LECTURE: 23

Title **T-AND B-LYMPHOCYTES COOPERATIONS**

LEARNING OBJECTIVES:

The student should be able to:

- **Enumerate the major types of T-helper cells surface molecules, and that expressed on the B-lymphocytes which have direct interactions.**
- **Explain the function of each receptor and it cognate molecule on the other cell.**
- **Enumerate generally the different types of cytokines produced by both cells.**
- **Explain the action of each cytokine.**
- **Determine which type of B or T-lymphocyte activated first.**
- **Enumerate the different types of T-cells that activate the B-lymphocytes in different ways.**
- **Determine B-T interaction either activate or inactivate both cells.**
- **Determine the different types of cytokines involve in:**

- **B-lymphocytes activation.**
- **B-lymphocytes division.**
- **B-lymphocytes differentiation.**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 8. pg. 131-141.

2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter 8. pg 163-188. Chapter 9 .pg 189-214.

3. TEXTBOOK:LAUREN SOMPAYRAC. PP 32-34.

T AND B LYMPHOCYTES COOPERATIONS

Lymphocytes

Activation of both T, and B lymphocytes leads to two partially competing processes: cell proliferation, and differentiation, such as plasma cells, may become so specialized that they lose surface molecules such as class II and become unable to respond to regulatory signals or to proliferate. The fate of lymphocytes responding to antigen is varied. Some can persist for a long time as memory cells. The lifespan of memory cells can be over 40 years in man. Others have shorter lifespan that is nevertheless sufficient for generating effective cell-mediated and antibody responses.

Antigen-specific activation of lymphocytes involves the specific receptors on T and B cells

The TCR complex can transmit messages to the interior of the cell. Molecules involved are chains, and the enzyme p56lck (is a lymphocyte-specific kinase of 56kDa) that is attached to the intracellular portion of CD4 and CD8. B cells are also recognized to have a family of molecules that attached to the surface IgM and IgD, and that are o involved in signal transduction (**mentioned in the previous lecture**). To stimulate a T-cell hybrid an effective APC (e.g., macrophage) must carry over 60 MHC class II-antigen complexes. A weak APC, such as a class II transfected fibroblast, needs 5000 such complexes. This would suggest that, in normal circumstances, a few tens to a few hundred TCR need to be activated for effective triggering. This type of information is not available for Tdep antigens and B cells. For T_{ind} antigens, binding to a single receptor are not sufficient for triggering, but binding to tens or hundreds probably is.

T and B cells can be mitogens, a class of molecule that can activate lymphocytes in non-antigen-specific manner. The mitogens molecules such as phytohaemagglutinin (PHA) and concanavalin A (Con A) activate the T cells by binding to T cell surface molecules TCR and CD2. Superantigens are another group of antigen which activates T cells non-specifically. Most are of bacterial origin, and they include the staphylococcal enterotoxin, toxic shock syndrome toxin. These Superantigens bind to MHC class II on APCs and are recognized by TCRs, but not in the same way that MHC class II-antigen complexes are recognized. They bind only to the V beta chain of the TCR, but this sufficient to activate the T cells.

The cooperation between T and B cells is well organized. T cells are activated when they recognize antigen presented to them by an antigen presenting cell. Th cells interact with B cells that present antigen fragments to them. Activated B cells proliferate and differentiate into antibody-forming cell. Antibody is produced and various immune responses follow.

ANTIGEN PRESENTATION TO T AND B CELLS

Antigen processing

Antigen processing refers to the degradation of antigen into peptide fragments (< 1% of antigen are involved in the immune response, the rest are degraded and excreted), which become bound to MHC I or MHC II molecules. These are the critical fragments involved in the triggering of T cells, whose receptors recognize **sequences** of amino acids in peptides in the MHC groove**,** rather than the protein **shape**, **('conformational determinants), which is recognized by B cell immunoglobulins**. A store of antigen complexed with antibody and complement is maintained in the **germinal centers of lymph nodes**, adherent to **follicular dendritic** cells, and **is used** to **trigger B cells**.

Interaction with antigen-presenting cells is essential for T-cell activation

The interactions between the T cells and the antigen-presenting cells (APCs) are the first interactions occurs after the antigen challenge (**B cells is one type of the several kinds of the APCs**), and its outcome largely dictates the subsequent course of events: if a sufficient number of $CD4^+$ T-helper (TH) cells are triggered, then the activation of B cells or the development of delayed hypersensitivity almost certainly follows. If TH cells are not triggered, or are subject to a form of immunological tolerance known as 'clonal energy', then no other immunological events follow.

HOW B CELLS ARE ACTIVATED

In order for B cells to produce antibodies, they must first be "activated". Roughly speaking, there are two ways this can be accomplished. One way definitely depends on T cell help. The other is called T cell independent activation, but it is not clear exactly how independent of Tcell help this activation pathway really is. When we talk about activation here we are talking about activation of B cells that have never before encountered antigen. This kind of B cell is usually called a naïve or virgin B cell. The rules for activating virgin B cells and for re-activating experienced B cells (those that have already encountered antigen) are somewhat different. For now we'll focus on virgin B cells that are recognizing their cognate antigens for the very first time.

The molecules involved in the interaction between T cells and APCs.

B-T CELL INTERACTION

Interaction of B cells and T cells also involves multiple surface molecules

The interaction between B and T cells is a two way process, in which **B** cells **present** antigen to **T** cells, and also **receive signals** from the **T** cells for **division** and **differentiation**. The **central** interaction is that between the **MHC class II antigen complex** and the **TCR**; it is augmented by interactions between **LFA-3** and **CD2**, and ICAM-**1** and **LFA-1.** Other cell-surface molecules are also involved in interactions. The **B7-1** and **B7-2** surface antigens on **B cell**s interact with **CD28** which caused **stabilization** of **mRNA** for **IL-2** and other cytokines in the **T cells** and thereby prolongs the **delivery** of the **activation signals. CD5** on **T cells** binds **CD72** on the **B cell surface,** further **promoting** the **interaction** between the cells. **CD40** delivers the **most potent activating signals** to **B cells**, even more potent than signals transmitted via immunoglobulin. Activated T cells transiently express a ligand that interacts with CD40. IL-1 and IL-6 released by some B cell enhance expression of IL-2 receptor on T cells.

Different T-cell subsets activate B cells in different ways

$CD4^+$ T cell can be divided into different subsets depending on their cytokine profile:

1. **$CD4^+$ T cells** that produce **IL-2** and **IFN gamma but not** IL-**4** are designated **TH1** and are responsible for delayed type hypersensitivity responses. They help B cells to produce IgG2, but not much IgG1 or IgE in the mouse.
2. **$CD4^+$ T cells** that produce **IL-4** and **IL-5**, **but not** IL-**2 or IFN gamma** are designated **TH2.** They are very efficient helper cells for antibody production, especially of IgG1 and IgE.
3. Many T cells, especially in human are **intermediate** in their cytokine profile and are known as **TH0**.

T cell cytokine secretion and action is important for B cell activation

During the T-B cell interaction, T cells secrete a number of cytokines that have a powerful effect on B cells. These include IL-2, a proliferation inducer for B cells as well as for T cells, IL-4 which acts early in B cell activation of proliferation, IL-5 which in mouse but not in man is a powerful cell activator, and IL-6 which

is a strong signal for B cell differentiation. T cells also produce TNF alpha and TNF beta. These molecules have also been reported to be important for B cell growth.

B cells are activated by antigen on APCs such as macrophages, in the presence of IL-4 and IL-1. This causes expression of receptors for IL-2 and other cytokines. IL-2, IL-4, and IL-5 (in the mouse) drive cell division. Only one cycle of cell division is illustrated although many cycles will usually occur. Differentiation into antibody-forming cells (AFCs) is effected by IL-4, IL-5 (in mouse), IL-6, and IL-10 and IFN gamma.

B-T interaction may either activate or inactivate (energize)

APC-T cell interaction may yield two opposing results, activation and inactivation (clonal anergy) of T cells. In the same way, B cells frequently become anergic. This is important because affinity maturation of B cells during the immune response, due to rapid mutation in the genes encoding the antibody variable regions, could easily result in high-affinity autoantibodies. Clonal anergy in the periphery is important device for silencing these potentially damaging clones.

T-independent antigens do not require T-cell help to stimulate B cells.

The immune response to most antigens depends on both T and B cells recognizing that antigen. These types of antigens are called T-dependent (T_{dep}). There are some antigens capable of stimulating B cell without T cell help, referred to as T-independent antigens (T_{ind}). T_{ind} antigens have a number of properties in common. **All are large polymeric molecules with repeating antigenic determinants.** Many have the ability when they are in high concentration to activate B-cell clones that are specific for other antigens, a phenomenon known as polyclonal B cell activation; however, at low concentrations they only activate B cells specific for themselves. Many T_{ind} antigens are particularly resistant to degradation.

Primary antibody responses to T_{ind} antigens in vitro are generally slightly weaker than those to T_{dep} antigens; they peak fractionally earlier and generate mainly IgM. However, the secondary responses to T_{dep} and T_{ind} antigens differ greatly. The secondary response to T_{ind} antigens is very similar to the primary response; the secondary response to T_{dep} antigens is far stronger than the primary, and has a larger IgG component.

The secondary response to T_{dep} antigens is stronger and induces a greater number of IgG-producing cells.

It seems there fore that T_{ind} antigens do not induce the maturation of response seen with T_{dep} antigens that leads to class switching to IgG and increase in antibody affinity. Memory induction to T_{ind} antigens is also relatively poor.

The mechanism by which T_{ind} antigens trigger B cells without requiring T_H cells is not fully understood. It is likely that their polymeric structure enables T_{ind} antigens to cross-link B-cell receptors; this process would be facilitated by their resistance to degradation.

Many T_{ind} antigens are products of bacteria, for example, the endotoxin, dextrans, and levans found in bacterial cell walls, and the polymerized flagellin found in flagella. There are potential survival advantages for an organism whose immune response to bacteria does not depend on complex cell interactions and may therefore be more rapid. Many bacterial antigens bypass T-cell help because they are able to induce the production of cytokines IL-1 and IL-6 and TNF alpha by macrophages. The short-lived response and lack of IgG may be due to the lack of IL-2, IL-4 and IL-5, normally produced by T cells in response to T_{dep} antigen. T_{ind} antigens often activate a subset of B cells expressing CD5.

T cells and B cells recognize different parts of antigens

Antigen enter the body is processed by cells that present the antigen in a highly immunogenic form to the TH cells and B cells. The T cells recognize determinants on the antigen different to those recognized by B cells. The T cells deliver help to appropriate B cells, which are stimulated to differentiate and divide into antibody-forming cells. Thus the two processes are required to activate a B cell:

1. Antigen interacting with B cell Ig receptors.

2. Stimulating signal (s) from TH cells.

T cell stimuli are needed for optimal growth and differentiation of B cells (**figure**).

T CELL-DEPENDENT ACTIVATION

Co-stimulating signals are needed for activation

Interaction at the TCR or membrane immunoglobulin alone cannot mediate a positive activation signal for T or B cells. A number of interactions, each with a potential signaling function are involved in the cellular activation. These interactions (co-stimulatory signals) do not involve antigen-specific receptors. They may involve either **secreted molecules**, such as **cytokines,** or **cell-surface molecules**, which increase binding affinity and are collectively known as **"adhesion molecules"** (not only involve in binding, but also in signaling by their cytoplasmic domain). Cytokines such as IL-1 and IL-6, made by certain APCs, are co-stimulatory signals for T cell activation. It is likely there are other co-stimulatory cytokines. For example; IL-7 is more potent than IL-1 or IL-6 in inducing T cells to express IL-2 receptor, but it is not known whether IL-7 is produced by any APCs.

T cell-dependent activation of a naïve B cell requires two signals. The first is specific: recognition of cognate antigen by BCRs on the surface of the B cell, and the clustering of these BCRs and their associated signaling molecules. This clustering sends the "receptors engaged" message to the nucleus of the cell. However, this signal alone is not enough to activate the B cell, a second signal is required. Immunologists call this the "co-stimulatory" signal, and it is usually provided by a helper T cell (Th cell). This is where the T cell-dependent part comes in. the most important co-stimulatory signal involves direct cell-cell contact between the B cell and the helper T cell. On the surfaces of activated Th cells are proteins called **CD40L**. When **CD40L** plugs into (ligates) a protein called **CD40** on the surface of the B cell, a co-stimulatory signal is sent (**Figure-1)**.

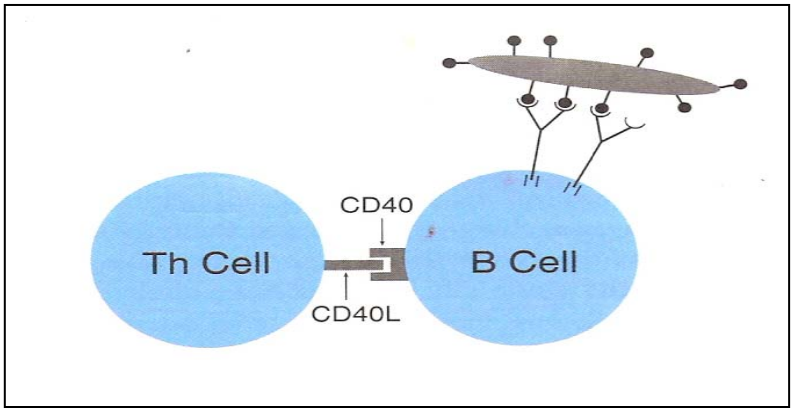


Figure-1 T AND B CELL CO-OPERATION CD40 AND CD40L

The interaction between these proteins, **CD40** and **CD40L**, is clearly very important for B cell activation; humans who have a genetic defect in either of these proteins are unable to make a T cell-dependent antibody response. Up until now, we have been talking about virgin B cells, but I need to say a few words about the activation requirements for experienced B cells. Once a B cell has been activated, it remembers that experience, and when it recognizes its cognate antigen again, the requirements for re-activation are less stringent than for the initial activation. How this memory works is not clear. The latest thinking is that re-activation definitely requires recognition of cognate antigen, but that in at least some cases, physical contact between B and Th cells is not necessary.

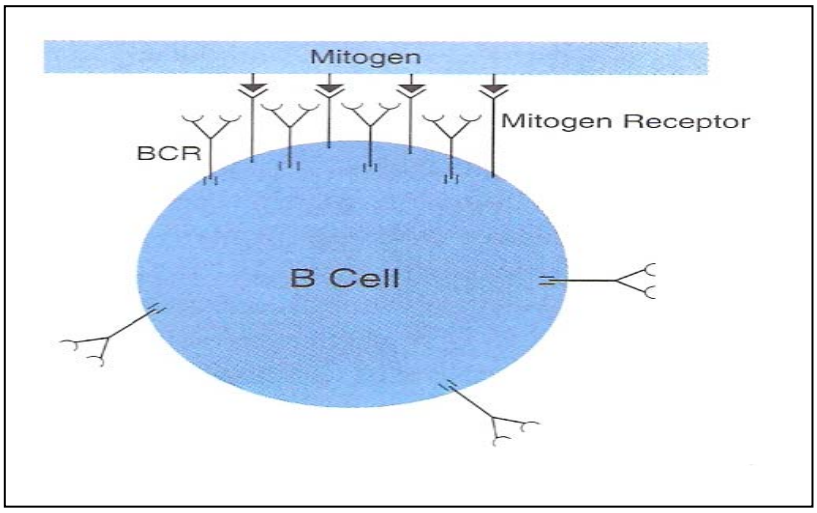
Now why would you want to have a system in which it is difficult to activate a B cell, and relatively easier to re-activate it? Well, one of places you find a lot of experienced B cells is in the collection of memory cells that persists after your first exposure to an invader (e.g., the smallpox virus). These are "legit" B cells that

have been through the stringent two-key selection for primary activation, and as a result are likely to be useful for protection against a second attack. In fact, these are the very B cells that you would like to activate quickly if you are attacked again. So making it easier for them to be re-activated makes perfect sense.

When B cells have been activated, they express new proteins on their surfaces. One of these is the receptor for IL-2, a growth factor that stimulates B cells to proliferate. So activation of B cells makes them able to receive cytokine signals that trigger proliferation. This coupling of activation to proliferation forms the basis for clonal selection, only those B cells that have recognized their cognate antigen and have been activated (the selection part) will react to growth factors, proliferate, and , and form a clone of B cells with identical BCRs. The major supplier of growth factors like IL-2 is the helper T cell; another reason T cell help is usually needed for B cell activation and proliferation.

T CELL INDEPENDENT ACTIVATION OF B CELLS

In response to certain antigens B cells can be activated with little or no T cell help. What these antigens have in common is that they are able to crosslink a ton of B cell receptors. In fact, clustering such a large number of BCRs appears to substitute for co-stimulation by CD40L. There are roughly two kinds of antigens that can do this. The first is an antigen that has repeated epitopes. A good example of this kind of antigen is a carbohydrate molecule of the type found on the surface of many bacterial cells. A carbohydrate molecule is made up of many repeating units, which if recognized by the BCRs together and trigger activation. Of course, this type of activation is antigen specific: only those B cells whose receptors recognize the repeated epitope will be activated. There is another quite different way that B cells can be activated, independent of Tc ell help. In this case the antigen, usually called a "mitogen", binds to molecules on the B cell surface and clusters these molecules, dragging the BCRs along (**Figrue-2)**.



Figrue-2 T CELL INDEPENDENT ACTIVATION OF B CELL

This "polyclonal activation" is independent of the specificity of the BCR; the BCR just comes along for the ride. In this way, many different B cells with many different specificities can be activated by a single mitogen. Two keys are required to activate the adaptive immune system. Just as with a safe deposit box, one of these keys is specific. For B cells, this specific key is the crosslinking of B cell receptors. The second key required for activation is nonspecific in that the same key works for all B cells. In the case of T cell-dependent activation, this key takes the form of the CD40L co-stimulatory molecule that plugs into the CD40 protein o n the B cell surface. By making this "two key rule", a fail safe mechanism of activation is established in which the decision to activate is made by a "committee", not by just one cell. But what about T cell-independent activation of B cells? Doesn't that violate the two-key rule for activation? We talked about crosslinking of the B cell receptors either by a specific antigen or by a mitogen, but where is the second key? This missing second key has bothered immunologists for some time, because it just seemed too

dangerous for B cells to be activated simply by recognizing a target with repeated epitopes. So it was a great relief when it was recently discovered that T cell-independent activation really does require tow keys!

In careful experiments, immunologists have now shown that when a B cell's receptors are crosslinked, the B cell begins to proliferate. However, after it proliferates, the B cell won't secret any antibodies, not unless it receives a second signal. And what is this second key? For T cell-independent activation, the second key is a battle signal like the cytokine IFN-γ. What this means is that if a B cell recognizes a molecule with repeated epitopes like, for example, your own DNA, it may proliferate, but fortunately, no anti-DNA antibodies will be produced, because your immune system is not engaged in a battle either your own DNA.

On the other hand, if the innate immune system is battling a bacterial invasion and a B cell recognizes a carbohydrate antigen with repeated epitopes on the surface of a bacterial invader that B cell will produce antibodies, because battle signals generated by the innate response will function as the second signal needed for complete B cell activation. So in response to T-cell independent antigens, B cells can take their cue directly from the innate immune system and jump right into the ballet without having to wait for T cells to be activated. But there is something even more important going on here. Since T cells only recognize protein antigens, if all B cell activation required T cell help, the entire adaptive immune system would be focused only on proteins. This wouldn't be so great, since many of the most common invaders have carbohydrates or fats on their surfaces that are not found on the surfaces of human cells. Because these carbohydrates and fats are unique to invaders, they would make excellent targets for recognition by antibodies. So by allowing some antigens to activate B cells without T cell help, Allah increases the universe of antigens that the adaptive immune system can react against to include not only proteins, but carbohydrates and fats as well.

ACTIVATED B CELLS

The third professional APC is the activated B cell. A virgin B cell is not much good at antigen presentation, because it expresses only low levels of MHC II and little or no B7. However, once a B cell has been activated, the levels of MHC II and B7 on its surface increase dramatically. As a result, an experienced B cell is able to act as an antigen presenting cell for Th cells. The current thinking is that B cells are not used as APCs during the initial stages of in infection, because at that time they are still naïve—they haven't been activated. However, later in the course of the infection or during subsequent infections, presentation of antigen by experienced B cells is thought to play an important role. Indeed, **B cells have one great advantage over the other APCs – B cells can concentrate antigen for presentation**. Here is how this work:

When B cell receptor binds to its cognate antigen, the whole complex or BCR and antigen is removed from the surface and taken into the cell. Once inside the cell, the antigen is processed, bound to MHC II molecules, and transported to the cell surface for presentation.

Because BCRs have a high affinity for antigen, the B cell acts like a magnet, collecting antigen for presentation to Th cells. Because a minimum number of T cell receptor must bind to antigen for a Th cell to be activated, it is estimated that B cells have a 100-10,000 fold advantage over other APCs in presenting antigen when there is relatively little of it around. So the first time an invader is encountered, the B cells are **all virgin**, and the important APCs are dendritic cells and macrophages. If this same invader is encountered again, however, **the experienced, memory B cells become very important APCs, because they can get the adaptive immune response cranked up quickly by concentration small amounts of antigen for presentation**. Finally the cooperation between T and B cells is well organized. T cells are activated when they recognize antigen presented to them by an antigen presenting cell. Th cells interact with B cells that

present antigen fragments to them. Activated B cells proliferate and differentiate into antibody-forming cell. **Antibody is produced** (**Figure-3-4**) and various immune responses follow.

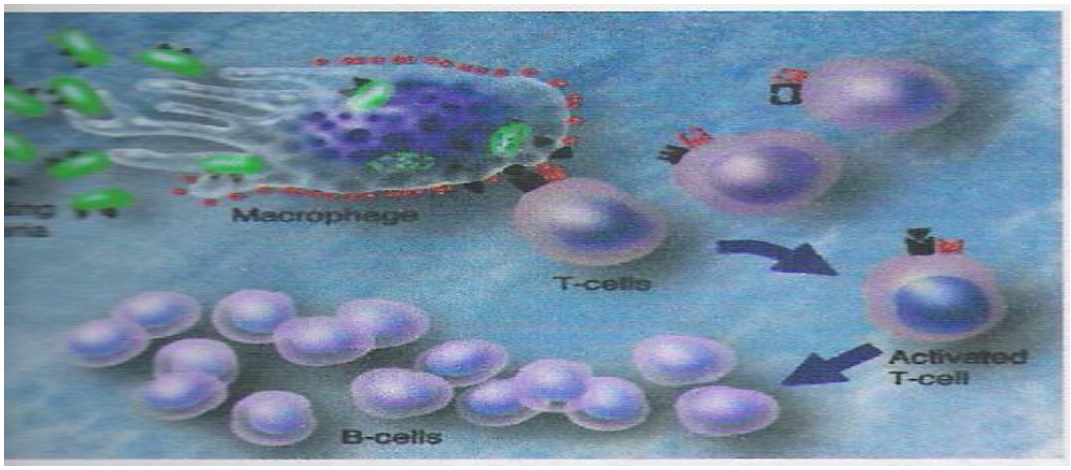


Figure-3 Invading bacteria are phagocytosed by the macrophage. The macrophage then removes the specifics identifying markers, or antigens. of the invading bacteria. It places these antigens on its own surface. Helper T-cell lymphocytes which are specially designed to fight this invading bacteria have on their own surface a complementary antigen marker, much like a puzzle piece. When these specialty T-cell lymphocytes notice a macrophage presenting the complement antigen they initiate a response which results in B-cell proliferation.

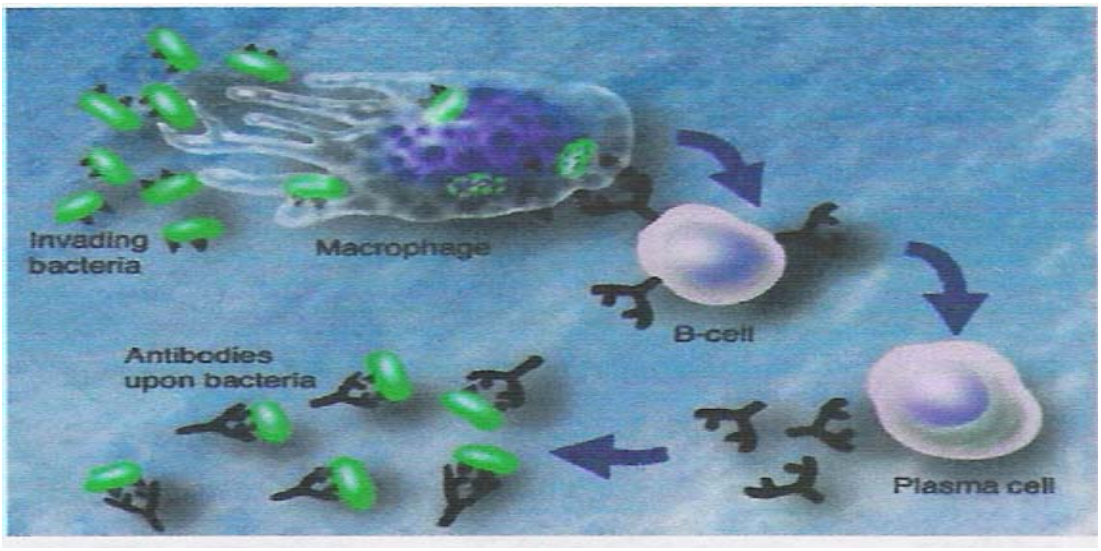


Figure-4 Macrophages engulf - invading bacteria. The macrophage will then remove the identifying markers, or antigens, from the invading bacteria and present them on its own surface. B-cell lymphocytes will notice that the antigens have been presented on the macrophage and will begin to secrete large quantities of antibodies. These antibodies bind to the invading bacteria, which will then become ineffective and more easily destroyed by macrophages.