PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction

A.J. McMichael and P.C. Doherty

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THE ROYAL SOCIETY

Introduction

Memory is a central feature of the immune response. The second and subsequent exposures to an antigen, typically a micro-organism, provoke a more rapid response of greater magnitude. Gowans first demonstrated that immunological memory was carried by small lymphocytes (Gowans & Uhr 1966), and the clonal selection theory provided a framework to understand this (Burnett 1960). However, many questions remain, for instance whether continuous or occasional stimulation by antigen, cross-reacting antigen or cytokines is required. How memory is regulated, preventing the responding population from taking over the entire immune system, is also poorly understood.

These basic questions are tackled in this issue, which reports on a symposium held at The Royal Society in March 1999. There were many new insights and the whole gives a comprehensive review of the current understanding of immunological memory. Some of the difficulties in the subject relate to the terminology and definition of memory cells. However, there is general agreement about what a naive lymphocyte is: a peripheral B or T cell that has not yet encountered antigen. Although such cells may have met weakly cross-reacting antigen, the more stringent requirements needed to prime an immune response keeps these cells distinct, although they can be hard to enumerate. Differential expression of certain cell-surface glycoproteins, such as CD44 and CD45 isoforms (in humans), can be used to discriminate naive and memory T cells, although these are not totally reliable (Beverley & Maini, this issue). Surface IgM identifies naive B cells.

There are different views on what memory cells are. Some would define a memory cell as any lymphocyte that had seen antigen, once it had started to divide, activate synthesis and release of cytokines, or change its surface phenotype. If antigen persists, as in a chronic virus infection, some would call the specific reacting lymphocytes memory cells even if they can no longer divide and are destined for programmed cell death. It is unlikely that such cells could maintain memory if antigen were suddenly removed. Others would restrict the term memory cell to lymphocytes that can be reactivated long after antigen has completely disappeared. In some definitions, memory lymphocytes are those that can divide and function when required to do so. Most authors do not normally define what they mean by memory. In this issue, Doherty makes a useful attempt to clarify the terminology according to the methods used to identify the populations (for example, surface markers, limiting dilution assay, tetramer staining, cytokine release, expression of particular T-cell receptors).

What was clear from the meeting is that memory is different for each of the main lymphocyte subpopulations. B-cell memory is recognized by the antibodies they produce. Zinkernagel (this issue) argued that the main evolutionary reason for immunological memory is the importance of transfer of anti-pathogen antibody from mother to foetus and infant (which is not just transplacental and through milk, but also occurs in egg-laying vertebrates). This is certainly remarkable; in humans a mother can protect her infant for as long as six months in this way, even though she might have been infected with the pathogen (e.g. measles virus) over 30 years ago. As antibody has a half-life in plasma of around 20 days, and antibody-producing plasma cells are relatively short lived, this implies some continuous turnover of specific B memory either in the absence of cognate antigen or because of stimulation by very long-lived sequestered antigen. A feature of memory that is special to B cells is the affinity maturation of the antibody. Together with class switching, this is a key feature of B-cell development after antigen stimulation and development of memory; it in part enables the B cells to maintain a steady level of a function, such as virus neutralization, as the antigenic stimulus becomes progressively weaker. The importance of class switching and affinity maturation is explored in detail in this issue (Neuberger *et al.*; MacLennan *et al.*).

$CD8^+$ (cytotoxic) T cells (CTL) are the most-studied lymphocytes in the context of memory, particularly those that are virus or transplantation antigen specific. For assessment of memory, the methodological endpoints are critical. Resistance to reinfection is an obvious and relevant measurement of memory, but how do we deal with the immune response to persisting viruses? Superinfection may be resisted (e.g. in the attenuated SIV vaccine experiments reported by Kaur *et al.* (this issue)), but it is arguable whether this is due to memory or just an ongoing immune response. In some studies, measurements of $CD8^+$ T-cell memory are made *in vitro* by restimulating the T cells under conditions where primary T-cell responses are not seen, thus any response indicates memory. These assays have been extensively used but do not tell whether such responses would actually protect from reinfection, an important distinction that could account for some of the disagreements in this area, particularly whether T-cell memory is antigen dependent. Protective $CD8^+$ T cells might require a state of activation that requires recent antigen stimulation (Zinkernagel, this issue). The life span of memory T cells indicates that they are long lived and (as discussed by Sprent *et al.*, Tanchot *et al.* and Beverley & Maini, this issue) this could be maintained by cytokine stimulation, particularly type I interferon and IL-15.

It is hard to compare long-term memory in the mouse, which means one to two years, with that in humans of 30–50 years when *in vitro* the T cells behave in a very similar fashion with similar life spans. The size of the naive and memory $CD8^+$ T-cell compartments may be relevant. Using T-cell receptor transgenic mice, Tanchot *et al.* (this issue) show that these populations are tightly regulated for size. Slow accumulation in a size-limited compartment of more and more memory T cells, specific for different pathogens, in a long lifetime may in part account for the fading of immunological T-cell memory seen in humans, rather different from inbred mice kept in relatively clean environments for short periods. Regulation of compartment size is clearly essential given the enormous expansions of $CD8^+$ T cells that occur during primary infections (discussed by Whitmire *et al.*, Doherty, McMichael *et al.*, Rickinson *et al.* and Beverley & Maini, this issue).

$CD4^+$ T-cell memory is less well understood and there are undoubted differences (Swain and Van Essen *et al.*, this issue). Unlike $CD8^+$ T cells, antigen is not so widely distributed: class I MHC is present on most cells and these can present antigen; class II MHC is restricted to specialist antigen-presenting cells which can also deliver specialist co-signals. Antibody influences antigen presentation and therefore there is a close relationship with B-cell memory. Conversely $CD4^+$ T cells affect both B-cell and $CD8^+$ T-cell memory. The extent of clonal expansion for $CD4^+$ T cells in acute infections seems to be smaller than for the $CD8^+$ set and they are less likely to be maintained in some persistent infections. There are fewer oligoclonal expansions compared to those in $CD8^+$ T cells in humans (Beverley & Maini, this issue). Whether there are differences in memory in Th1 compared to Th2 cells is not yet clear; any differences could profoundly affect memory in B cells and $CD8^+$ T cells.

The evolutionary significance of immunological memory deserves some attention. If mother-to-infant antibody transfer is the driving force: Is immunological memory in males just an accident, and why have memory in $CD8^+$ T cells? The latter must have a value. More rapid responses upon second encounter with a virus should offer real advantages, for instance, in an encounter with a new pandemic influenza strain where antibody offers no protection because of the shift in haemagglutinin type. The persisting viruses Epstein–Barr virus (EBV) and cytomegalovirus (CMV), which infect most humans, are demonstrably lethal when $CD8^+$ T-cell responses are removed (e.g. by immunosuppressive drugs); memory T cells (at least according to most definitions, see above) are essential for continuing health.

Do we know enough about immunological memory to attempt mathematical modelling? Within agreed parameters this may be possible and Wodarz *et al.* (this issue) make a brave attempt. However, additional factors such as those regulating compartment size (Tanchot *et al.*, this issue) and non-specific cytokine stimulation may have to be factored in. Such models may give a rationale for therapeutic approaches to treating damaging persistent infections in humans, such as those with HIV and hepatitis C virus.

Finally, understanding immunological memory is essential for the rational design of vaccines. Empirical vaccines (particularly attenuated viruses and formalin-inactivated viruses) have controlled many serious infections but have so far failed to deliver vaccines for malaria, HIV and some other infections. We now have good ways of stimulating strong B-cell, Th1, Th2 and $CD8^+$ T-cell responses. But often it is not known what are the real correlates of protection. There could be a big difference between the ability of a highly activated immune response, two weeks after vaccination, to repel an invader (in an experimental challenge system) compared with a primed but resting and contracted memory lymphocyte population. The latter could have difficulty dealing with a rapidly infecting pathogen. How a potent and effective memory population can be maintained is likely to be a crucial issue for designed vaccines. This will be specially important when the aim is to induce T-cell immunity or high titre neutralizing antibody immunity.

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A. J. McMichael
P. C. Doherty

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