Hidden lymphocytes

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THE prevailing view of autoimmune diseases is that they represent failures of immunological tolerance to self-antigens. On page 77 of this issue[1], a group at Kyoto University (Murakami *et al.*) report observations with anti-erythrocyte immunoglobulin-expressing transgenic mice demonstrating that autoantibody production can result from atypical B lymphocytes that evade self-antigen-induced elimination. These experiments illustrate the importance of tolerance induction in the B-lymphocyte compartment, and point to a mechanism by which autoimmunity might arise.

Transgenes

Transgenic mice have provided a powerful window into the underlying basis of immunological tolerance[2,3]. New protein antigens can readily be expressed from transgenes, and their effects on antigen-specific lymphocytes assessed. These neo-self-antigens are typically expressed continuously, and so are much more akin to true self-antigens than are injected antigens. Perhaps even more powerful is the approach of introducing functionally rearranged genes encoding lymphocyte antigen receptors (membrane immunoglobulin or T-cell antigen receptor) into the mouse germ line to create mice with large numbers of B cells or T cells of identical specificity. This technique makes it possible to follow the fate of antigen-specific lymphocytes that encounter antigen in various contexts.

The Kyoto group used genes encoding an anti-erythrocyte antibody to create transgenic mice with B cells that were almost all specific for an erythrocyte self-antigen[4]. About half of the mice exhibited an autoimmune haemolytic anaemia, indicating that immunological tolerance was only partially able to protect the animals from attack by the self-reactive B cells. The mice did exhibit a dramatic reduction in the numbers of B cells in spleen and lymph node; presumably, tolerance mechanisms caused death of most of the self-reactive B cells. In the peritoneum, very few B cells were present soon after birth, but the numbers increased with age until by adulthood roughly normal numbers had been attained. In addition to conventional B cells, the peritoneum is rich in a second type of B cell called the Ly-1$^+$ B cell or, more recently, the B-1 cell[5]. B-1 cells arise early in development and in adoptive transfer experiments have the potential to renew themselves[6,7]. They may represent a lineage separate from the conventional B cells of spleen and lymph node (see box).

An attractive hypothesis is that a few B-1 cells are somehow spared elimination at the hands of the self-antigen, and that they migrate to the peritoneum, where, in the absence of the self-antigen, they can proliferate until normal numbers are achieved. To test this hypothesis, Murakami *et al.*[1] injected red blood cells into the peritoneal cavity of their transgenic mice. The results were dramatic — within 12 hours almost all of the transgenic B-1 cells had died. The presence of fragmented DNA suggests that these cells had undergone programmed cell death by apoptosis. Thus, contact with the self-antigen induced rapid death of those cells that had previously avoided inactivation. They were not inherently resistant to immunological tolerance mechanisms such as clonal deletion, but rather were resident in a privileged environment in which the self-antigen was absent.

Some of the B-1 cells from the peritoneum of the transgenic mice differentiated into cells secreting antibody at a high rate. The number of these autoantibody-secreting cells in the peritoneum correlated with the severity of the haemolytic anaemia, and such cells were not found elsewhere[1]. These autoantibody-secreting cells disappeared following weekly injection of red blood cells into the peritoneal cavity, and the autoimmune haemolytic anaemia rapidly cleared up. Thus, the conclusion that B-1 cells in the peritoneum are responsible for autoantibody production seems likely to be correct.

Autoantibodies

Although the involvement of B-1 cells in autoimmune diseases has not been firmly established, it has been shown that these cells from non-transgenic mice secrete a number of autoantibodies and that in certain autoimmune diseases of man and mouse[6] their numbers are increased. The

How do B-1 cells originate?

THE CD5$^+$ or B-1 lymphocytes[5] exhibit a distinctive cell-surface phenotype, a characteristic homing to the peritoneal and pleural cavities, and an unusual capacity for self-renewal[6,7]. A number of self-reactive antibody specificities are greatly enriched in this B-cell subpopulation.

Two models have been proposed to explain the origin of B-1 cells. First, they could derive from developing B-lineage precursors that are committed to become B-1 cells. According to this hypothesis, B-1 cells and conventional B cells represent separate cell lineages, as is believed to be the case for T cells expressing either αβ T-cell receptors or γδ T-cell receptors[11]. This view has received strong support from adoptive transfer experiments, which showed that adult bone-marrow progenitors repopulate the B-1 cell population only poorly, whereas fetal liver or omentum progenitors do so quite effectively[12,13]. The lymphoid progenitors in fetal omentum, unlike those in fetal liver or adult bone marrow, only reconstituted B-1 cells and did not reconstitute conventional B cells in SCID mice[13]. Moreover, cotransfer of immunoglobulin allotype-marked adult bone-marrow stem cells and fetal liver stem cells resulted in B-1 cell production from the fetal liver precursors but not from the adult bone marrow precursors, demonstrating that the ability to generate B-1 cells is an intrinsic property of the fetal B-cell precursors[12].

Although a great deal of evidence favours the separate-lineage hypothesis, it is possible that B-1 cells instead represent a long-lived physiological state that results from a particular type of contact with antigen of a previously uncommitted B cell. Evidence for this view comes from experiments in which *in vitro* treatment of conventional B cells with anti-immunoglobulin antibodies and interleukin 6 leads to a cell-surface phenotype that closely resembles that of B-1 cells[14]. The observation that certain immunoglobulin-transgenic mice seem predominately to express either conventional B cells[15] or B-1 cells[16] also seems to fit this hypothesis better, as different membrane immunoglobulins might differ in their interactions with environmental antigens.

Nonetheless, the adoptive transfer experiments cannot be readily explained by a model invoking one B-cell lineage, and more information will be required to reconcile the current data with one or other of the two hypotheses. A possible explanation is that B-1 cells could derive from committed progenitors that require contact with antigen in a particular form to enter the long-lived, self-renewing B-1 cell population. In any case, a greater knowledge of the origin and properties of B-1 cells is important for understanding normal and autoantibody immune responses. **A.L.DeF.**