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B–cell memory and the persistence of antibody responses

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THE ROYAL SOCIETY

B-cell memory and the persistence of antibody responses

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Antigens such as viral envelope proteins and bacterial exotoxins induce responses which result in the production of neutralizing antibody. These responses persist for years and provide highly efficient defence against reinfection. During these antibody responses a proportion of participating B cells mutate the genes that encode their immunoglobulin variable regions. This can increase the affinity of the antibody, but can also induce autoreactive B cells. Selection mechanisms operate which allow the cells with high affinity for the provoking antigen to persist, while other B cells recruited into the response die.

Keywords: B cell; memory; germinal centre; affinity maturation

1. THE PERSISTENCE OF B-CELL MEMORY AND ANTIBODY RESPONSES

Virgin B cells are recruited into T-cell-dependent antibody responses in the periods immediately following antigen administration (Gray *et al.* 1986; MacLennan *et al.* 1990). Once established, these responses can continue indefinitely without the recruitment of further B cells (Askonas *et al.* 1970; Gray *et al.* 1986; MacLennan *et al.* 1990). This can be shown by the transfer of genetically marked mature lymphocytes from an animal in the established phase of a secondary response to a congenic recipient. If the recipient is immunized after the transfer, the donor memory B cells make a contribution to the response, which is maintained for the life of the animal (Askonas *et al.* 1970; Gray *et al.* 1986; MacLennan *et al.* 1990); functional B-cell memory does not appear to persist for more than a few weeks following cell transfer unless the recipient is immunized (Gray & Skarvall 1988).

2. RENEWAL OF PLASMA CELLS AND MEMORY B CELLS

The transfer experiments cited in §1 (Gray & Skarvall 1988) show that B-cell memory is sustained, at least in part, by continued antigen-dependent activation of memory B-cell clones. In addition, bromodeoxyuridine labelling studies show slow renewal of at least a proportion of memory cells during established antibody responses (Liu 1989; Liu *et al.* 1991; Schittek & Rajewsky 1990). The details of how and where long-term memory B-cell activation occurs are still largely a matter for speculation. Recent studies indicate that the very long-term production of neutralizing antibody to viral envelope proteins or bacterial exotoxins may be achieved through the persistence of certain plasma cells (Slifka *et al.* 1998; Manz *et al.* 1998). Although many of the plasma cells found in the bone marrow in established responses have a life span of around a month (Ho *et al.* 1986), indicating that there is plasma cell renewal, a proportion of plasma cells live for much longer (Slifka *et al.* 1998; Manz *et al.* 1998). These studies have successfully transferred bone marrow plasma cells without antigen and have shown that some of plasma cells establish themselves in the recipient's bone marrow and spleen and continue to produce antibody indefinitely (Slifka *et al.* 1998; Manz *et al.* 1998).

3. AWAKENING MEMORY

On re-exposure to antigen, both memory B cells and a fresh cohort of virgin B cells are rapidly recruited into the new response. On this occasion the presence of antibody 5w›at the time of immunization reduces the threshold for B-cell activation by cross-linking CD21 to the B-cell receptor (BCR) (Dempsey *et al.* 1996). This and the immediate availability of primed T cells, results in a lower stringency for B-cell activation. The effect of these conditions is particularly apparent in mice primed with carrier protein and that are subsequently challenged with hapten-carrier. The range of immunoglobulin (Ig) variable regions used is much greater, and the affinity of the anti-hapten antibody produced is lower than in a primary response to hapten-protein (Sze 1998).

4. THE DISTRIBUTION AND PHENOTYPE OF MEMORY B CELLS

The distribution of most virgin B cells differs from that of memory cells (figure 1). Immature B cells are produced throughout life in the bone marrow and these gradually replace the mature recirculating and marginal zone B-cell pools (reviewed by MacLennan 1998). Contrary to early conclusions, the recirculating B-cell pool is largely or perhaps entirely virgin (Küppers *et al.* 1993); recirculation in this context is used in the classical sense defined by Gowans (Gowans & Knight 1964) of small non-dividing lymphocytes that migrate from blood to

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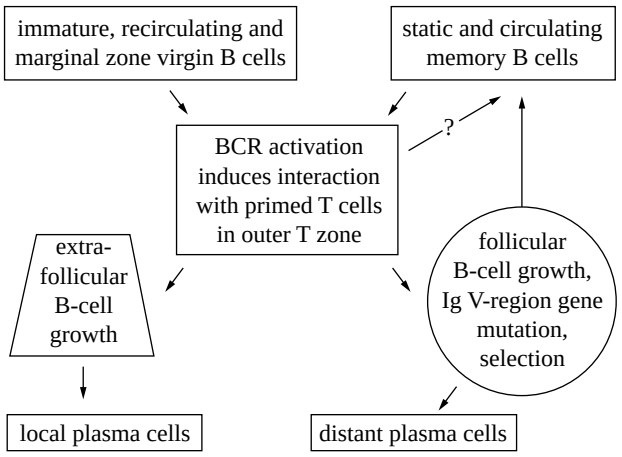

Figure 1. The relationship between non-dividing virgin and memory B cells and those cells that have been recruited into antibody responses.

secondary lymphoid tissue and from there back again to the blood either directly or via efferent lymph. The cells of the marginal zones of the spleen and equivalent areas in other secondary lymphoid tissue comprise a variable mixture of virgin (MacLennan & Liu 1991) and memory B cells (Liu *et al.* 1988; Dunn-Walters *et al.* 1995). These are sessile cells (Kumararatne & MacLennan 1981), but on activation by antigen they leave the marginal zone and move to the outer T zone where they are very efficient at eliciting T-cell help (Toellner *et al.* 1996; Slifka *et al.* 1998). Although the recirculating B cells are characteristically virgin there is a significant memory B-cell population which circulates in the blood (Klein *et al.* 1997).

The virgin and memory B cells of the marginal zone share a substantially common phenotype, which differs from recirculating cells in several ways. The marginal zone cells are larger, have less condensed chromatin and markedly more messenger RNA in their cytoplasm. They generally express IgM with little or no IgD (Stein *et al.* 1980; Gray *et al.* 1982), or have switched Ig class (Gray *et al.* 1982). Importantly a significant proportion of memory B cells have not undergone switch recombination (Pascual *et al.* 1994; Klein *et al.* 1997). Marginal-zone B cells lack CD23 expression (Ling *et al.* 1987), which characterizes recirculating follicular B cells; they express high levels of CD21 (Timens *et al.* 1987), a feature which reduces the threshold for B-cell activation through the BCR when C3d is linked to antigen (Dempsey *et al.* 1996). Recently, the work of Klein *et al.* (1998) has shown that the memory B-cell component in humans differs from virgin cells by the expression of CD27.

Until activated through the BCR, marginal-zone B cells are sessile, but these splenic B cells and those in equivalent areas in other secondary lymphoid organs occupy sites which give them direct access to antigen. Thus marginal zone B cells are perfused by blood sinusoids and consequently have direct access to antigen in the blood (Herman 1980). Memory B cells in the tonsil are located in the sponge-like epithelium that lines the tonsilar crypts. These crypts open directly to the oropharynx and so provide access to antigen in the mouth and nose (Liu *et al.* 1995). Memory cells in Peyer's patches lie in the dome directly under the epithelium with its antigen-transmitting M cells (Spencer *et al.* 1985). In lymph nodes, memory B cells accumulate in patches on the inner surface of the subcapsular sinus (Casamayor-Palleja *et al.* 1995).

The splenic marginal zones in most strains of mice are relatively small compared with those found in human and rat spleen. In mice, marginal-zone B cells usually comprise about 10% of splenic B cells, but in rats and humans around one-third of splenic B cells are located in the marginal zone. The larger numbers of marginal-zone memory B cells in rats result in a more substantial accumulation of B blasts in the outer T zone during recall immune responses in the T zone (Liu *et al.* 1991), compared with that seen in mice (Toellner *et al.* 1996).

On engaging antigen, both memory and virgin B cells become efficient at finding primed T cells. In doing this they migrate to the outer T zone, where primed T cells accumulate (figure 1) (Casamayor-Palleja *et al.* 1995; Gulbranson-Judge & MacLennan 1996). The efficiency of this process is shown by the rapidity with which memory B cells from the marginal zone of mice relocate to the outer T zone and respond to cognate interaction with primed T cells. Within 12 h of intravenous challenge the production of switch transcripts can be detected—a product of B cells specifically activated by T cells (Toellner *et al.* 1996).

5. THE GENERATION OF MEMORY B CELLS AND AFFINITY MATURATION

Memory B cells are produced in follicular responses to protein-based antigens (Klaus *et al.* 1980; Coico *et al.* 1983). The follicular response is started by cognate interaction in the outer T zone between primed T cells and B cells that have taken up and processed antigen (Claassen *et al.* 1986; Liu *et al.* 1988, 1991; Jacob *et al.* 1991*a*; Toellner *et al.* 1996). This cognate interaction can result in immediate Ig class switch recombination (Toellner *et al.* 1996). It also induces the B cells to migrate to one of two sites where they undergo exponential growth—the follicles or extra-follicular foci (Jacob & Kelsoe 1992; Toellner *et al.* 1996).

(a) *The extra-follicular response*

In extra-follicular foci B cells proliferate in the absence of T cells and differentiate into plasmablasts within 24 h (Gulbranson-Judge & MacLennan 1996; Luther *et al.* 1997). After a further 24–48 h they come out of cell cycle and differentiate *in situ* into plasma cells, which are mainly short lived (Ho *et al.* 1986). This extra-follicular growth occurs without the activation of Ig variable region gene-directed hypermutation (Jacob & Kelsoe 1992). About 10–20% of the plasma cells generated in extra-follicular foci live for much longer (Liu 1989; Liu *et al.* 1991; Schittek & Rajewsky 1990; Sze 1998). The long-lived plasma cells in spleen are partially produced in extra-follicular foci and partially in follicles (Sze 1998). It is not obvious that memory B cells result from extra-follicular growth, although this possibility has not been excluded.

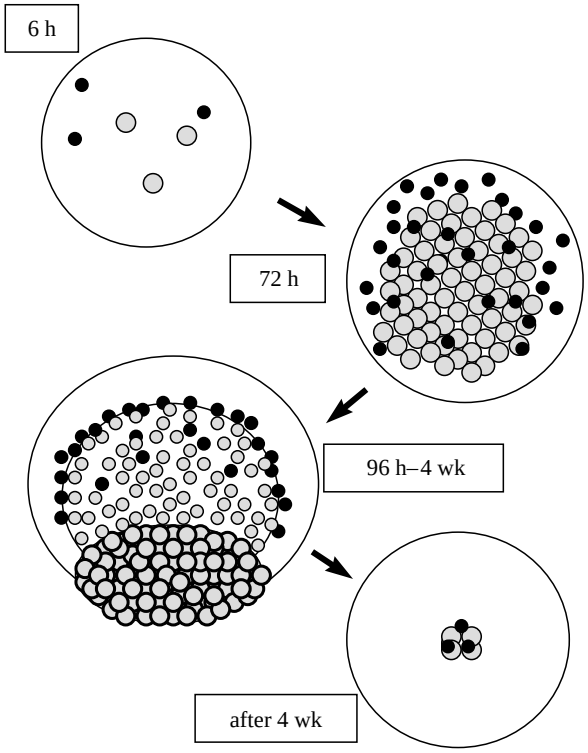

Figure 2. The phases of germinal centre reactions. The timings relate to the interval after cognate interaction in the outer T zone between primed T cells and B cells that have taken up antigen. Grey spheres represent B blasts, centroblasts and centrocytes; black spheres represent germinal centre T cells. From top to bottom the first panel shows early colonization of the follicle with T and B blasts. Panel 2 shows the position at the end of the exponential growth phase when the follicle centre is filled with B blasts while the CD4 T cells are mainly located towards the outside of the follicle. Panel 3 shows the stage when a lower dark zone is formed filled with centroblasts; the centroblasts give rise to centrocytes which enter the light zone. This stage is shown in greater detail in figure 3. Panel 4 shows the end stage after the germinal centre reaction has ceased when small numbers of B blasts and T cells can be found in the centre of some follicles.

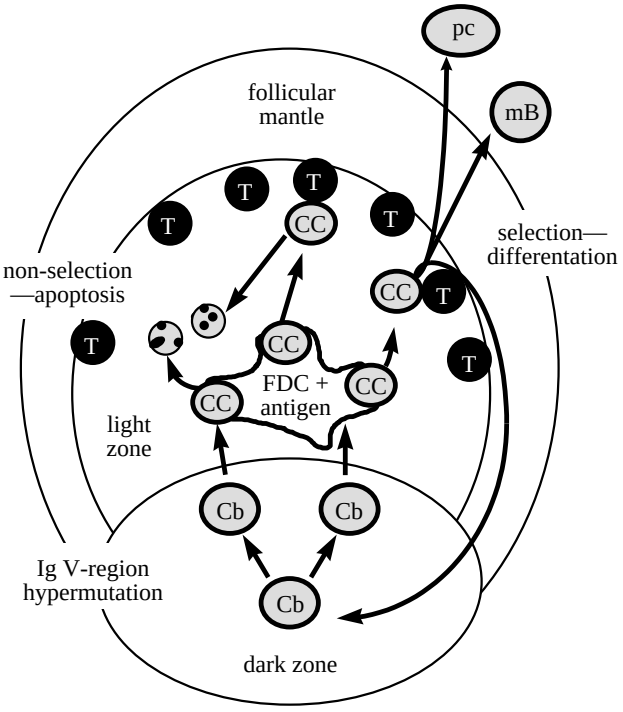

Figure 3. The relationship between different cell types in a germinal centre. Centroblasts in the dark zone proliferate and undergo somatic mutation in their Ig variable region genes. After a few divisions they give rise to centrocytes which are selected on their ability first to bind antigen held on FDC, and second to present this to and make cognate interaction with germinal centre T cells. Non-selected centrocytes die by apoptosis. Selected cells either leave the germinal centre as plasmablasts or memory B cells or they differentiate into centroblasts, returning to the dark zone of the germinal centre. This cyclical differentiation from centrocyte to centroblast is essential for maintaining the centroblast population and the germinal centre reaction.

(b) *Memory B-cell formation in germinal centres*

Small numbers of B cells seed the follicles and go through repeated rapid cell cycles increasing some 4000-fold in around three days. In doing this they fill the spaces between the network of follicular dendritic cells (FDC) (MacLennan *et al.* 1990; Slifka *et al.* 1998) (figure 2). After this period of exponential growth the cells differentiate to form a mature germinal centre. This involves the proliferating cells moving to one pole of the FDC network and becoming centroblasts.

Hypermutation mechanisms are activated in centroblasts, which act selectively on Ig variable-region genes (Jacob *et al.* 1991*b*; Neuberger *et al.*, this issue). Hypermutation can result in increased affinity of the BCR for antigen—a critical event in affinity maturation and the production of high-affinity neutralizing antibodies against viruses and toxins. Conversely hypermutation can cause loss of affinity, or altered specificity with the acquisition of autoreactivity—many pathogenic auto-antibodies have variable region mutations indicating that the cells that produce them have been through a germinal centre reaction. Not surprisingly, stringent selection mechanisms exist which act on cells that have undergone variable region gene mutation.

(c) *Selection in germinal centres*

Centroblasts continually give rise to non-proliferating centrocytes, which enter the FDC network where they are subjected to selection (figure 3). The selection is based on the ability of a centrocyte first to bind antigen held on FDC (Liu *et al.* 1989), and second to present this in a processed form to CD4 T-effector cells (MacLennan 1994), which are sited towards the outer edge of the FDC network (Casamayor-Palleja *et al.* 1995). Cells that fail to undergo positive selection die by apoptosis—the default pathway for centrocytes that do not receive selection signals. Selection eliminates those cells that have lost antigen-binding activity, or have acquired autoreactivity.

Available evidence indicates that centrocytes on selection can differentiate in three main directions—to memory cells (Klaus *et al.* 1980; Coico *et al.* 1983), to plasma cells (Dilosa *et al.* 1991; Smith *et al.* 1997) or to centroblasts (Casamayor-Palleja *et al.* 1996). The possibility that centrocytes give rise to centroblasts was suggested by Kepler & Pereslon (1993). They calculated on mathematical grounds that cells undergoing somatic mutation in

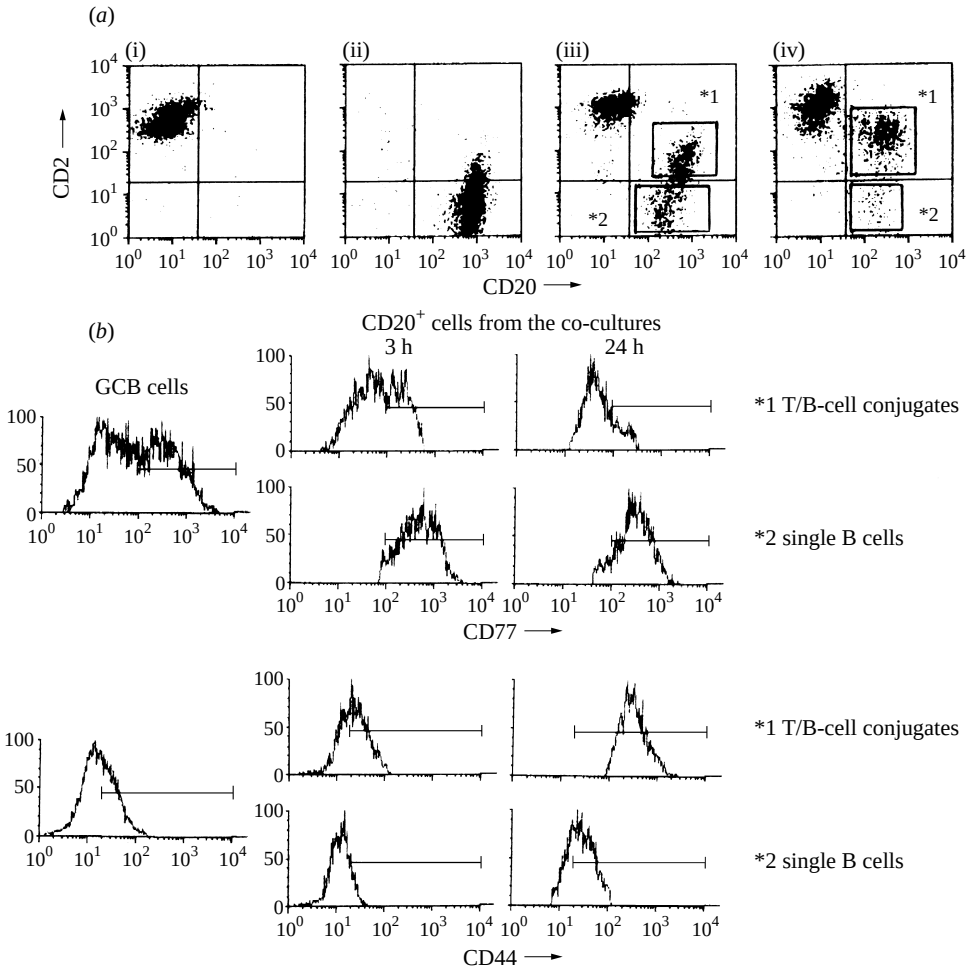

Figure 4. The formation of centroblasts and memory B cells in co-cultures between germinal centre (GC) B cells and autologous germinal centre T cells which had been induced to express CD40–ligand on their surface. (*a*) The top row shows flow cytometry plots stained for CD20 (a pan-B-cell marker) and CD2 (a pan-T-cell marker). From left to right (i) the starting germinal centre B-cell population obtained by negative selection of tonsil B cells from which $CD39^{+}$ and IgD^{+} B cells had been removed; (ii) autologous $CD45RA^{-}$, $CD8^{-}$ tonsil T cells washed after 4 h culture in phorbol dibutyrate and ionomycin to induce surface CD40–ligand expression; (iii) the germinal centre T- and B-cell populations after co-culture for 3 h, and (iv) 24 h; double-positive cells have formed T-cell–B-cell conjugates. The conjugate cells in gate *1 in the upper right quadrant and B cells not in conjugates in gate *2 in the lower right quadrant are analysed in the histograms in (*b*) for CD77 and CD44 expression, as are the starting germinal centre B cells. The B cells in the conjugates are $CD44^{high}$ and $CD77^{low}$ (features of memory B cells), while the B cells that are not in conjugates are $CD44^{-}$ and $CD77^{high}$ (features of centroblasts). Full phenotypes of these cells and details of the experiments are described by Casamayor-Palleja *et al.* (1996).

germinal centres would have to go through successive rounds of selection during germinal centre reactions to achieve the levels of mutation observed in plasma cells during T-cell-dependent antibody responses.

Both centroblasts and memory B cells are produced from centrocytes co-cultured with autologous germinal centre T cells. This occurs within the physiological time-scale of 24 h (figure 4) (Casamayor-Palleja *et al.* 1996). Although this result is dependent on CD40 ligation, CD40 ligation alone is not sufficient to induce the differentiation to either the centroblast or memory B-cell phenotype. Germinal centre cells subjected to prolonged CD40 ligation are protected from entering apoptosis, but acquire a phenotype not identified among any physiological B-cell subset (Casamayor-Palleja *et al.* 1996). Further evidence to support recycling from centroblast to centrocyte and, after, selection back to centroblast was provided by the finding that blocking CD40 ligation causes loss of established germinal centres (Han *et al.* 1995). An alternative interpretation is that blocking CD40-ligation inhibits an autocrine growth stimulus of centrocytes; CD40 ligand expression by B blasts has been described (Grammer *et al.* 1995; Wykes *et al.* 1998). Recent data (García de Vinuesa *et al.* 2000) favour the conclusion that centrocyte differentiation to centroblasts is essential if the germinal centre reaction is to be maintained. In these experiments germinal centres were established without T-cell help by unphysiologically strong BCR signalling. The germinal centres developed normally (figure 2) until the stage when centrocytes were produced; then, in the absence of T cells, the reaction ended abruptly with all B-lineage cells in the germinal centre dying within a few hours.

6. CONCLUDING REMARKS

Viral and toxin neutralization by antibody is at the forefront in providing defence against reinfection. The mechanisms that result in the production of high-affinity

antibody and the persistence of its production sustain this protection for many years, while retaining the capacity to mount fresh responses against micro-organisms that alter the molecules they use to adhere to and gain entry to cells.

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