LECTURE: 12
Title: B-LYMPHOCYTES SURFACE RECEPTORS AND THEIR ACTIVATION

LEARNING OBJECTIVES:

The student should be able to:

- Identify the percentage of B-lymphocytes in comparison to other circulating lymphoid cells.
- Distinguish B-lymphocytes in regards to their cell surface receptors.
- Identify the definitive B-cell surface markers.
- Determine to which type of immune receptor families this B-cell definitive marker belongs?
- Identify the surface immunoglobulin isotypes (antigen-receptors).
- Describe the B-cell receptor complex (BCR).
- Enumerate some B-cell markers, and indicate their importance in immunity.
- Explain the reason that, CD5⁺ B-lymphocytes are considered as a distinctive cell subset.
- Indicate which B-cell marker(s), enhance B-cell phagocytosis.
- Enumerate some of the B-cell receptors which are important in B-cell-cooperations.
- Explain the serological technique used to visualize the immunoglobulins in the cytoplasm of the plasma cell.
- Describe the mechanism of signal transduction is performed by BCRs.

LECTURE REFERENCE:


2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter. 7 .pg 129-151.

B-LYMPHOCYTE SURFACE RECEPTORS AND B CELL ACTIVATION

INTRODUCTION

B cells are characterized by their surface immunoglobulins. They represent about 5-15% of the circulating lymphoid pool, and are classically defined by the presence of surface immunoglobulins. These immunoglobulin markers are made by the B cells themselves, and are inserted into the surface membrane where they act as specific antigen receptors.

Generally lymphocytes represent about 20% of blood leukocytes. The peripheral blood B cells represent approximately 10-15% comparing to peripheral blood T-cells (70-80%), and natural killer cells (10-15%). In bone marrow B cells represent about 80-90% comparing to T-cells and NK-cell which each both represent about 5-10%. In the thymus the majority of lymphocytes are the T-cells which represent about 99%, while each of the B-cells and NK-cells represents about <1%. Also in the lymph node T cells represent about 70-80% of the lymphocytes, while B cells represent about 20-30%, and NK cells percentage is less than 1% (NK=-cells represent <1% of lymphocytes). Finally in the spleen slight increase in the percentage of the B cells (50-60%) comparing to the T-cells percentage which is around 30-40%, and the NK-cells represent about 1-5% (Table-1).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>T-lymphocytes</th>
<th>B-lymphocytes</th>
<th>Natural killer cells (NK-cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood</td>
<td>70-80 %</td>
<td>10-15 %</td>
<td>10-15 %</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>5-10 %</td>
<td>80-90 %</td>
<td>5-10 %</td>
</tr>
<tr>
<td>Thymus</td>
<td>99 %</td>
<td>&lt;1 %</td>
<td>&lt;1 %</td>
</tr>
<tr>
<td>Lymph node</td>
<td>70-80 %</td>
<td>20-30 %</td>
<td>&lt;1 %</td>
</tr>
<tr>
<td>Spleen</td>
<td>30-40 %</td>
<td>50-60 %</td>
<td>1-5 %</td>
</tr>
</tbody>
</table>

Table-1: Proportions of lymphoid cells types in normal human tissues, includes cells at all recognizable stages of development in each lineage.

Definitive b-cell surface markers

B-lymphocytes posses distinguished cell surface receptor that is the exhibition of integral surface immunoglobulin. Most of the peripheral blood B cells express two immunoglobulin isotypes on their surfaces IgM and IgD. Very few cells in the circulation express IgG, IgA, or IgE. But these cells bearing immunoglobulins are found in large quantity in specific location, for example; B lymphocytes bearing IgA are found in large quantity in intestinal mucosa. The B-lymphocyte complex is formed by the association of the surface immunoglobulins with other molecules on the B cell surface as well (Figure-1, 2). These molecules are disulphide-bonded heterodimer of:

A. Igα (CD79a, a 34 kDa molecule and a product of the mb-1 gene)
B. Igβ (CD 79b, a 39 kDa molecule and product of the B29 gene)
Figure-1 B cell antigen receptor

Figure-2 Schematic representation of Ig on a cell membrane
OTHER B CELL (BCR) RECEPTORS

1. MHC class-II antigens, (which is important in the cooperative interaction with T cells). The class II molecules consist of HLA- DP, DQ, and DR antigens.

2. Complement receptors for C3b (CR1, CD35), and C3d (CR2, CD21)

3. Fc receptors for IgG (Fcγ RII, CD32)

4. The main markers currently used to identify human B cell are CD19, CD20, and CD22.

5. CD 72-78 is markers found in both human and murine

6. CD40 is an important marker on B cells and is involved in the cognate interaction between T and B cells.

7. CD5 is usually present on the T cells but it was detected on one set of the B cells as well. B cells expressing CD5 is called B1a cell, where the conventional B cell is called B2 cells. Some B cells bind to (and form a rosette with) mouse erythrocytes (ME-R).

ACTIVATION MARKERS ON LYMPHOCYTES

Activation of T and B cells triggers the expression of certain surface molecules and also enhances the expression of others, this activation occurs in different stages (Figure-3). The activation markers include:

B-lymphocytes activation markers:

1. IL-2R (high affinity IL-2R).
2. The IL-3, IL-4, IL-5, and IL-6 receptors for growth and differentiation factors.
3. CD71 a transferrin receptor.
4. Elevated level of class II MHC molecules.
5. CD23 (FcεRII, a low-affinity IgE receptor) is present on activated B cells and is involved in driving B cells into proliferation.
6. CD38 is not found on mature B cells is found on terminally differentiated plasma cells (as well as in the very early stages of B-cell development).
7. PCA-1 molecules are found at the plasma cell stage of human B-cell differentiation.
8. Memory cells within the germinal centers of secondary follicles do not express surface IgD or CD22.
DIFFERENT PHASES OF B CELL RESPONSES IN B-CELL ACIVATION

Resting T lymphocytes are essentially inert until they have been activated by antigens or mitogens, when they develop into blast cells, proliferate, and become able to carry out their specific functions, e.g., help B cell responses, activate macrophages, or kill target cells. Roughly speaking, the same principles apply to B lymphocytes. Resting B cells are also immunologically inert, but when they are activated by antigens or polyclonal B cell activators, they can develop into blast cells, proliferate, and secrete antibodies which mediate their effector function.

Figure-3 Terminology for stages of B cell responses
B cell responses therefore can be divided into several stages, activation, proliferation, and antibody secretion. These antigen-dependent events are entirely unrelated to the antigen-independent development of mature resting B cells from their precursors, and correspond to different stages of the cell cycle (Figure-4).

1. The term activation can be used to describe the earliest events preceding B cell proliferation and/or Ig secretion. This corresponds to progression of the cell from G₀ into G₁ of the cell cycle, when small resting B cells develop into B lymphoblasts (blastogenesis).

2. Proliferation can be divided into DNA synthesis or replication (S phase), and mitosis (M phase) or growth, which may or may not include DNA synthesis depending on the assay. A cell undergoing several cycles of proliferation is sometimes called a "cycling cell".

3. Antibody secretion is often referred to as "terminal differentiation" of the B cell, or sometimes as "differentiation". This can be divided into the induction phase, when class switching occurs and a new class of membrane Ig is expressed by the cell, and maturation in which the soluble form of that Ig is secreted.

Resting B cells must be activated before they can proliferate and secrete Ig. However, B cells can proliferate without secreting antibodies, and proliferation and Ig secretion can be mutually exclusive, at least within the same cell cycle. Class switching seems to depend upon proliferation having occurred, but whether a B cell can secrete antibodies without first proliferating is controversial.

Figure-4 The cell cycle
Cytokines and other stimuli

As general rule, it is thought that when B cells are triggered by certain stimuli, they start to express membrane receptors for particular cytokines. These soluble molecules produced by T cells, accessory cells, and perhaps in some cases by B cells. When a particular cytokine bind to its specific receptor, the B cell is thought to progress to another point in the cell cycle (e.g., it may become a cycling cell) and then begins to express receptors for other cytokines. These receptors can mediate a different set of cellular responses (e.g., class switching may occur and the cell might begin to secrete Ig). In this way it is envisaged that there must be several control points for B cell responses. One difficulty has been to define these points precisely and to determine the precise effects of any particular cytokine on the B cell.

B-LYMPHOCYTES ACTIVATION

Stages in B cell activation

The G₀ and G₁ phases can be divided into several "Subcompartments" of the cell cycle (Figure-5). Truly resting B cells are sometimes said to be in the most senescent form of G₀ called G₀Q. When a resting B cell is activated from G₀ into the G₁ phase of the cell cycle, a number of changes occur. These include a dramatic increase in cell size (i.e. it becomes a lymphoblast), increased RNA synthesis, and the expression of a number of new molecules such as membrane receptors for cytokines that are required for subsequent proliferation and/or Ig secretion by the B cell.

**Figure-5 Subcompartment of G₀ and G₁ for B cells**

Within a few hours of leaving G₀Q, the cell begins to express new antigens, such as CD23 (the Fcε RI receptor) and the cell is said to be in the G₀A phase. Sixteen hours or so later, the G₀B phase, the chromatin
in the nucleus decondenses, i.e., it becomes looser in structure, presumably to allow new gene transcription. Subsequent entry to G\textsubscript{1} is marked by the loss of membrane IgD and greatly increased expression of MHC class II molecules, and the cell is said to be in G\textsubscript{1A} phase of the cycle. In later G\textsubscript{1}, G\textsubscript{1B}, the cells express transferrin receptors which mediate the uptake of transferrin bound iron that is required in part for subsequent DNA synthesis. Some of these stages can also be defined by the expression of other surface antigens identified by monoclonal antibodies. It is important to note that different stimuli may cause the B cell to progress to different points of G\textsubscript{0} and G\textsubscript{1}, and in many cases these points have not yet been well defined.

**Activation of B cells via membrane-bound Ig molecules**

B cells can be activated by a variety of routes. Under physiological circumstances, B cells are thought to become activated after they recognize specific antigen via their membrane-bound Ig molecules. Legation of these delivers an activation signal to the B cell and blastogenesis is initiated. It is possible to activate B cells experimentally by this route by using anti-Ig or anti-idiotypic antibodies to cross-link membrane-bound Ig. Of course, anti-Ig will activate B cells polyclonally, while anti-idiotypes only activate specific clones of B cells. Anti-IgM is most commonly used to activate B cells experimentally. At low concentrations, soluble IgM causes the cells to become responsive to certain cytokines, while at higher concentrations some IgM antibodies alone can stimulate B cell proliferation. Thus, the degree to which the B cells are activated, and their progress through the cell cycle depends on the concentration of anti-IgM, as well as the particular determinants that are recognized, and it also makes a difference whether soluble or immobilized anti-IgM is used. B cells can also be activated via membrane-bound Ig by using the bacterium staphylococcus aureus Cowan 1 strain (SAC), which can bind to the Fe and Fab portion of antibodies.

**Mechanism of B cell activation via membrane-bound Ig**

Intact antibodies against membrane-bound Ig on B cells, or their F(ab')\textunderscore 2 fragments, stimulate phosphatidylinositol turnover in the cell, and lead to a rapid increase in the concentration of intracellular free Ca\textsuperscript{2+} ions, and activation of protein kinase C. These events are very similar to those following legation of T cell receptors. Depending on the particular specificity of the anti-Ig antibody, and whether or not it is immobilized on beads, both anti-μ and anti-δ chain antibodies can be effective (a soluble beads). In contrast, monomeric Fab fragments of these antibodies do not stimulate these intracellular events, suggesting that some degree of receptor cross-linking is important.

**Other routes for B cell activation**

There are other ways of activating B cells, independently of their membrane Ig molecules. Cognate interactions with T cells can also result in B cell activation, and it seems likely that MHC class II molecules mediate delivery of a signal to the B cell when they are recognized, in association with a foreign peptide, by T cell receptors. This is accompanied by a number of intracellular changes, associated with lymphocyte activation, which can also lead to proliferation of primed B cells. Resting B cells also express receptors for IL-4, and when this cytokine binds to the cell it initiates activation. None of these stimuli triggers the intracellular events outlined above of B cell activation via membrane-bound Ig, and how they act is something of a mystery at present (although LPS may be able to activate protein kinase C directly). A number of other membrane molecules on B cells are also involved in signaling events and B cell activation. It seems most likely that LFA-1, and its ligand ICAM-1, play an important role in interactions between B cells and other B cells, and between B cells and different cell types such as T cells. In addition, some of these interactions may be required for the capacity of B cell blasts to activate resting T cells (immunostimulation). Activation of T cells by this route may involve binding of the B cell molecule B7/BB1 to CD28 or CTLA-4 on T cells, as well as binding of CD72 (Lyb2) to its ligand CD5 (mouse Ly1) on T cells. Monoclonal antibodies specific for CD72 can augment antibody responses to TD antigens as well as B cell proliferation.

One molecule that appears to be coordinately regulated with LFA-1, and to have a role in B cell activation, is CD22; another important molecule in B cell activation is the complement receptor type 2, CR2 (CD21),
which associates with CD19 complex. Activation signals can also be delivered via the CD40 molecule, a glycoprotein with homology to the verve growth factor receptor and TNF receptors.

Dr. MUSATAF HASAN LINJAWI