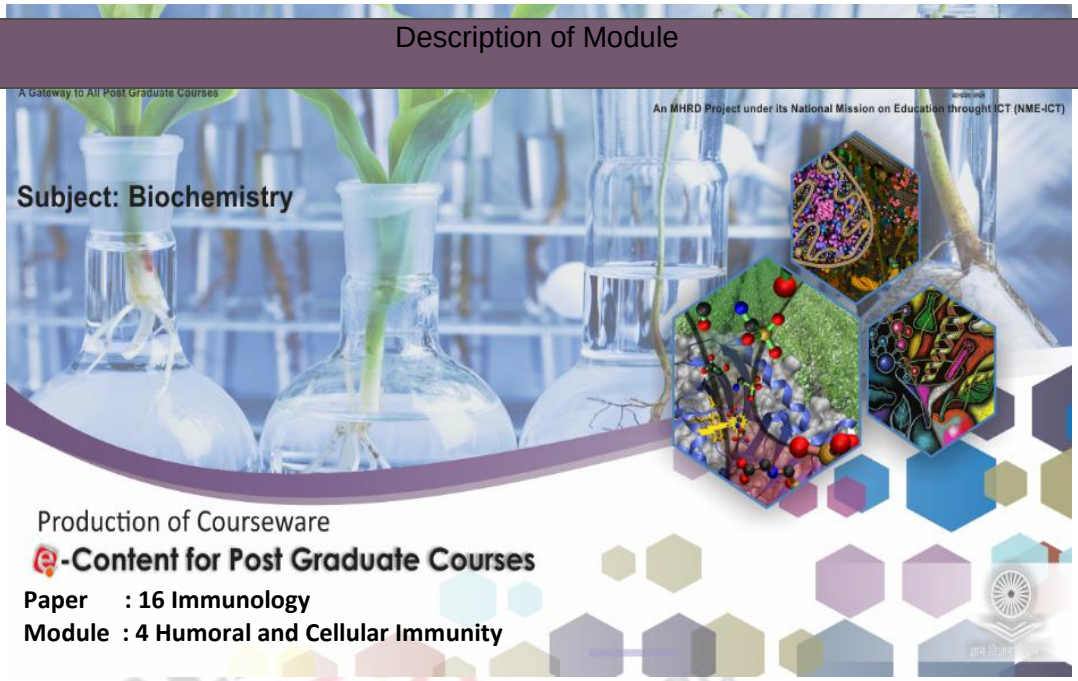
Description of Module



Principal Investigator

Dr. Sunil Kumar Khare, Professor
Dept. of Chemistry,
I.I.T. Delhi

Paper Coordinator and Content Writer

Dr. M.N.Gupta, Emeritus Professor
Dept. of Biochemical Engg. and Biotechnology, I.I.T. Delhi

Content Reviewer:

Dr. Prashant Mishra, Professor
Dept. of Biochemical Engg. and Biotechnology, I.I.T. Delhi

Subject Name	Biochemstry
Paper Name	16 Immunology
Module Name/Title	4 humoral and Cellular Immunity

1. Objectives

- To understand how acquired immunity evolved to make innate immunity more versatile
- To understand how innate immunity cooperates with humoral and cellular immunity
- To understand the respective roles of humoral and cellular immunity

2. Concept Map

3. Description

Humoral Immunity means the protection offered by antibodies. Antibodies themselves are produced by B-lymphocytes. Cellular Immunity means the immune mechanism is mediated by T-cells which are another class of lymphocytes.

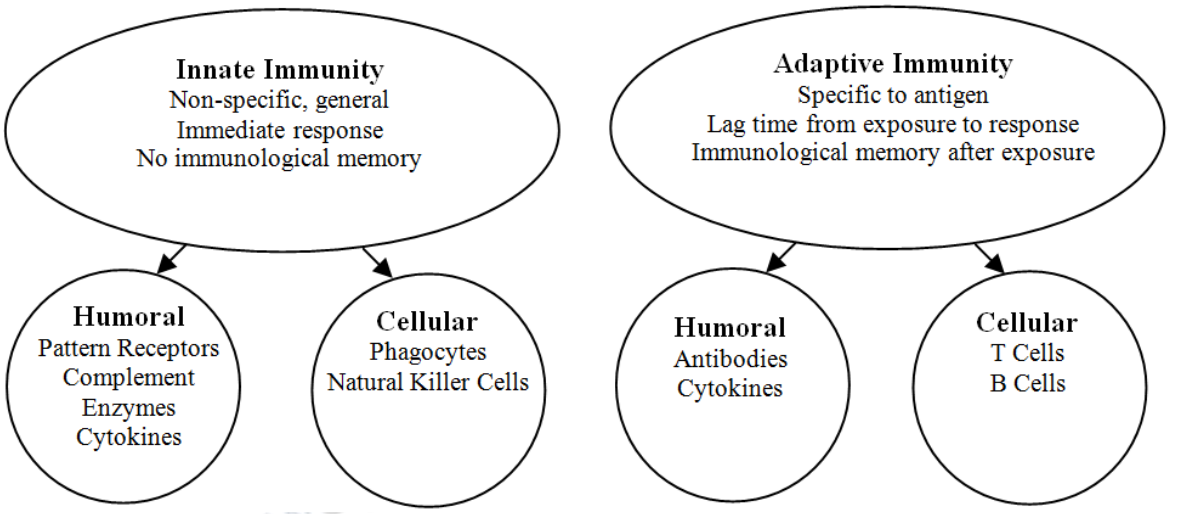


Figure 1

It is the right time to talk of the relative role of the innate and the acquired or adaptive immunity. With regards to the latter, we need to look at the broad level of how these co-operate.

What does the immune system achieve?

- It protects against pathogens
- It distinguishes between self and nonself
- It has a “memory”
- The protection against pathogens involve different locations

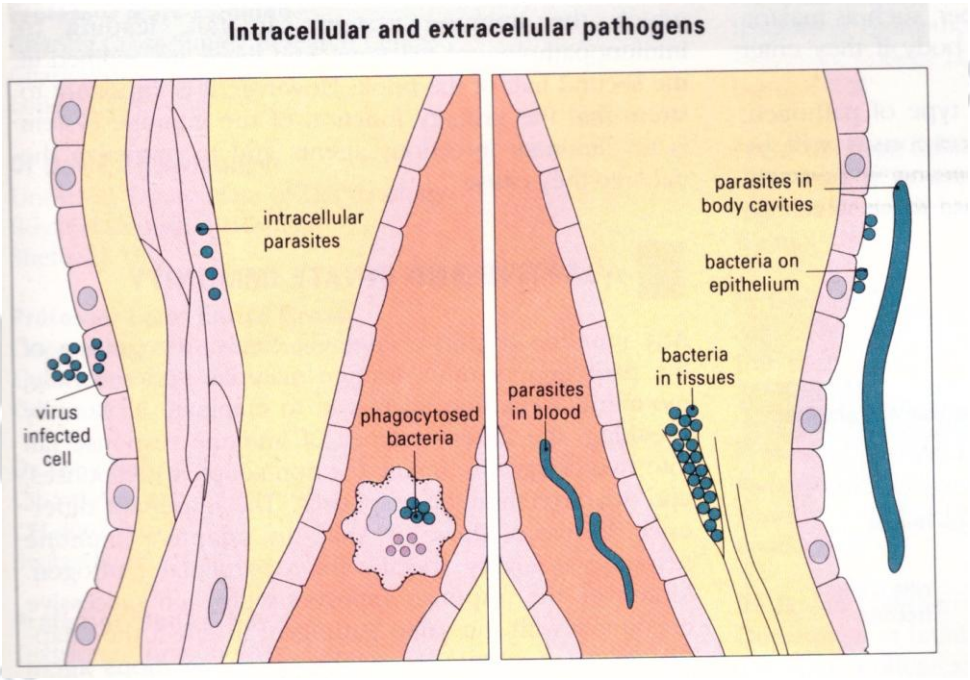


Figure 2

- Some protozoans parasites like african trypanosomes live in the blood
- Other multicellular parasites like tapeworm live in tissues/organs
- Many bacteria try colonization of the epithelial surfaces and multiply inside the tissues

- Protozoa (eg. *Plasmodium* species which cause malaria and *Trypanosoma cruzi* which cause Chaga's disease) have intracellular phases
- Virus enter cells to multiply

Whether the pathogen is inside the cell or outside the cell is important. To destroy the microbe inside the cell, humoral response (antibody mediated) does not work, we need cell mediated immune response.

The disease causing agents are viruses, bacteria, fungi, protozoa and helminths (worms)

So, the immune system has to meet the following challenges:

- Diversity of the pathogens has to be matched by the immune mechanisms
- Life cycle of the pathogens and their location in the body has to be countered by these mechanisms
- Many pathogens mutate, some very rapidly. These "evading tactics" have to be taken care of.

Invasion of both extracellular and intracellular spaces is further illustrated in the table. It may be added that to a varying degree, nearly all pathogens have an extracellular phase.

Different arms of the Immune system

Extracellular bacteria can be killed by phagocytes

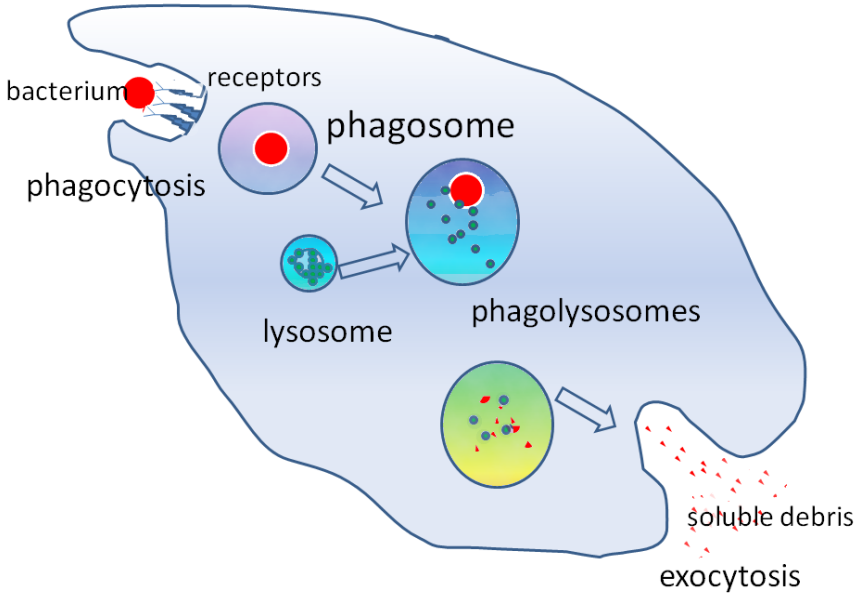


Figure 4

The encapsulated gram positive cocci are major examples of bacteria which can resist phagocytosis because of the protective action of the polysaccharide capsules. Opsonization by specific antibody (humoral immune response) or by complement (innate immunity) facilitates their phagocytosis. So, we see the need for multiple arms of the immune system.

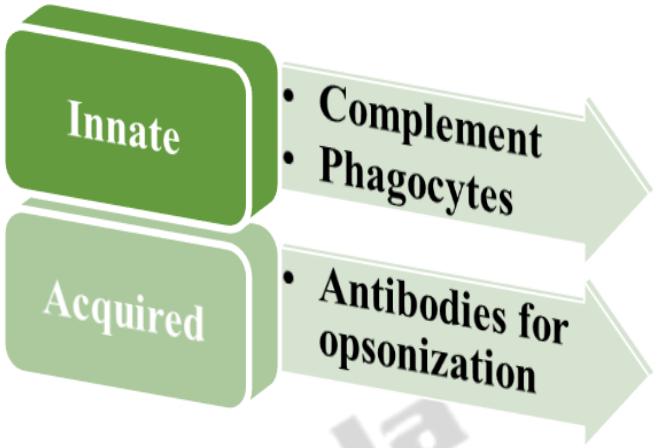


Figure 5

Virus can be neutralized by antibodies. Production of such antibodies depend upon T_h2 cells (cell mediated immunity). Once inside, T_c cells (cell mediated immunity) kill the infected cells.

Intravascicular pathogens infect macrophages which are helped by T_h cells to destroy these (cell mediated immunity).

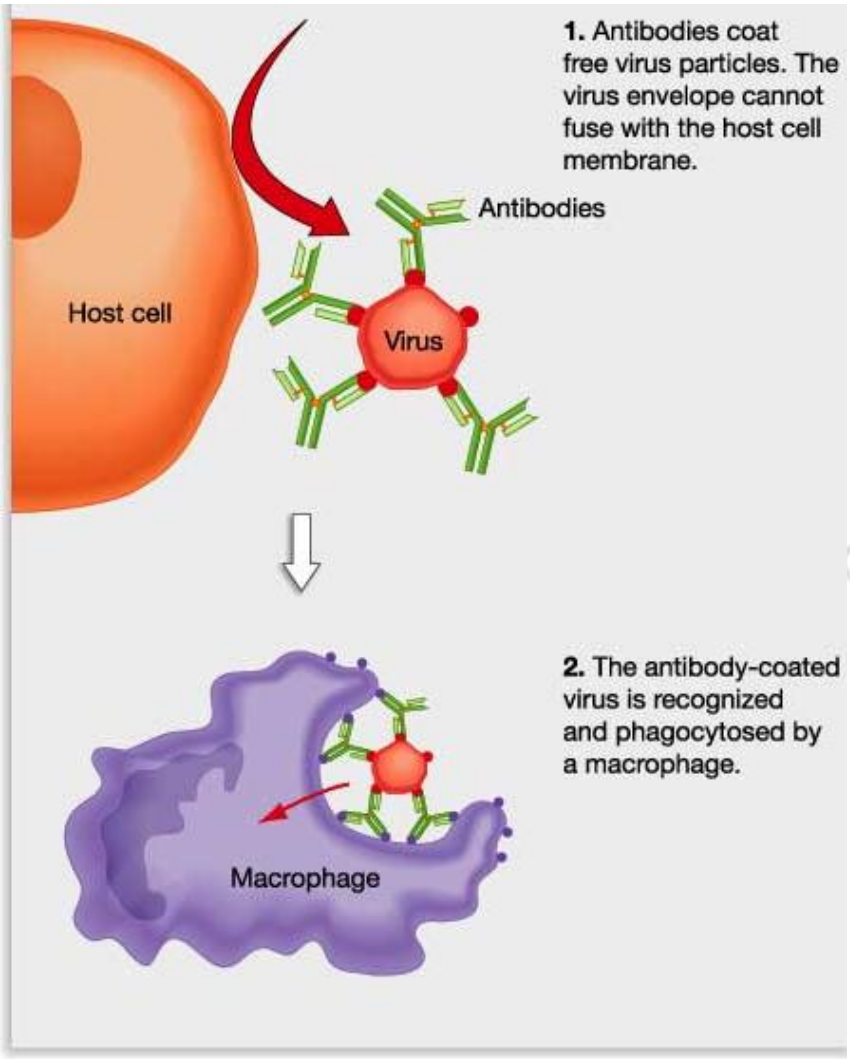

1. Antibodies coat free virus particles. The virus envelope cannot fuse with the host cell membrane.
Antibodies
Host cell
Virus
2. The antibody-coated virus is recognized and phagocytosed by a macrophage.
Macrophage

Figure 6

Need for both Innate and Acquired Immunity

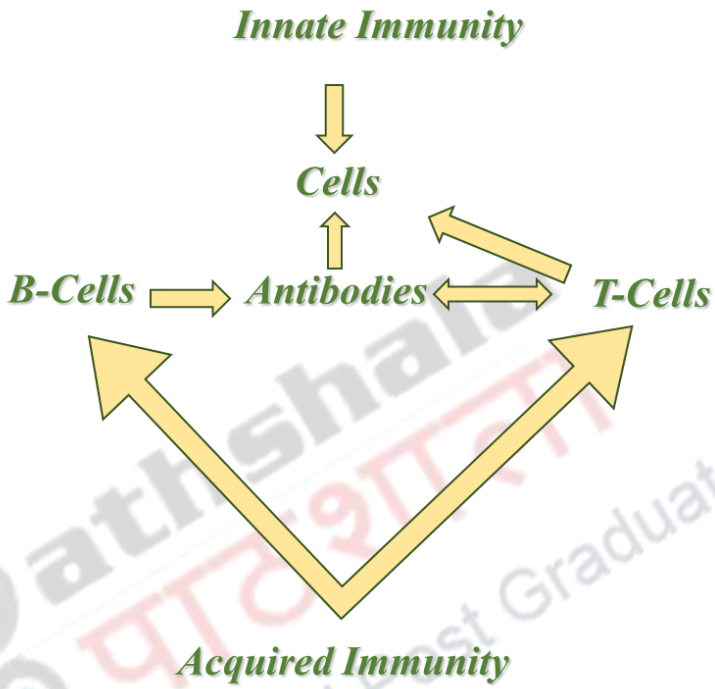


Figure 7

So, we begin to see the need for innate as well as acquired immunity. We also see the need for both humoral and cell mediated arms of acquired immunity

Ways of tissue damage

The way different organisms cause tissue damage also differs. Many extracellular microbes act by releasing toxins. Neutralizing antibodies are induced and try to overcome the effect.

Intracellular pathogens infect cells. T-cells not only destroy the damaged cells, it thereby prevents the spread of viral infection. In some cases, this itself leads to pathological situations. The site of infection is important. *Streptococcus pneumoniae* in the lung causes pneumonia. If the organism infects blood, it leads rapidly to total systemic failure

The immune response in many cases is localized. For *vibrio cholera*, the intestinal pathogen, mucosal system of the gastrointestinal tract uses acquired immunity mechanism to respond.

One general feature is that innate immunity comes into play first. If it is overcome, acquired immunity takes over. The latter also takes effect after several days. Immune responses, as we will see in more detail are well orchestrated phenomenon.

The Timeline of Infection

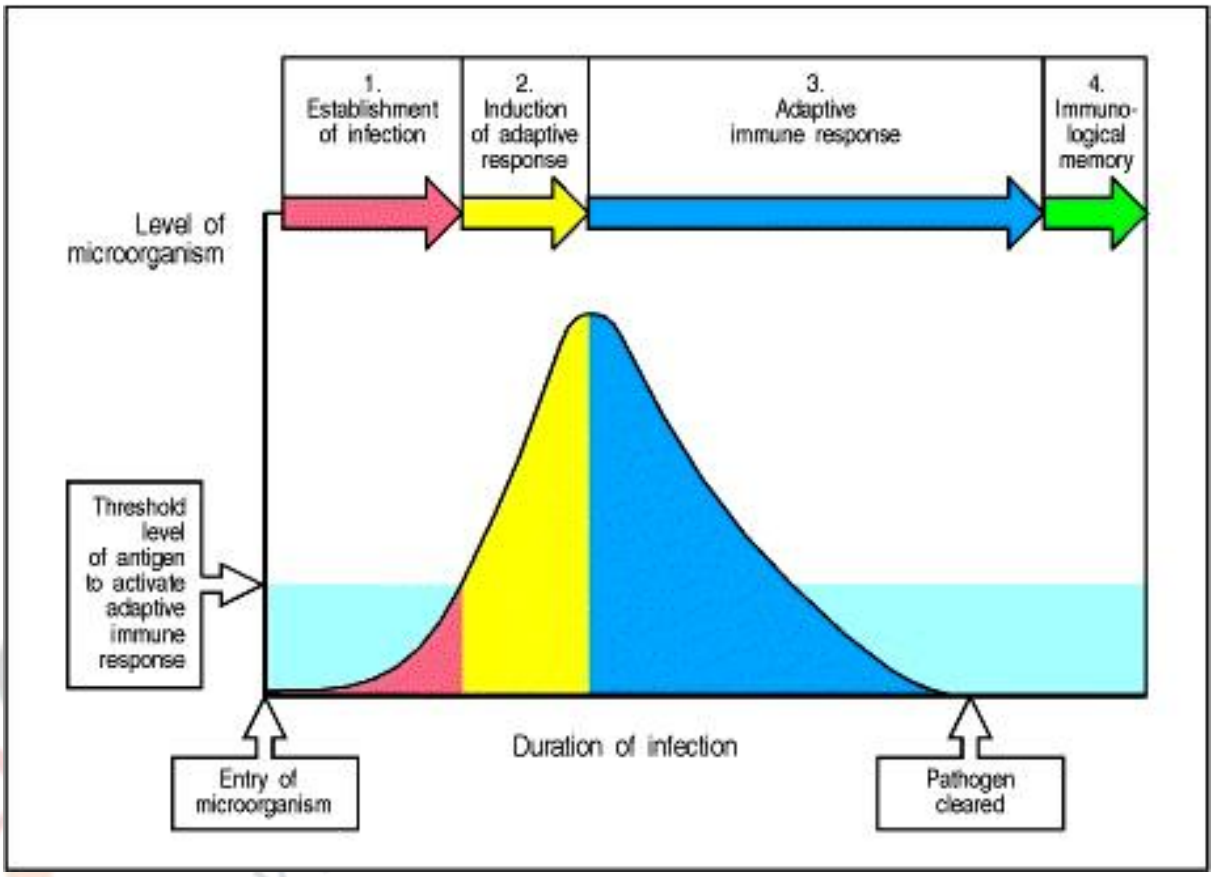


Figure 9

1. The level of infectious agent increases as the pathogen replicates. 2. When numbers of the pathogen exceed the threshold dose of antigen required for an adaptive response, the response is initiated; the pathogen continues to grow, retarded only by the innate and nonadaptive responses. At this stage,immunological memory also starts to be induced. 3. After 4–5 days, effector cells and molecules of the adaptive response start to clear the infection. 4. When the infection is cleared and the dose of antigen falls below the response threshold, the response ceases, but antibody, residual effector cells, and also immunological memory provide lasting protection against reinfection in most cases.

The infection first of all has to overcome physical, chemical and biological barriers of entry.

Innate immunity fights the colonization of the pathogen. This includes local inflammation and cytokines inducing other effector cells and molecules to gather at the site of infection.

Even theses innate immune responses distinguish between self and nonself surfaces through a number of germline coded receptors.

At best, these innate immune mechanisms are able to deal with the infection. There are few deficiency diseases related to non-adaptive immune responses known. So, we never know the success rate of these mechanisms.

At worst, these innate immunity mechanisms are not able to contain the infection. If the level of infection reaches a threshold level, adaptive immune responses get initiated. Again, in this period and even subsequent time period, innate immunity, first alone and then in co-operation with acquired immunity fights the infection.

The infection is either contained by the lymphoid tissue by the immune mechanisms. If the infection overwhelms these, it spreads through blood.

In both phases, humoral and cell mediated immunity participate together.

Once the level of infection falls below the threshold required to operate acquired immunity, the response ceases but effect cells and memory cells prolong the protection.

In the last phase of infection clearance, humoral response clears the extracellular infectious particles and cell mediated immunity clear the residual intracellular infection.

At the end, the following consequences emerge:

- No residual sign of infection remains
- Some tissue damage is caused
- Infection is contained but remains in the latent form. For example: by *mycobacterium tuberculosis*
- If the acquired immune responses are severely compromised such as in AIDS, the diseases reappear as systemic infection

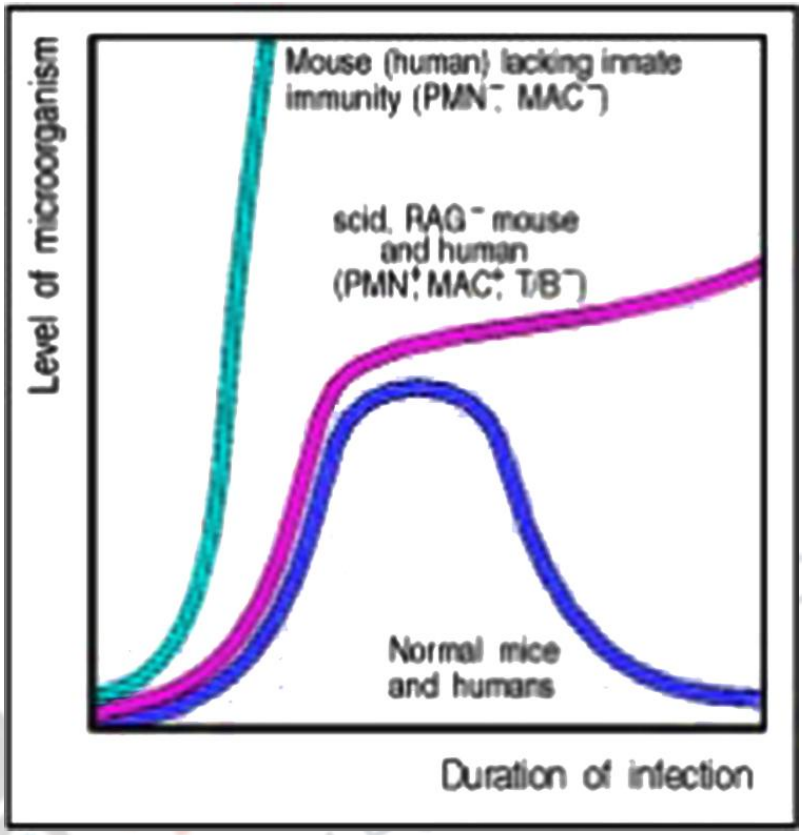


Figure 10

The time course of infection in normal and immunodeficient mice and humans.

The red curve shows the rapid growth of microorganisms in the absence of innate immunity, when macrophages (MAC) and polymorphonuclear leukocytes (PMN) are lacking. The green curve shows the course of infection in mice and humans that have innate immunity but have no T or B lymphocytes and so lack adaptive immunity. The yellow curve shows the normal course of an infection in immunocompetent mice or humans.

The innate immunity response is essential for the initiation of acquired immune response.

To start with, in the absence of innate immunity, the level of infection can rise unchecked and very rapidly.

If innate immunity was the only arm of the immune mechanisms, it will not be able to deal with any infection at a serious level. It will fight for a while, try to contain it and then the

infection will overcome it. It is the time period around which acquired immunity comes into play and contains the infection. The innate immunity first of all creates responses around the local environment of infection.

In the case of bacterial infection, inflammation is the first visible response. The macrophages residing locally have Toll like receptors through which they recognize the lipopolysaccharide coat of the bacteria.

The cytokines and chemokines are released by the macrophages and bring in PMN leucocytes, such as neutrophils and monocytes.

So far, this is all relatively non specific in nature.

The second important phase is the activation of the local dendritic cells.These dendritic cells ingest the antigens and move from the lymph in the infected tissue to enter the secondary lymphoid tissues. At this point, the fight against infection moves beyond the local site of infection. It is here that T-cells come into play. This is the antigen specific response. The first response is thus cell mediated in this case. Clonally selected T-cells enter the circulation and can reach back the local site of infection.

It is in the lymphoid tissue that these activated T-cells run into B-cells. Clonally selected, B-cells release specific antibody. Humoral response, thus initiated, further ensures containment of the infection.

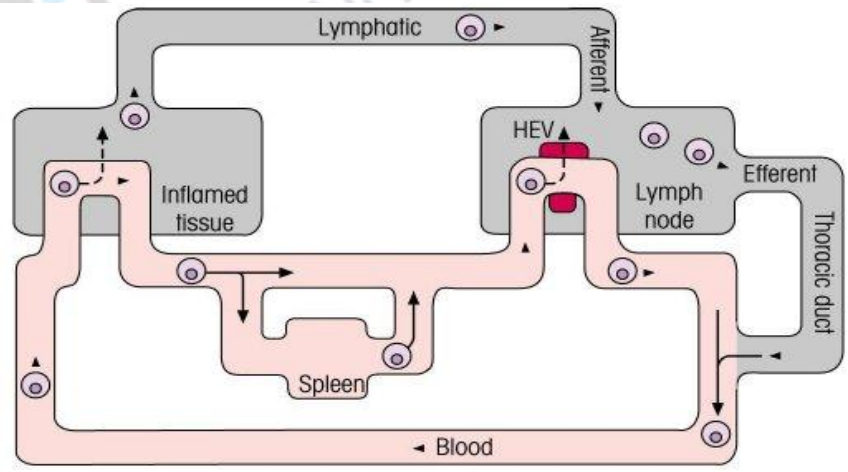


Figure 11

Why antibodies may have been necessary?

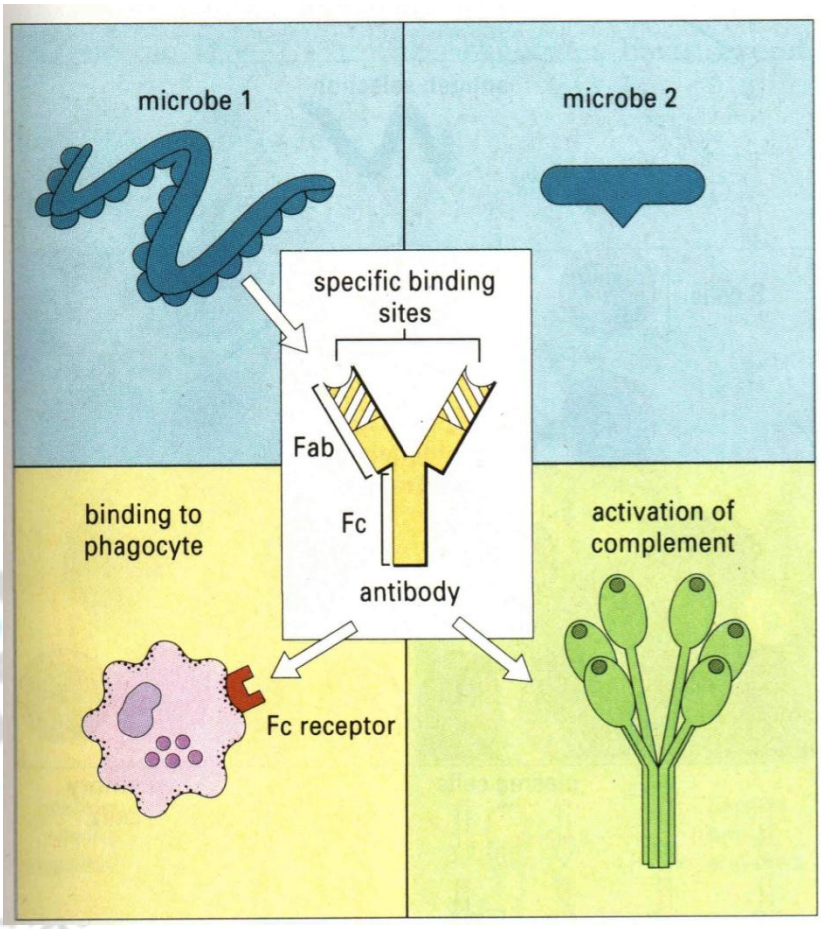


Figure 12

Antibodies play the role of an adapter molecule in many ways. When a micro-organism lacks the inherent ability to activate complement or bind to phagocytes, the body provides antibodies as flexible adapter molecules. The body can make several million different antibodies able to recognize a wide variety of infectious agents. Thus the antibody illustrated binds microbe 1 but not microbe 2, by its antigen binding portion (Fab). The Fc portion may activate complement or bind to Fc receptors on host cells, particularly phagocytes.

An antigen binds to the antigen binding site in F_{ab} of the antibody

The other parts of the same antibody molecule have effector functions. These are mediated by other components of the immune system binding to these other parts of the antibody.

These include:

- Phagocytes
- One of the complement component

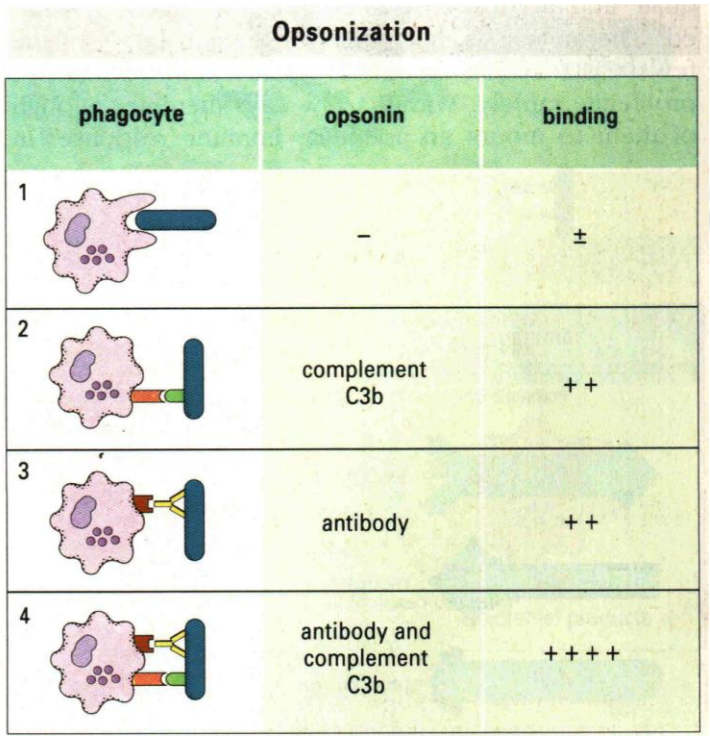


Figure 13

Phagocytes have an intrinsic ability to bind directly to bacteria and other micro organisms (1), but this is much enhanced if the bacteria have activated complement. They will then have bound C3b so that the cells can bind bacteria via C3b receptors (2). Organisms which do not activate complement well, if at all, are opsonized by antibody (Ab) which can bind to the Fc receptor on the phagocyte (3). Antibody can also activate complement and if both antibody and C3b opsonize the microbe, binding is greatly enhanced (4).

Neutrophils, macrophages and other mononuclear phagocytes have F_c receptors. The antibody molecule simultaneously binds to the antigen and phagocytes. In this, the antibody has acted as opsonin, attracted the phagocyte and initiated the phagocytosis of the pathogen antigen.

While either antibody or C3b (activated complement component) can act as opsonin, phagocyte acts more efficiently when both antibody and C3b are linked to the pathogen.

The design of the antibody as an adaptor has to be appreciated. Its F_{ab} part has specific recognition tailored to diverse antigenic substances. That is why this is fashioned out of the variable part of the molecule. This is where the humoral response comes in.

The other part relates to the interaction with components of innate immunity. These interactions are common, that is, these are not dependant upon the specific antigen which is bound to F_{ab}. Hence, these do not need tailoring for any antigen. This structural part is thus made up of constant region of the antibody.

At the same time, antibody during evolution allowed a strategy where evolution did not discard innate immunity. Antibody is the means by which evolution neatly segued the humoral response to the existing innate immunity. Antibody mediates the classical complement pathway (which is a misnomer since it evolved later than the alternative pathway but was discovered earlier!)-another route to complement activation which requires antibody presence and thus differs from the earlier evolved pathway which could act in the absence of antibody. This is in line with the above picture about the evolutionary progress. Classical pathway broadened the scope of action by the complement on pathogens which otherwise escaped innate immunity.

Many C3b coated pathogens bind to the phagocytes but are unable to initiate phagocytosis presumably due to weak binding.

The antibody provides stronger binding by acting as the adapter. This is confirmed by the fact that a single antibody molecule is not enough. This adapter action which crosslinks the pathogen and the phagocyte requires multiple linkages. So, either few Ig has to bind close to each other on the pathogen cell surface or multivalent IgM is necessary.

These facts have been experimentally verified. In cases where C3b opsonization does not trigger phagocytosis, adding antibodies initiates phagocytic action.

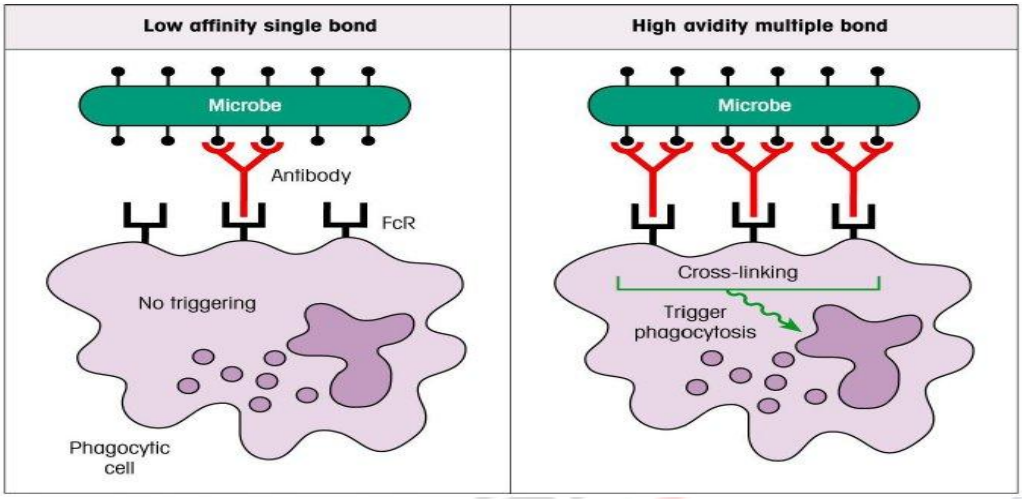


Figure 14

Binding of bacterium to phagocyte by multiple antibodies gives strong association forces and triggers phagocytosis by cross-linking the surface receptors for antibody

Evolution of the Acquired or Adaptive Immunity

Adaptive immunity, as we know now has two arms: antibody mediated humoral response and cell mediated immunity

The structure of IgG strongly suggested that it evolved from a single domain polypeptide. That itself must have evolved from a pre-existing molecule of the immunoglobulin super gene family.

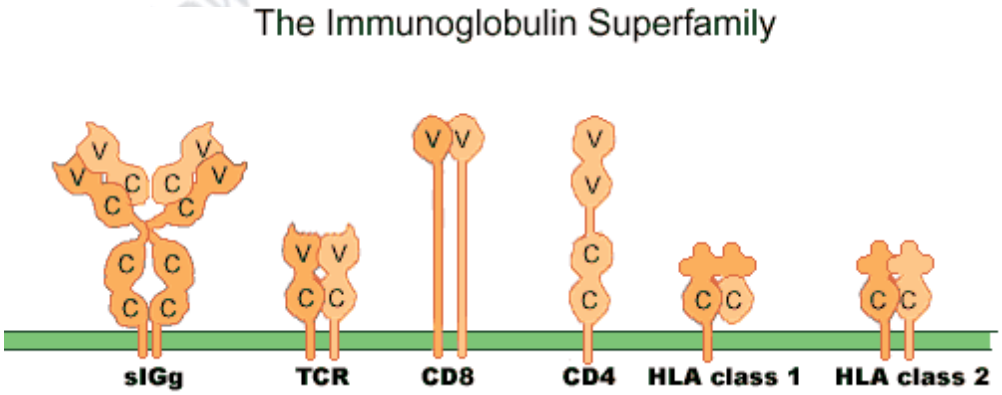


Figure 15

Here did the receptor molecule which later evolved into Ig initially get expressed. It may have been a cell similar to cells involved in innate immunity which later evolved into B-lymphocyte as we know.

All jawed fish have the capacity for adaptive immune response

Jawless vertebrates hag fish and lampreys do not have adaptive immune response

Cartilaginous fish, the earliest jawed fish have all the elements of adaptive immune response (of both kinds) in a primitive form

All metazoan have cell surface recognition systems which are capable of distinguishing between self and nonself.

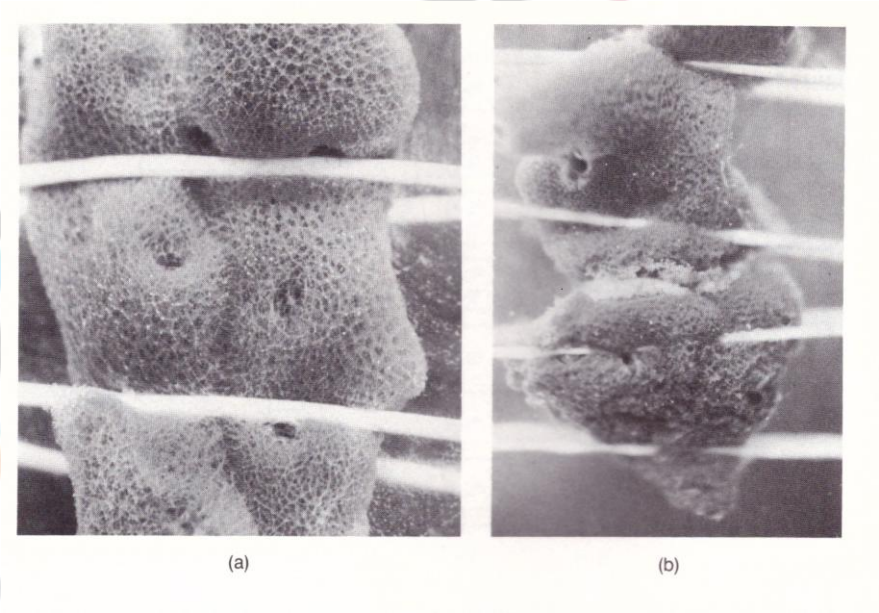


Figure 16: The grafting of genetically identical (a) and genetically disparate (b) sponges of the species Callyspongia diffusa

The Hawaiian sponge *Callyspongia diffusa*, a very primitive metazoan has three fundamental traits of the immune system of the vertebrates

- Self and nonself recognition systems operate via cell surface molecules

- Nonself are destroyed by some effector mechanisms
- Individuals within a given specie have distinct self markers

Juxtaposition of 900 pairs of genetically different Callyspongia have exhibited incompatibility with a zone of dead cells separating the two and indicating rejection.

Higher invertebrates such as colonial ascidian botryllus also has similar self-nonself recognition mechanisms.

Gene rearrangement has been the crucial step in evolution. Not all animals carry gene arrangement to create diversity of antibodies in similar way

In chicken and rabbits the recombined v gene is diversified in bursa or intestinal lymphoid organ respectively. In carcharine sharks, multiple rearranged vL segments occur in the germline.

Summary

- Innate immunity initially tries to contain the infection
- Persistance of the infection leads to the acquired immune response
- Both antibody mediated and cell mediated immunity are necessary and plays a complementary role
- It is likely that during evolution, innate immunity existed and later on acquired immunity evolved. Simultaneously a synergy between the two also evolved.