months after clearing an acute infection, antigen levels may drop below an immunological threshold of activation and the immune response would then return to homeostasis. Memory B cells enter a resting state and no longer differentiate into plasma cells, unless restimulated by antigen. Our model proposes that plasma cells in the bone marrow, and a subpopulation of plasma cells in the spleen and lymph nodes, have a long life span, and that serum antibody levels may be sustained for several years in the absence of antigenic restimulation. M, memory B cells; P, plasma cells.
**Hypothesis: long-lived plasma cells**

We propose a model of how long-lived humoral responses may be maintained following acute viral infections or vaccination (Box 1). This model emphasizes what may be the most overlooked component of long-term humoral immunity: long-lived plasma cells. After migrating to the bone marrow, which contains a rich source of growth factors and cytokines, plasma cells may settle into a stable microenvironment, sheltered from the radical changes that occur in peripheral lymphoid follicles following new, or recurrent, viral and bacterial infections. Moreover, as plasma cells lose nearly all surface-bound immunoglobulin, they are presumably insensitive to antigenic stimulation and therefore would be unaffected if antigen became a limiting factor.

Soon after rats have been immunized, it is apparent that most plasma cells have a very short half-life, with estimates ranging from 8 hours to ~3 days \(^{58-61}\). This may be misleading, however, because after the initial immune response has peaked and then subsided, the remaining plasma cells appear to survive for several weeks \(^{62}\) to more than 6 months \(^{64}\). If just early timepoints are studied, this biphasic response could go unrecognized and could easily be interpreted as an indication that plasma cells are short-lived. It is only by examining late timepoints after immunization that a stable subpopulation of long-lived ASC can be readily identified \(^{65}\). In addition to these studies, preliminary data indicate that long-lived plasma cells in mice are induced after acute LCMV infection (M.K. Sifk and R. Ahmed, unpublished).

In the context of an acute viral infection, there are three general stages of antibody production (Box 1): (i) antigenic stimulation of B cells to proliferate and differentiate into ASC (Box 1a). During this period, most, if not all, antibody is produced by short-lived ASC in the periphery and few virus-specific ASC will have migrated to the bone marrow. The second stage occurs after the viral infection has been resolved (Box 1b). By this time, a potentially long-lived population of antigen-specific ASC have migrated to the bone marrow and appear to be responsible for maintaining most serum antibody production. During this stage of the immune response, any remaining antigen–antibody complexes on FDC may continue to stimulate memory B cells to differentiate into more ASC. The third stage is marked by long-term, immunological homeostasis (Box 1c). When antigen levels are very low, or nonexistent, memory B cells enter a resting state, unless restimulated by antigen. The plasma cells in the bone marrow are then no longer replenished and serum antibody levels may decline as a function of the number of plasma cells remaining over time.

A fundamental difference appears to exist between the longevity of mucosal and serum antibody responses (Table 1). Mucosa-associated lymphoid tissues, e.g. tonsils, Peyer’s patches and the lamina propria, have the same basic architecture as the spleen and lymph nodes, including follicles that give rise to germinal centers that produce memory B cells and plasma cells. After antigenic stimulation, these lymphoid tissues show similar kinetics to the spleen (Box 1), including a short-lived local ASC response that peaks early and is followed by ASC migration to the bone marrow as local antibody production subsides. For mucosal immunity, however, this may be a ‘one-way street’ because once ASC migrate to the bone marrow they are unlikely to continue to contribute to mucosal antibody production. This may, in part, explain why local mucosal antibody responses tend to decline more rapidly than serum antibody responses.

**Questions for future research**

- What determines the half-life of plasma cells during the humoral response to vaccination or viral infection? Are there separate lineages of short-lived and long-lived antibody-secreting cells (ASC)?
- How many antigen–antibody complexes on follicular dendritic cells (FDC) are required to stimulate a memory B cell to proliferate and/or differentiate into a plasma cell?
- Are antigen–antibody complexes consumed during the stimulation of memory B cells during an ongoing antibody response?
- As FDC are thought to play an essential role in maintaining humoral immunity, what is the life span of these important antigen-retaining cells and how does this affect the longevity of antigen-specific antibody responses?
- What induces the migration and/or retention of plasma cells in the bone marrow? What homing receptors may be involved?
- What factors (cytokines, microenvironment, etc.) are involved in sustaining long-term antibody production at different anatomical sites? Why is mucosal antibody production generally short-lived by comparison with serum antibody production, and how could one increase the magnitude and duration of mucosal antibody responses?

**Conclusions**

To sustain humoral immunity against a pathogen for greater than half a century is a remarkable accomplishment. The question of whether the longevity of this response results from persisting antigen, long-lived plasma cells or a combination of both deserves further attention. To understand fully how persisting antigen continues to provide an immunogenic stimulus over the course of several years, it will be essential to gain a more extensive biochemical and biological understanding of how antigen–antibody complexes are maintained on the surface of FDC. In addition, determining the half-life of FDC, memory T cells, memory B cells and plasma cells will be essential to our understanding of how long-term humoral responses are maintained. It is hoped that a greater knowledge of these mechanisms will allow better design of adjuvants and vaccines that are potentially capable of providing long-term protective immunity.

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