Lec (4)

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B cell
Generation /Activation / Differentiation

B cells originate in the bone marrow of adult mammals, and their development is dependent upon the stromal cell environment.
Interaction of VLA-4 on Pro-B cell with VCAM-1 on stromal cell.

Interaction of c-Kit on Pro-B cell with stem cell factor (SCF) on stromal cell.
Signal to express IL-7 receptor, and secretion of IL-7 by stromal cell.
Detachment of Pre-B cells from stromal cells and proliferation.
Immunoglobulin (Ig) gene rearrangement begins in Pro-B cells DH to JH rearrangement begins .Productively-rearranged V-D-J gene is transcribed and translated, and H chain appears on the cell surface together with the **surrogate light chain** .Consists of the products of two genes, **λ5** and **VpreB**. They act much like the **preT**α that appears together with the TCRβ chain in T cells. The complex signals to the cell to stop rearranging the H chain locus. The cell then divides 5-6 times and then begins to rearrange the **kappa (κ) light chain locus**. If the **λ5** gene is knocked out, B cell development is blocked at the pro-B stage.

Negative selection of immature self-reactive B cells

The bone marrow (probably mouse) produces 5 x 10^7 B cells per day, 10% of which survive to join the circulating B cell pool, and the rest die of apoptosis. That negative selection occurs was shown by an experiment by Nemazee and Burki.
Transgene encoding fully-rearranged immunoglobulin specific for H-2Kk was crossed into H-2d and H-2d/k mice. The transgenic anti-H-2Kk antibody was observed both on the surface of mature B cells and in secreted form in the H-2d mice, but not in the H-2d/k mice. This suggests negative selection.
Cross-linking of IgM on immature b cells leads to apoptosis. This probably happens upon recognition of H-2Kk on stromal cells by the transgenic IgM.
A B cell recognizing a self-epitope can be saved from death by **light chain editing** caused by up-regulation of RAG-1 and RAG-2 and further rearrangement of light chain loci. If a second rearrangement occurs and the resulting IgM no longer binds self, the B cell can survive.

B cell activation
Cross-linking of surface IgM is crucial. Certain so-called **thymus-independent (TI) antigens** do so directly and appear to activate in absence of true TH cell.
•**TI-1** antigens are polyclonal activators like lipopolysaccharide (this may act through Toll-like receptors).
•**TI-2** antigens like polymeric proteins or capsular polysaccharides truly cross-link surface IgM and appear to require some cytokines that likely come from T cells. Response is weak, IgM only, and there is no memory.

Activation by conventional, non-polymeric antigens also requires cross-linking of B cell receptors. Bringing their associated Ig-α/Ig-β complexes into proximity begins a chain of reactions. There are many parallels with T cell activation.
Src family kinases Fyn, Blk and Lck associated with the plasma membrane are dephosphorylated by CD45, and they phosphorylate the cytoplasmic domains of the Ig-α/Ig-β complex.

Role of the B cell co-receptor complex

This is a complex of three proteins:
•**CD19 –** a transmembrane protein with three Ig-like domains;
•**CD21 –** a transmembrane protein that was previously referred to as **complement receptor 2 (CR2)**. It binds **C3d**, a cleavage product of complement that can be associated with antigen;
•**TAPA-1** (CD81) **–** a protein that crosses the plasma membrane four times and binds to CD21.
Interaction of the antigen associated with the co-receptor complex with surface IgM greatly amplifies the signal through the IgM complex. Makes cells as much as 100 times more sensitive to stimulation.

The CD22 protein is also associated with the B cell receptor complex on resting B cells, and its signal is negative, working against activation.
Role of TH cells in humoral (i.e., antibody) responses

TH cells are initially activated on APCs. To aid in activating B cells, they must physically interact with the B cell whose surface IgM binds to the antigen.

- The B cell binds to the antigen via its surface IgM. The antigen is internalized by receptor-mediated endocytosis, degrades the antigen via ubiquitination and the proteasome, and within 60 minutes displays peptide/class II complexes on the cell surface. It also up-regulates B7.

- The T-B conjugate form, with the TCR recognizing peptide/class II and CD28 binding B7.
- CD40L is up-regulated on the T cell, and it binds CD40 on the B cell. This interaction is typical of the TNF receptor family, and we'll see others like it in later chapters. B cells now are stimulated to express cytokine receptors.
- Cytokines secreted directionally by the cell bind to the receptors on the cells, and stimulate the B cell to progress to DNA synthesis and proliferation.

Negative selection of mature self-reactive cells in the periphery

The text describes an interesting experiment by Chris Goodnow that illustrates negative selection of self-reactive B cells in the periphery. It involves a transgene for hen egg lysozyme (HEL) whose expression.

The Humoral Immune Response

The increases in IgM and IgG antibodies seen in the serum following primary (1o) and secondary (2o) exposures to antigen. Note that the ordinate is a logarithmic scale, showing that the amounts of antibody produced in the 2o response is greatly increased over that seen following the 1o immunization. Note the steeper rise in antibody response following the 2o immunization than was seen after the initial immunization. The more rapid synthesis and greater amounts of antibody seen following the 2o immunization are largely due to the presence of large numbers of memory B cells that were generated following the initial immunization. Note also that the predominant antibody isotype produced in the 2o response is IgG due to the switch.

The role of TH cells as illustrated by responses to hapten-carrier conjugates

The important features of the **so-called carrier effect** are summarized in the following statement:

For a 2o antibody response to a hapten to occur, an animal must have experienced an effective 1o immunization to **both the hapten and the carrier to which the hapten is conjugated in the 2o immunization**. At the time of the 2o immunization, the animal must contain primed B cells specific for the hapten, and it must have primed T cells specific for the carrier to which that hapten is conjugated when presented in the 2o immunization.

The *in vivo* site of for induction of the humoral response

Antigen enter the lymph nodes by way of the afferent lymphatics, alone or in association with dendritic cells. Lymphocytes enter the lymph nodes by extravasation from high endothelial venules. Initial activation of both B and T cells appears to be in the **paracortex**in association with macrophages and interdigitating dendritic cells. Activation occurs over a period of 3-4 days following antigen entry. Most of the antibody produced in the primary response comes from plasma cells that differentiated in foci at the edge of the T-cell rich paracortex. (An analogous set of events occurs in the PALS of the spleen.)

Several days later, a small number of activated B cells and TH cells migrate to **primary follicles** in the **cortex** .The primary follicles mature to **secondary follicles** that provide an environment favorable for interactions between B cells, TH cells and **follicular dendritic cells** (**FDCs**). The latter do not arise in the bone marrow, are class II-negative and do not present antigen to the CD4+ cells. They bind antigen-antibody complexes on the long extensions of their surfaces by means of **Fc receptors**, and may do so for periods as long as months.

The FDCs release small membrane-derived particles 0.3-0.4 microns in diameter called **iccosomes**(for **immune complex-coated bodies**) that are endocytosed by B, resulting in processing and display of antigen-derived peptides on class II molecules. As both B and T cell activation continue, these cells migrate to the center of the secondary follicle where they form a **germinal center.** Germinal centers arise approximately 7-10 days after initial exposure to a thymus-dependent antigen, they reach a peak of activity during the next 4-10 days, and they disappear by 4 weeks after immunization.

Within the germinal center, B cells (called **centroblasts**) proliferate extensively and accumulate in an area termed the **dark zone** near the edge of the follicle. Centroblasts are large cells with much cytoplasm, no surface immunoglobulin and diffuse chromatin.

Centroblasts give rise to **centrocytes**– non-dividing cells containing surface immunoglobulin that move to the **light zone** of the germinal center and encounter antigen on the long extensions of FDCs

As a result of interaction with FDCs and TH cells, some centrocytes give rise to **plasma cells** that migrate to the medulla of the lymph node and secrete antibodies. Memory B cells are also generated. Antibodies and some memory B cells leave the node by way of the efferent lymphatics. However, **most centrocytes die by apoptosis** and are phagocytosed by so-called **tingible body macrophages**. The next question is:

Affinity maturation

Most centrocytes die because of a process of **somatic hypermutation**which alters the structure of the immunoglobulin that they express. That immunoglobulin was able to bind antigen and enabled the cell to be activated. However, now the rearranged genes encoding that immunoglobulin are mutated by a poorly-understoodmechanism, and a stringent test of binding affinity is applied. Some mutations increase the affinity of the antibody, and cells with such mutations survive. Other mutations decrease the affinity of binding, and these cells die. Only the strongest binders survive.

It appear that as centroblasts proliferate in the dark zone, point mutations, insertions and deletions occur in rearranged H and L chain genes. Mutations can be demonstrated from a region 0.5 kilobases 5’ to the V(D)J coding region to 1.5 kilobases 3’ to the coding region. In the light zone, the centrocytes expressing the now-mutated genes are selected for high affinity, and the failures die by apoptosis. The rate of mutation is such that almost every centroblast should accumulate a mutation in the H or L chain every two divisions. This is a very high frequency (10^{-3}/cell/generation), and this high rate and precise targeting to a chromosomal region is unique to the immune system.

The immunoglobulin class switch

The immunoglobulin class switch, involving homologous recombination of the switch regions preceding heavy chain constant region genes, occurs in the germinal centers **under the influence of cytokines secreted from TH cells**. Defects in CD40L lead to problems of B cell activation and failure to class switch. In individuals with the **X-linked hyper-IgM syndrome**, an immunodeficiency disorder in which TH cells do not express CD40L, IgM is produced but not the other isotypes. Patients do not form germinal centers or memory B cells, and immunoglobulin chains do not undergo somatic hypermutation.

Generation of plasma cells and memory cells

Centrocytes that survive selection and therefore produce high affinity antibody give rise to **plasma cells** that secrete antibody and **memory cells** that persist I the body and are responsible for the stronger and more rapid antibody responses seen upon a secondary encounter with antigen. With the exception of lower IgD levels and the expression of IgG, IgA or IgEisotypes, there are no good surface markers to distinguish memory B cells from naïve B cells.