Plasma cells for a lifetime?

Rudolf A. Manz and Andreas Radbruch

Deutsches Rheumaforschungszentrum Berlin, Berlin, Germany

Antigen-specific serum antibodies are protective for long periods of time [1, 2]. These serum antibodies, the “humoral memory”, are secreted by plasma cells derived from activated, antigen-specific B lymphocytes. Given their crucial role in immunity, surprisingly little is known about the biology of plasma cells. One of the fundamental questions is whether persisting protective serum antibody responses are maintained by long-lived plasma cells, or by short-lived plasma cells generated continuously from activated memory B cells. Here, we review some recent experiments suggesting that plasma cells have the capacity to live for unlimited time if rescued by specific factors provided in a limited number of survival niches in the body.

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Lifespan of plasma cells

The lifetime of plasma cells has been a matter of debate for as long as 50 years. Initially, plasma cells of secondary lymphoid tissue, *i.e.* of those organs where activated B lymphocytes differentiate into plasma cells were analyzed. Using the state-of-the-art technology for that time, proliferating cells were labeled with pulses of radioactive thymidine, and the frequency of radioactively labeled plasma cells was determined at various intervals afterwards. From this work, it was proposed that some plasma cells might be as long-lived as a few weeks, or even longer [3], while others concluded that most plasma cells have a lifetime of only a few days [4, 5]. This conclusion was based on the observation that the numbers of labeled plasma cells drop rapidly within a few days after antigenic stimulation. Basically there was consensus that most of the plasma cells in secondary lymphoid organs are short-lived and few, if any, long-lived. In 1964 Miller [6] observed that „in stimulated lymph nodes there was a rapid early decrease in plasma cell numbers, but a small percentage (8%) of labeled plasma cells persisted through the 6-month time point”. Although these experiments suggested that some plasma cells might be long-lived, the observed drastic early drop in numbers of plasma cells in the secondary lymphoid organs led to the commonly held view that most plasma cells are short-lived.

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In 1972 McMillan [7] described that high numbers of antigen-specific plasma cells can be detected in the bone marrow at later time points after immunization, when their counts are already running low in the secondary lymphoid organs, suggesting that antigen-specific plasma cells might migrate from secondary lymphoid organs to the bone marrow [8, 9]. Consequently, persistence of these plasma cells in the bone marrow was tracked for a 10-day period by MacLennan and collaborators [10], who concluded that some bone marrow plasma cells are short-lived, while others may survive for up to 3 weeks. This lifetime is much too short to explain the persistence of protective antibody titers in the serum by persistent, long-lived plasma cells. The idea that plasma cells would have to be generated constantly *de novo* from activated memory B lymphocytes became popular. This hypothesis was based on the observation that antigen is presented for long periods after immunization by follicular dendritic cells in secondary lymphoid tissue, and that cultures of secondary lymphoid tissue from immunized mice spontaneously produce specific antibodies *ex vivo,* without need for added antigen. The concept of long-lived immune reactions as a source of short-lived plasma cells would predict that, since specific serum antibody titers and the number of plasma cells in the bone marrow are stable over long periods [11, 12], the continuous differentiation of memory B lymphocytes into plasma cells would either result in a drop in frequency of specific memory B lymphocytes, or require their proliferation to maintain their numbers. Unfortunately, neither is the case [13]. We are back at the original question: how long do plasma cells live?

Several groups have taken up the challenge and have recently reinvestigated the lifespan of plasma cells. Our

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group originally used bromo-deoxyuridine (BrdU) pulse chases to determine the lifetime of plasma cells in a secondary murine immune response to ovalbumin [14]. About 70% of the plasma cells in the bone marrow had stopped proliferating within about 3 weeks after immunization and survived for at least another 3 months, without further proliferation, but continuously secreting antibodies. The remaining 30% of plasma cells or their precursors had proliferated between week 3 and week 6 after immunization but stopped proliferating then and survived at least for another 2 months, the period of observation. This experiment showed that the entire population of antigen-specific plasma cells in the bone marrow consisted of long-lived plasma cells, surviving without cell division over long periods and continuously secreting antibodies. It also became evident that the extrapolation of results from the initial phase of the immune reaction would have led to the wrong conclusion that the population of plasma cells would be completely replaced by proliferated, newly derived cells within 3 months. In the older experiments, such extrapolations had led to the hypothesis that plasma cells are short-lived.

Continuing our analysis of plasma cells, we were able to show that persisting titers of protective antibodies can be transferred with the fraction of $CD45R^-$ ($B220^-$) bone marrow cells, containing plasma cells but not B memory cells, and that the activity of the plasma cells is not dependent on antigen [15]. In absolute numbers, the bone marrow contained approximately half the number of antigen-specific plasma cells that had been present in the secondary lymphoid organs at the peak of the response.

The latter experiments showed that in a secondary immune response against a non-replicating protein antigen, the formation of plasma cells is restricted to the initial phase of the immune response, *i.e.* within about 2 months after rechallenge. Slifka et al. [16] analyzed the lifespan of plasma cells in the immune response against lymphocytic choriomeningitis virus (LCMV), a replicating antigen. They depleted reactive memory B lymphocytes in mice with an established response to LCMV *in vivo* by irradiation, or inhibited their proliferation by treatment with mitomycin C, prior to adoptive transfer into congenic recipients. Nevertheless, specific serum antibodies and high numbers of plasma cells persisted for more than 1 year, suggesting that activation and proliferation of memory B cells are not required for the persistence of plasma cells.

This observation contrasts with a recent report by the group of Hengartner and Zinkernagel [17], suggesting that, in the immune response to LCMV and vesicular stomatitis virus (VSV), most plasma cells might be short-lived and derived from the continuous activation of memory B lymphocytes. In this analysis, high titers of persisting serum antibodies were only obtained with active, replicating but not with inactivated virus. Persistence of serum antibodies seemed to be correlated with the persistence of antigen. An alternative explanation would be that the difference in stability of serum antibody titers reflects a primary versus a secondary immune response, the non-replicating virus being able to generate just a primary response. In contrast, immunization with the replicating virus would initiate a primary immune response, and later an overlapping secondary response, resulting in five- to tenfold higher numbers of persisting plasma cells in the bone marrow as compared to a primary response [18]. In a second line of experiments, Ochsenbein et al. [17] showed that spleen and bone marrow cells from VSV-immunized mice, when transferred into non-irradiated hosts, generated only low titers of persistent antibody titers in the absence of antigen. Cotransfer of antigen enhanced the specific antibody titers. In our analysis of murine ovalbumin-specific plasma cells, we were able to separate the antigen-dependent from the antigen-independent humoral memory according to expression of CD45R (B220) [15]. Murine memory B lymphocytes express CD45R, while murine plasma cells do not. Transfer of $CD45R^+$ bone marrow cells only resulted in antibody secretion if antigen was cotransferred, while transfer of $CD45R^-$ bone marrow cells, *i.e.* plasma cells, resulted in persistent serum antibody production which was independent of cotransferred antigen.

In both the VSV- and ovalbumin-specific immune responses, transfer of bone marrow plasma cells yielded rather low persistent titers, arguing that longevity of the plasma cells is not an intrinsic feature, but depends on stimuli other than antigen, provided by their natural microenvironment, *i.e.* specific survival niches, and that transferred plasma cells may have difficulties in reaching such niches and surviving in the host, after having been removed from them experimentally.

Plasma cell survival niches

Where to look for survival niches for plasma cells? An obvious choice is the bone marrow. A second argument for the existence of distinct survival niches for plasma cells in the bone marrow is that the capacity of the bone marrow for plasma cells seems to be limited. The total number of plasma cells in the bone marrow of mice increases over time, reaching a plateau after about 1 year of age of approximately 400,000 plasma cells in the entire bone marrow, *i.e.* about 0.4% of all mononu-

cleated cells in this tissue are plasma cells [19]. In the bone marrow of adult humans, the percentage of plasma cells among mononucleated cells is relatively invariable and similar to that in mice, *i.e.* 0.2–0.4% [20]. A third and more direct argument for the existence of distinct survival niches comes from the attempt to characterize these niches at a molecular level. It has been known for a long time that isolated plasma cells from bone marrow or spleen do not survive *ex vivo*. Essentially, they can only survive if fused to a myeloma cell [21]. In recent, as yet unpublished, experiments we have attempted to identify those factors that can sustain the survival of isolated bone marrow plasma cells *ex vivo*. While antigen is dispensable, a combination of secreted cytokines, including IL-6, and plasma cell-stroma cell and plasma cell-extracellular matrix signals, is able to dramatically prolong the survival of plasma cells *ex vivo* (Cassese et al., manuscript in preparation).

In accord with the early studies of Miller [6], the experiments of our and Rafi Ahmed's group suggest that survival niches for plasma cells may be present not only in bone marrow but also in spleen, as a secondary lymphoid organ, although at lower numbers. MacLennan and coworkers [22] have used BrdU pulse chasing to detect non-proliferating long-lived plasma cells after activation of naive B cells and activation of memory B cells. Apparently, both plasma cell types can enter the pool of long-lived plasma cells in the spleen, but the number of persisting cells depends on the restricted capacity of this organ to sustain these cells. Recently, we have reported that long-lived plasma cells can persist in the inflamed kidneys of (NZB×NZW)F1 mice, a murine model for systemic lupus erythematosus, in numbers roughly equivalent to bone marrow [23]. Do inflamed tissues in general provide survival niches for plasma cells? Plasma cells have been identified in many other types of inflamed tissue, *e.g.* rheumatoid synovium [24, 25], but their lifespan there still has to be determined.

In the concept of plasma cell survival niches, the balance between short- and long-lived plasma cells in a given immune reaction will depend on the instruction of freshly generated plasma cells to express genes enabling it to leave the site of generation and home to survival niches and genes enabling it to respond to the survival signals. In addition, retaining signals of secondary lymphoid organs and attractive signals of the bone marrow may be involved. At present we know little about signals and genes involved in the generation, differentiation, homing and maintenance of long-lived plasma cells and, because of that, it is hard to access the relative contributions of short-lived and long-lived plasma cells in particular immune reactions. Reasoning teleologically, the generation of long-lived plasma cells should depend on the clearance of antigen, with plasma cells generated in that late phase of an immune reaction representing a successful humoral response and providing an antigen-independent protection with proven efficiency. Whether immune reactions follow this reasoning, and if so how, will have to be determined.

Adaptation of humoral memory

The hypothesis of "survival niches" for plasma cells, as compared to "intrinsic" survival programs, also offers a simple explanation for the gradual decline observed in many persistent serum antibody responses. Competition for survival niches would eventually lead to a replacement of old plasma cells by new ones, specific for more recent antigenic challenges. The plasma cell memory could adapt to the antigenic environment of the individual. An immune system might keep older memory plasma cells as long as there are niches available, and these plasma cells will provide serum antibody titers sufficient to protect the individual from antigenic challenges up to distinct concentrations of antigen, probably those not exceeding the original ones. Pre-existing serum antibody would inhibit the activation of specific memory B lymphocytes [26, 27].

In such a situation, memory B cells would be inert and would not participate in the clearance of the antigen. At higher concentrations of challenging antigen, or after long periods, when numbers of specific plasma cells have declined and not all antigen is neutralized, memory B lymphocytes would be reactivated and would differentiate into new plasma cells, which in turn would compete for plasma cell survival niches and adjust the concentration of specific serum antibodies to the new challenge by the old antigen (Fig. 1). This model implies a regulatory function of antigen in humoral immune responses, as postulated earlier [28]. In addition, the numbers of plasma cells originally formed, and most likely their migration potential, will depend on the amount and form of antigen that triggers the immune response, and the quality of help by other cells, as discussed above. By definition, persisting antigen has the potential to trigger continuously the generation of plasma cells, as long as it persists and appropriate help is available for the activation and differentiation of memory B lymphocytes into plasma cells. As discussed above, it would not make much sense for the immune system to generate persistently long-lived plasma cells against persisting antigen, unless this antigen is eventually cleared. During persistence of antigen, a significant part of persistent antibody titers might indeed be derived from short-lived plasma cells, which are continuously generated in an ongoing immune reaction.

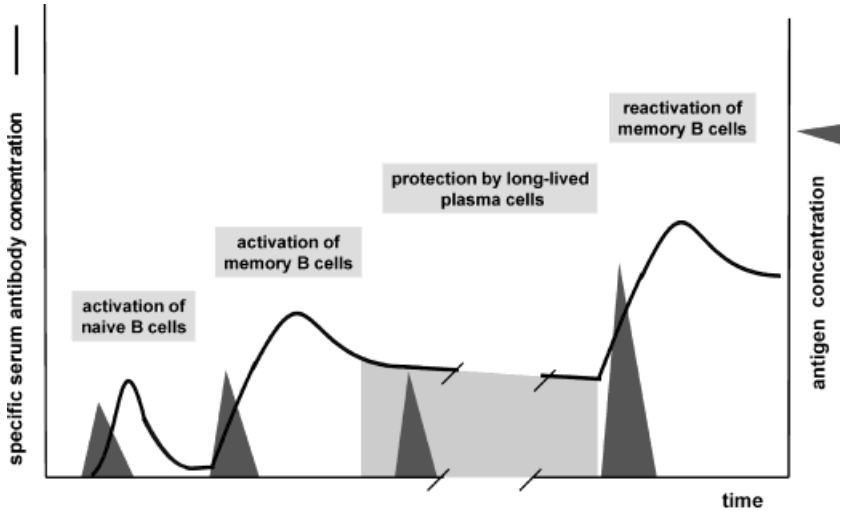

Fig. 1. Humoral memory adapts to the environment.

The new view on survival and life-span of plasma cells and their essential role in the generation of persisting serum antibody titers offers intriguing options for our understanding and treatment of diseases with antibodies involved in immunopathology. In IgE-mediated allergy and autoimmune diseases like lupus erythematosus, the fate of the pathogenic plasma cells has to be determined, and to what extent they had been generated by long-past or chronic immune reactions. In those diseases where conventional immunosuppression aimed at inhibiting the generation of plasma cells fails, the challenge is to develop new therapeutic strategies targeting the survival of already generated plasma cells. Such therapeutic strategies might also be helpful for the treatment of plasmacytomas. Finally, the efficient generation of long-lived, antigen-independent plasma cells should become an essential aim in the development of successful vaccination strategies for protective humoral memory.

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Correspondence: Andreas Radbruch, Deutsches Rheumaforschungszentrum Berlin, Berlin, Schumannstrasse 21/22, D-10117, Germany
radbruch@drfz.de