Immunological Memory

Secondary article

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Immunological memory is defined functionally as the event that occurs when the immunologically experienced individual re-encounters antigen through infection or immunization, and as a result develops a greater and faster response than after the first encounter.

Article Contents

Introduction

In its various manifestations memory is the essence of life. Central nervous system memory is the link between the present and the past, for the individual and the species. Similarly, immunological memory is the link between present and previous antigenic experiences. In both cases our understanding of the anatomical, cellular or structural correlates is still primitive. As to immunological memory, its significance is best understood in the light of the role this plays in the survival of the individual and the species. In this respect immunological memory can be categorized broadly into two types: transmissible memory and acquired memory.

Transmissible memory refers to memory that protects against cytopathic, life-threatening pathogens during the perinatal period when immunocompetence has not yet developed in the individual. In mammals and birds this type of memory is mediated only by antibodies transmitted from mother to fetus. In humans transmission occurs transplacentally and, as in cattle, by intestinal absorption of maternal colostrum, while in birds it occurs through the egg. The evolutionary advantage of transmissible memory for the individual and the species needs no further comment.

Acquired memory, the type of memory that most people recognize as immunological memory, is defined functionally as the event that occurs when the immunologically experienced individual re-encounters antigen through infection or immunization, and develops as a result a greater and faster response than after the first encounter. As discussed below, immunological memory is equated to an increase in the frequency of specifically reactive precursor lymphocytes and heightened sensitivity to antigen, characteristics that may or may not be dependent on periodic antigen exposure.

Before getting into particulars it is important to realize that the value of acquired immunological memory in protection against pathogens is dependent on a number of factors including differences among pathogens with respect to their portal of entry, localization in tissues, and manifestation of disease. It is well appreciated that B and T lymphocytes participate in these processes in various forms and proportions. For instance, pathogens that initially come into contact with external secretions, or enter the bloodstream in a cell-free form, can be deflected by humoral mechanisms (antibody). Pathogens that have become established intracellularly require in turn cell-mediated immunity.

Immunological memory induced by vaccination needs also to be measured against the length of the incubation period of specific pathogens. For instance, for diseases of longer incubation period, such as paralytic poliomyelitis (which requires more than 3 days for central nervous system invasion from the primary site of infection in the intestinal tract), immunological memory responses are sufficient to prevent paralysis. In contrast, for diseases of short incubation period (less than 3 days, as for influenza) memory responses are insufficient to afford protection, and adequate levels of serum neutralizing antibodies need to be present at the time of exposure to prevent the establishment of infection. Therefore, the lesson learned from pathogens is that the effectiveness of immunological memory established by infection or vaccination is regulated by the incubation period of the particular infectious agent.

Cellular and Molecular Characteristics of Memory

Cellular memory among B and T lymphocytes is characterized by a large pool of cells, which can recognize and react with the particular antigen, and which persist as a population for long periods of time. The initial (primary) response to antigen starts with so-called naive or virgin B and T cells which have not previously encountered the antigen with which their specific receptors react (B-cell or immunoglobulin receptor (BCR) and T-cell receptor (TCR)). These cells expand in numbers, becoming highly activated, and then a proportion persists and returns to a resting state (memory cells) where they remain ready to initiate a secondary response upon re-encounter with antigen (e.g. infection or vaccination). As discussed below, each individual memory cell may not necessarily live for an extended period, but sporadic cell division may allow the population as a whole to persist for months and in some

Table 1 Features of memory B and T cells

B cells	T cells
Rearranged receptor genes	Rearranged receptor genes
Have undergone somatic hypermutation	No somatic hypermutation
Frequency of antigen-reactive cells is moderately increased compared with the primary response	Frequency of antigen-reactive cells is dramatically increased compared with the primary response
Certain markers may help to show memory status	Certain markers may help to show memory status
Moderate survival (population or individual cells)	Long-term survival (population or individual cells)
Memory function is also carried out by long-lived plasma cells in bone marrow	No functional equivalent to plasma cells exists for differentiated T cells

cases years. In becoming memory cells, B and T lymphocytes share some common features, but are also distinct in many respects. Some of the differences are listed in **Table 1**.

In both instances memory cells are those cells that have effectively escaped death and complete elimination, a common sequel to cellular activation events. Also common to both lymphocytes is that when they re-encounter antigen they react more promptly and a greater number of cells participate in the anamnestic response than during the initial response when antigen is first encountered. Differences do, however, exist in the activation requirements and the topology of memory B- and T-cell development. A major distinction is that during the progression from the primary response to a memory status the BCR continues to accumulate mutations, a mechanism of adaptation to changes in a pathogen's antigenic characteristics and also a way to generate antibodies with increasingly higher affinity (reactivity) for antigen (Griffiths *et al*., 1984). T cells do not undergo somatic hypermutation and appear to compensate with the selection and expansion of high-affinity clonotypes whose TCRs are able to recognize peptide–major histocompatibility complex (MHC) complexes more efficiently as shown by analysis of CDR3 regions (McHeyzer and Davis, 1995). In addition, the triggering requirements for memory T cells are lower than those for T cells that are activated during the primary immune response. This in part reflects a greater ability to respond to lower levels of antigen because of enhanced TCR signalling, and in part is due to a lower requirement for costimulatory signals. In addition, cytokines are secreted with increased kinetics by memory T cells, and the range of cytokines that can be produced initially is larger when compared to the naive T cell (Croft and Dubey, 1997; Dutton *et al*., 1998). Both mechanisms allow memory cells to counteract antigen quickly and efficiently once it is re-encountered.

Memory T and B cells can potentially be delineated from naive cells based on the expression of several cell surface antigens. However, in many cases recently activated naive cells and effector T and B cells also bear similar phenotypes, making the distinction between these cell types difficult. In addition, caution should be exercised when defining memory cells by surface markers because many proteins appear to cycle depending on length and type of antigenic encounter (Bell *et al*., 1998). It is widely believed that memory cells are in a resting state for much of the time (i.e they are small in size with little granularity), and this parameter should be used in combination with phenotypic analysis in order to identify them. Memory B cells are thought to have high levels of expression of immunoglobulin (Ig) M, other immunoglobulin isotypes such as IgG and IgA, CD21, CD39, CD27 and CD148, and low levels of IgD (Tangye *et al*., 1998). Memory T cells are thought to be characterized by low levels of CD45RA/B and CD62L, and high levels of CD44, CD2, CD11a/CD18 and CD29 (Swain *et al*., 1996). Naive cells bear the reciprocal phenotypes.

Because memory B and T cells do have distinct characteristics, they are best discussed separately.

Generation of Memory

The phase of generation of memory cells revolves around a central theme: expansion in cell numbers and escape from death (**Figure 1**). For B cells this occurs in a specialized environment, the germinal centre (GC), requiring interaction with T cells and cells termed follicular dendritic cells (FDCs). The GC favours cell activation and rescue from untimely death through upregulation of antiapoptotic molecules. For T cells, rescue from cell death may require similar antiapoptotic molecules, although there is no evidence that a specialized environment or interaction are required.

B cells

Memory B cells are formed in the GCs of secondary lymphoid organs. From the GCs they migrate to the marginal zone where they wait to be activated by antigen. The path to these simple events is as follows. Recirculating naive B cells enter secondary lymphoid organs through the

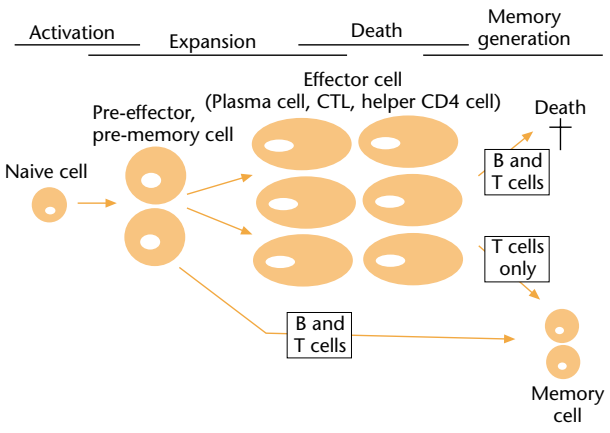

Figure 1 Scheme of generation of memory lymphocytes. The pathway indicated is common to B and T cells. In both cases, upon encounter with antigen, naive cells differentiate into pre-effector pre-memory cells in a phase of expansion that includes contact with antigen, upregulation of costimulatory and antiapoptotic molecules, and cytokine production. In the next phase, distinct populations of effector cells (plasma cells and effector T cells) are generated, followed in a matter of days by massive cell death. Only plasma cells and memory T cells survive and either go to the bone marrow (plasma cells) or recirculate throughout the lymphatic and blood systems (T cells). As indicated, while memory T cells can derive from either the pre-effector or effector T-cell population, memory B cells derive only from the pre-memory cell population as plasma cells differentiate terminally and cannot revert back to the resting B-cell stage. CTL, cytotoxic T lymphocyte.

marginal zone and localize in the B cell-rich follicle. If they encounter antigen in the blood draining into the marginal sinus, they move towards the edge of the T cell-rich zone to meet with T cells that have already been activated by antigen presented on interdigitating dendritic cells (IDCs) (Clark and Ledbetter, 1994). Signals from the BCR and CD40 induce proliferation that is rapid and lasts for 2–3 days. During this time a small number of the B-cell blasts give rise to GCs, the site where clonal expansion, somatic hypermutation, affinity maturation and possibly isotype switch take place during the adaptive immune response. A fraction of activated B cells go on to become plasma cells which secrete the first wave of antibodies (mainly IgM). Other B cells are arrested in their terminal differentiation and fail to secrete antibodies. It appears that a prolonged engagement of CD40 is responsible for the arrest of terminal differentiation and persistence at the memory stage (Arpin *et al*., 1997; Randall *et al*., 1998).

Selection of B cells destined to become memory cells takes place in GCs and is controlled by the expression of intracytoplasmic molecules (Bcl-2 and Bcl-x) which prevent a form of cell death termed apoptosis, together with the concomitant suppression of signals from cell surface proteins that lead to death (e.g. CD95 or tumour necrosis factor receptor (TNFR)). The protracted upregulation of antiapoptotic molecules is effective in counteracting CD95-mediated death, and hence promoting further cell differentiation.

In their path to survival, differentiating B lymphocytes come to an important checkpoint: the choice between becoming a plasma cell or a memory cell. The signals that drive these two pathways are presently considered to be different. From *in vitro* studies it is appreciated that triggering the cell surface molecule CD40, during cognate interaction between B cells and T cells, directs B cells towards the memory rather than the plasma cell pathway. However, if ligation of CD40 is transient, B cells die by apoptosis. Conversely, signalling through cell surface molecules such as CD23 and CD134L, in the absence of CD40 triggering, may direct B cells to differentiate terminally into plasma cells. However, as seen above, continuous engagement of CD40 freezes terminal differentiation into plasma cells. Although not formally proven, it would appear that the continuous expression of the BCR, and binding of antigen, constitutes another prerequisite for survival. Inference is drawn from the fact that, while mature B cells are selected into the long-lived peripheral pool for their ability to express the BCR, nonfunctional B cells are rapidly eliminated (Lam *et al*., 1997).

Within 4 days of antigen activation, those cells selected to survive within the GCs become long-term residents. Most of them show somatic hypermutations, the unmistakable fingerprint of a GC origin. Once in the marginal zone memory B cells become resting and noncycling. An alternative to the paradigm that memory B cells derive from the pool of GC precursors is the notion that memory B cells may originate from cells that apparently exist before the encounter with antigen (Linton *et al*., 1989). These may be maintained in the potential repertoire by an innate regulatory network, a view supported by the fact that mature B cells that lose the ability to express the BCR are eliminated (Lam *et al*., 1997). Evidence for this alternative view remains limited.

T cells

The absolute mechanisms governing the generation of memory in the T-cell compartment are less well understood. Initial encounter with an antigen occurs in the T-cell zones of lymph nodes or in the spleen where naive T cells are directly adjacent to IDCs (Garside *et al*., 1998). The small number of naive T cells specific for the particular antigen (typically fewer than 1 in 10^5 CD4 T cells react with any antigen) are stimulated on these antigen-presenting cells (APCs) and receive signals to become activated and divide. These signals are obtained from recognition of peptide–MHC complexes by the TCR, with essential costimulatory signals received from the interaction of accessory molecules such as CD28 with CD86 on the APC, and from cytokines such as interleukin (IL)-1 and IL-6. T-cell expansion generally proceeds over the course of a week

and results in a massive increase in the number of antigen-reactive T cells by anywhere from 100- to 5000-fold. Variations in number may occur for several reasons. Assays differ in sensitivity and may produce aberrant numbers. Limiting dilution analysis, tetramer staining using peptide–MHC complexes, and intracellular cytokine staining have all been used to assess the frequencies of effector and memory cells (Bradley *et al*., 1993; Gallimore *et al*., 1998; Murali-Krishna *et al*., 1998). In addition, it appears that CD8 T cells expand tremendously to highly replicating viruses such that one in two or three cells may be specific for the virus during the peak of response (Murali-Krishna *et al*., 1998). The highest estimates for the frequency of CD4 T cells are in the 1 : 50–100 range, suggesting that intrinsic differences exist between the two responses (Bradley *et al*., 1993; McHeyzer and Davis, 1995; Whitmire *et al*., 1998). These so-called effector T cells are primed to clear the initial wave of the foreign antigen, being able to kill directly in the case of CD8 cells, or secrete cytokines and help other lymphoid cells in the case of CD4 cells. Once the effector cells have performed their function, the majority are subsequently eliminated, resulting in a dramatic decline in cell numbers such that as many as 95% of them die (Gallimore *et al*., 1998; Murali-Krishna *et al*., 1998). Apoptosis or activation-induced cell death (AICD) is governed by further signals from peptide–MHC complexes and from the cell surface molecules CD95 and TNFR. After the death phase of the response, which begins during the expansion phase (days 3–6) and continues for up to 3 weeks, a population of T cells persists, but at an increased frequency compared with the initial number of naive T cells specific for the antigen. Depending on several factors described above, this memory pool may be as little as three times, or up to 100 times, as large as the naive pool.

As defined, memory cells are resting whereas effector cells are highly activated and cycling. The precise relationship between a memory T cell and an effector T cell is, however, unknown. Both appear to arise from the naive precursor T cell and their development requires the presence of antigen. It is possible that memory T cells are derived directly from effector T cells and purely represent a cell that has reverted back to a resting state having avoided signals to undergo AICD (Swain, 1995). Alternatively, as for B cells, memory T cells may derive from recently activated naive T cells that are cycling but which do not receive appropriate signals to progress to the effector stage (Klinman, 1997). Unlike B cells, where differential responses may result depending on whether CD40, CD23 or CD134L is engaged (Liu and Banchereau, 1997), it is not known whether triggering particular molecules on a T cell will result in memory or effector cells. Regardless of the absolute pathway to a memory cell, a major factor that determines the size of the memory T-cell pool is the amount of antigen that is seen and the context in which it is presented, i.e. whether costimulatory or inhibitory signals from accessory molecules are received or signals from other inflammatory factors such as cytokines. For example, too much antigen and too high a level of stimulation during the initial phase of response may result in all cells reaching the effector stage with the majority dying by AICD. This was demonstrated in experiments with Lymphocytic choriomeningitis virus, where exhaustion of the CD8 pool specific for this virus was seen when high titres were achieved (Moskophidis *et al*., 1993; Gallimore *et al*., 1998). This antigen translates into a very good primary response but the development of poor memory. In contrast, less antigen and a lower level of stimulation may push fewer cells to the effector stage with less death by AICD. In this case, the primary T-cell response would be weaker, but a much greater number of T cells would survive resulting in the development of strong memory. Too little antigen or too low a level of stimulation during the initial stages of the naive T-cell response may have the opposite effect in that few cells will be pushed through the cell cycle and few effector cells will develop. Little if any memory will be achieved and, if the antigen is encountered in the absence of costimulatory signals, immune tolerance may result (Kearney *et al*., 1994).

Whether memory T cells arise in a specialized site like B cells in the GC is not known. Naive T-cell activation generally occurs in the T-cell zones (paracortex, periarteriolar lymphoid sheath) of lymph nodes and spleen and this is where the majority of the expansion phase takes place (Kearney *et al*., 1994; Garside *et al*., 1998). A small number of the expanded T cells do enter the GCs where they are involved in selecting high-affinity B cells, but the ultimate fate of these T cells is not clear (Gulbranson-Judge and MacLennan, 1996). If the amount of antigen and how often it is seen determines the development of memory T cells as discussed above, it is likely that a specialized site and specialized cells such as FDCs are not required.

Maintenance of Memory and Factors Determining Memory Longevity

Possibly more important than the induction phase of memory cells is their maintenance. In fact this forms the prerequisite of functional memory: the durable ability to mount a specific immune response upon re-encounter with antigen over time. Are there special conditions to ensure longevity of memory cells?

B cells

At the time of re-encounter with bloodborne antigen, memory B cells that were resident in the marginal zone migrate to the T-cell zone. Within 12 h, B-cell blasts are induced and rapid clonal expansion (five times greater than during the primary response) is visible. At the same time GCs expand in only a limited fashion, evidence that most memory B cells proliferate outside the GCs (Garside *et al*., 1998).

A question still debated is whether or not there are factors essential to persistence as noncycling memory cells. According to one school of thought, antigen is responsible not only for the initiation of the immune response (including clonal expansion of B cells and generation of antibody-producing plasma cells) but also for the selection of memory cells. Early cell transfer experiments showed that B-cell memory is short lived, approximately 12 weeks (Gray and Skarvall, 1988). The prevailing view is that maintenance of B-cell memory in the intact host is a function of the persistence of antigen on FDCs (Tew *et al*., 1980). In fact, following the production of antibodies, antigen–antibody complexes are formed and retained on the surface of FDCs with great efficacy. FDCs bind immune complexes through their Fc and complement receptors, and organize them spatially in a regular pattern in special structures termed iccosomes (immune complex-coated bodies). Similar to any local immune complex formation, antigen–antibody complexes on FDCs are also subject to a dynamic equilibrium depending primarily on the amount of free antibody. Thus, the immunogenicity of FDC-bound immune complexes is related directly to the antigen : antibody ratio. For instance, when antibody levels in the circulation decline, antigens are exposed and memory B cells are stimulated. Apparently only a few hundred picograms of antigen are retained in the long term on FDCs, but these small amounts are sufficient to sustain durable and efficient memory responses. Memory cells are periodically restimulated every time iccosomes are endocytosed by B cells, which then process the antigen, present it to T cells, and in turn receive signals from the T cell to respond. In addition, FDCs also provide B cells with costimulatory signals that augment cell proliferation.

Directly relevant to sustained antibody responses *in vivo*, and certainly close to the concept of B-cell memory, is the fact that maintenance of serum antibodies over time does not require the continuous proliferation and differentiation of memory B cells into antibody-secreting plasma cells. Long-lived plasma cells can persist in the bone marrow and in the spleen, without the need for replenishment by memory B cells, providing an alternative basis for long-term humoral immunity (Slifka *et al*., 1998). To maintain a stable population of memory B cells, the rate at which new memory B cells are generated must equal the rate at which they die, so long-lived plasma cells have the potential to fill any gap in the turnover of memory B cells. By working in concert, these two mechanisms ensure that humoral immunity is maintained in the long term. The advantage for the immunocompetent host is evident.

T cells

Two main theories exist regarding the persistence of memory T cells; they are not necessarily mutually exclusive. One theory is that these cells are intrinsically long lived, survive in a resting state for months or years, and do not need to receive stimuli for their persistence (Lau *et al*., 1994; Di Rosa and Matzinger, 1996). The second is that memory T cells are relatively short lived and require periodic stimulation for their long-term survival (Gray and Matzinger, 1991; Kundig *et al*., 1996). The second theory is more plausible but the nature of the stimulus is controversial. Specific antigen can be present in an individual for a long period of time, either from low-grade infection that is never fully cleared but is controlled, or by persisting on FDCs. Alternatively, the specific foreign antigen may not be needed, with the T cells responding to either self peptides or other foreign antigens. These have to be similar to the specific antigen and cross-react sufficiently for engagement of the MHC and TCR and to produce signalling through the TCR (Takeda *et al*., 1996; Tanchot *et al*., 1997; Markiewicz *et al*., 1998). In either way, memory T cells may be stimulated at intervals over time and receive sufficient signals that induce brief entry into cell cycle and promote survival for long periods. Cytokines may also participate in the survival of memory cells, with interferon α (type I interferon) and IL-15 playing some role in this process (Zhang *et al*., 1998).

Similar to the process of memory cell generation, the level of stimulus may determine the longevity of response. If memory T cells receive a high level of stimulation, the majority may be pushed into the effector stage resulting in a strong secondary response. These memory effector cells are susceptible to AICD and may die. If too many are eliminated, the frequency of antigen-specific cells will drop, in essence only leaving the naive T-cell pool intact and subsequently no evidence of memory. In contrast, low-level periodic stimulation may favour long-term survival as few cells ever become effectors, but survival signals are still received. The total absence of antigen (specific or cross-reactive) may also result in memory waning as most cells may die from neglect (programmed cell death) and only those few that are intrinsically long lived will remain.

Conclusion

Immunological memory represents a powerful aspect of the immune response and a key attribute of the immune system for protection of the individual (acquired memory) and the species (transmissible memory) against pathogens and other molecules able to cause injury. The general cellular and molecular characteristics of B- and T-cell immunity are clear with respect to the fact that, while T cells are limited in their function by MHC restriction, B cells and their antibody product are not. These characteristics of the adaptive response also influence acquired and transmissible immunological memory.

As to acquired immunological memory, the form the individual is most concerned with during life, some final

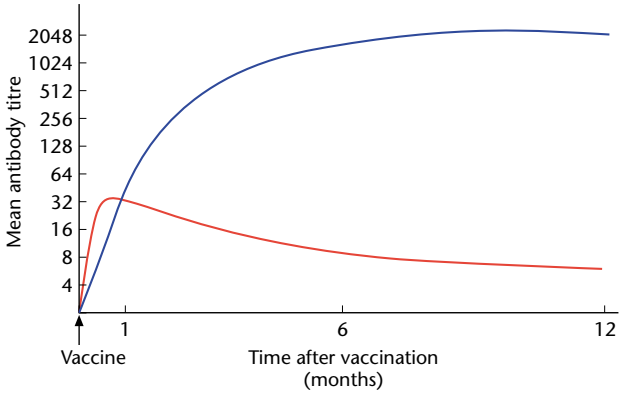

Figure 2 Development and persistence of serum antibody (red) and immunological memory (blue) following one dose of noninfectious poliovirus vaccine.

considerations can be made. While there may be differences in the longevity of memory B and T cells, a direct assessment of the status and degree of existing immunological memory is difficult. B-cell memory cannot be assessed merely by measuring antibodies in serum since persistence of antibodies can be due to long-lived plasma cells which do not expand upon re-encounter with antigen. Moreover, by studying the course of development and persistence of antibodies in serum and the course of development of immunological memory following a single dose of noninfectious vaccine in humans, it has become apparent that serum antibody levels do not reflect the degree of immunological memory and, conversely, immunological memory can be present in the absence of detectable antibodies (**Figure 2**).

T-cell memory can be assessed by determining the frequency of antigen-specific precursors by limiting dilution analysis or immunostaining using MHC–peptide polymeric units. Neither approach can, however, assess the functional status of T-cell memory or estimate the degree of clonal expansion cells will undergo *in vivo* after re-encounter with antigen.

As a phenomenon, immunological memory remains a functional event that cannot be defined solely through its major components, B and T cells. It should continue to be considered a property of the immune system as a whole.

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