• Immune response

• Lecture (9)
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• Ph.D. Microbiology
Primary Immune Response:

- Primary Immune Response to initial antigenic stimulus is slow, sluggish, short live with low antibody titer
- that do not persist for along time, antibody formed are IgM.
The antibody classes start with IgM followed by IgG and described as Antigen specific response.
Second exposure to antigen X, first exposure to antigen Y

Primary immune response to antigen X

Primary immune response to antigen Y

Antibodies to X

Antibodies to Y

Time (days)
• Secondary Immune Response to subsequent stimuli is prompt, powerful prolonged and with much higher level of antibody it vast for long time

• Antibody predominantly formed are IgG, prescence of memory cell which are specific for antigen, so always if we give multiple dose of the same Antigen to the same host will lead good immune response because of the regulatory mechanism, The antigen type is in these responses is B-dependent-Antigen.
T cell proliferation

- Resting T cell
- Activated T cell
- T cell proliferation
  - IL-2, IL-4, IL-7
  - IL-2R
- Decay of receptors

Cell cooperation in the antibody response

- Antigen
- T cell priming
  - APC
  - T
- T-B cooperation
  - TH
  - B
- Division
- Differentiation
  - AFC
  - Bm
  - AFC
After a macrophage engulfs and degrades a bacterium, it displays a peptide antigen complexed with a class II MHC molecule. A helper T cell that recognizes the displayed complex is activated with the aid of cytokines secreted from the macrophage, forming a clone of activated helper T cells (not shown).

A B cell that has taken up and degraded the same bacterium displays class II MHC–peptide antigen complexes. An activated helper T cell bearing receptors specific for the displayed antigen binds to the B cell. This interaction, with the aid of cytokines from the T cell, activates the B cell.

The activated B cell proliferates and differentiates into memory B cells and antibody-secreting plasma cells. The secreted antibodies are specific for the same bacterial antigen that initiated the response.
factors affecting antibody production

- Nutritional status
- Route of administration
- Size and Number of doses
- Multiple antigens
- Adjuvant
- Immunosuppressive agent
- Age
• **Cellular Immune Response:** -

• The term cell mediated immunity refers to the specific immune responses that do not involve antibodies, induction of cell mediated immune response (CMI) consists of specifically sensitizing T-lymphocytes comes against the antigen. When sensitized T-cell comes in contact with antigen determinant (epitopes) by the function of Antigen Presenting Cell (APC), so T-cell under goes blast transformation and clonal proliferations selectively in paracortical areas of lymph nodes.

• lymphokines: material required as secreted proteins from the activated T-cell, These Lymphokine have several biological function
T-cell recognition of antigen

- Infected cell
- MHC molecule presents peptide
- Antigen peptide bound to MHC molecule
- T-cell receptor recognizes MHC and peptide
Antigen challenge → Immature T cell → Differentiation into specialized T cells

- Helper T cell (T_H)
- Cytotoxic T cell (T_C)
- Memory T cell
After a dendritic cell engulfs and degrades a bacterium, it displays bacterial antigen fragments (peptides) complexed with a class II MHC molecule on the cell surface. A specific helper T cell binds to the displayed complex via its TCR with the aid of CD4. This interaction promotes secretion of cytokines by the dendritic cell.

Proliferation of the T cell, stimulated by cytokines from both the dendritic cell and the T cell itself, gives rise to a clone of activated helper T cells (not shown), all with receptors for the same MHC–antigen complex. The cells in this clone secrete other cytokines that help activate B cells and cytotoxic T cells.
lymphokines: material required as secreted proteins from the activated T-cell. These Lymphokine have several biological function.

- **1-Effect on Lymphocytes**: This role is done by:
  - a-Blastogenic factor (BF)
  - b-Potentiation factor (PF)
  - c-Cell co-operation factor (CE).
2-Effect on macrophage: This Lymphokine is function is covered out by the following:

- a-Macrophage inhibition Factor (MIF)
- b-Macrophage aggregation Factor (MAF)
- c-Macrophage chemotactic Factor (MCF)
- 3-Effect on granulocytes:
  - a- Inhibition factor
  - b- Chemotactic factor
4-Effect on tissue culture:

- a-Lymphotoxine
- b-Interferon
- C-Proliferation inhibition
- Antibody or Immunoglobulin

- Are glycoproteins present in the gamma-globulin fraction of serum. Immunoglobulin generally naturally present in blood without previous antigenic stimulation. However, antibody are the Immunoglobulin that

- Produce specifically by B-cell after antigenic stimulation. Thus all antibodies are Immunoglobulin while no all Immunoglobulin are antibody
• The characteristics of antibody are:

• glycoprotein in nature.
• specific to antigen induce them.
• React specifically with their own antigen
• Antibody are distributed in serum, body fluid, Urine, Saliva, Ear wax and tears.
A B cell receptor consists of two identical heavy chains and two identical light chains linked by several disulfide bridges.
• The basic structure of antibody Molecule
• the antibody molecule is a four chains molecule which are:
  • two light chain (L.C) consist of 214 amino acid, 106 amino acid respect the constant region of molecule while variable region consist of 108 amino acid for Kappa & Lambda
  • two heavy chain (HC) consist of 440 amino acid, residcus 322 amino acid occur in constant region (CH), 118 amino acid in the variable region (VH)
BASIC STRUCTURE OF IgG

- Variable region
- Fab region
- Antigen binding sites
- V = Variable
- C = Constant
- Disulphide bonds
-Heavy chain
-Hinge region
-Light chain
There are 5 Classes of (HC) Heavy chain content for five Classes of Immunoglobulines:

1. (Gamma) or lgG
2. (Mu) or lgM
3. (Alpha) or lgA
4. (Epsilon) or lgE
5. (Delta) or IgD

- The variable region in both heavy chain (HC) and light chain (LC) are consist the antigen combination site.

- Fab: It is amino acid terminal half of heavy chain & light, it act as Antigen binding fragment.

- Fc: It is carboxyl terminal half of heavy chain & determine biological properties of Immunoglobuline.
<table>
<thead>
<tr>
<th>Class</th>
<th>Basic structure</th>
<th>Principal functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td><img src="image" alt="Structure" /></td>
<td>Protects extravascular compartment from micro-organisms and their toxins</td>
</tr>
<tr>
<td>IgM</td>
<td><img src="image" alt="Structure" /></td>
<td>Pentameric: effective first line of defence against micro-organisms in the bloodstream. Produced in the primary response</td>
</tr>
<tr>
<td>IgA</td>
<td><img src="image" alt="Structure" /></td>
<td>Dimeric: protects mucosal surfaces</td>
</tr>
<tr>
<td>IgD</td>
<td><img src="image" alt="Structure" /></td>
<td>Influences lymphocyte functions</td>
</tr>
<tr>
<td>IgE</td>
<td><img src="image" alt="Structure" /></td>
<td>Protects against intestinal parasites. Responsible for many of the symptoms of allergy</td>
</tr>
</tbody>
</table>

**Secretory component**

**J chain**

**Disulphide bridge**
<table>
<thead>
<tr>
<th>Class</th>
<th>Heavy Chain Designation</th>
<th>Number of Subclasses</th>
<th>Mean Serum Concentration mg/dl</th>
<th>Molecular Weight daltons</th>
<th>Mean Survival days</th>
<th>Biologic Activity</th>
</tr>
</thead>
</table>
| IgG   | γ                       | 4                    | 1250                           | 150,000                 | 23               | 1. Opsonin  
        |                         |                      |                                |                      |                  | 2. Fix complement  
        |                         |                      |                                |                      |                  | 3. Crosses placenta |
| IgM   | μ                       | 1                    | 120                            | 890,000                 | 5                | 1. Fix complement  
        |                         |                      |                                |                      |                  | 2. Agglutination  
        |                         |                      |                                |                      |                  | 3. Antigen receptor |
| IgA   | α                       | 2                    | 280                            | 170,000                 | 6                | 1. Secretory antibody  
        |                         |                      |                                |                      |                  | 2. Agglutination |
| IgD   | δ                       | 2                    | 3                               | 160,000                 | 3                | 1. Antigen receptor |
| IgE   | ε                       | 1                    | 0.03                            | 196,000                 | 2                | 1. Anti-parasite antibody  
        |                         |                      |                                |                      |                  | 2. Allergy antibody |
Antigen and Antibody Reaction

• Antigen-Antibody reactions are useful in:

• 1-Laboratory diagnosis of various diseases

• 2- in the identification of infectious agents in epidemiological survey

• Antigen - antibody reactions in vitro are called serological reactions.

• The following are the important tests based on antigen-Antibody reactions
The important test based on the Antigen-Antibody reaction:

1. Agglutination
2. Precipitation
3. Redial — immunoassay
4-5. ELISA
6. Immune fluorescence
7. Neutralization
8. Haemegglutination
9. Antiglobuline test (comb’s test)
10. Complement fixation test and other tests using complement system
• The antigen -Anti body complex is not found firmly together and may dissociate spontaneously unless PH, salt concentration and temperature are properly adjusted the major forces that hold antigen - antibody complex together are their ionic attractions the antigen have three factors which affected the Antigen —Antibody Reaction:

• a-movement antigen
• b- Bivalent Antigens,
• c-multivalent. Antigens

• Precipitation:
• when soluble antigen combines with its antibody in presence of electrolytes (Nacl) at suitable temperature and PH the antigen — antibody complex forms insoluble Precipitate.
Antigen-binding sites

Antibody A

Antibody B

Antibody C

Epitopes (antigenic determinants)
• Lattice hypothesis:

• It is multivalent antigens combine with bivalent antibody in varying proportions, depending antigen antibody ratio in reaction mixture. Precipitation results.

• When large lattice is formed consisting of alternating antigen and antibody molecules.

• This possible only in the zone of equivalence. zone of antigen and antibody excess lattice does not enlarge as valiancy of antigen and antibody is fully satisfied.

• In general precipitation is maximum when optimal proportions of

• antibody combine. precipitation can be produced in solutions or in semisolid (agar gel) medium. precipitation in solution can be shown by adding these two on a slide and mixing well or in small narrow tubes one -
(b)
The Complement system

A plasma protein with 25 fraction these protein heat sensitive at 56 C for 30 minutes, the chemical structure of complement is polypeptide chains interconnected by disulfide bound.

The complement system plays a major role in host defense and the inflammatory process. The complement component synthesized in liver, spleen as well as enterocytes with 25 fractions. Nine fraction of which are involve in complement pathways which act as sequential manner and can be activated or inhibited. Properdin is important on first exposure to Microorganism (first Immune Response)
The classical and alternative pathways join at the point of the conversion of C3 to C3b by the C3 convertases. Either of the two C5 convertases, which are subsequently assembled, act on C5 to produce C5b (and C5a) to which C6 to C9 are added to form the membrane attack complex.
Classical complement system pathway

$C_1 \rightarrow C_1$

$C_4,2 \rightarrow C_4,2$

$C_3 \rightarrow C_{3b} + C_{3a}$

$C_{5,6,7} \rightarrow C_{5,6,7} + C_{5\alpha}$

$C_8 C_9 \rightarrow C_{8,9}$

Occur in Plasma

Activates alternate pathway

Opsonin

Anaphylotoxin & Chemotaxin

Membrane damage by Phospholipase activity

On the surface of cell
Properdin
Aggr. IgA
Zymogen

P+ C3

opsonin

C3 → C3b + C3a

Anaphylotoxin & chemotaxin

C5,6,7 → C5,6,7 + C5a

C5,6,7 + C5a

C8,9 → C8,9

Membrane

damage by phospholipase activity

Occur in Plasma

On the surface of cell
<table>
<thead>
<tr>
<th>Activators of classical pathway</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td>Complexes containing IgG1, IgG2 or IgG3</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td>None (but see below for lipid A from LPS)</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td>Some, for example murine retroviruses</td>
</tr>
<tr>
<td><strong>Other micro-organisms</strong></td>
<td>Mycoplasmas</td>
</tr>
<tr>
<td><strong>Macromolecules</strong></td>
<td>Polyanions</td>
</tr>
<tr>
<td></td>
<td>DNA, Lipid A, cardiolipin (all contain $\text{PO}_4^{3-}$)</td>
</tr>
<tr>
<td></td>
<td>Dextran sulphate, chondroitin sulphate and heparin</td>
</tr>
<tr>
<td></td>
<td>(all contain $\text{SO}_4^{2-}$)</td>
</tr>
<tr>
<td></td>
<td>Mannan proteins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activators of alternative pathway</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td>Complexes containing IgG, IgA or IgE (less efficient than classical pathway)</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td>Many Gram-positive and Gram-negative bacteria</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td>Some virus-infected cells, for example Epstein-Barr virus</td>
</tr>
<tr>
<td><strong>Other micro-organisms</strong></td>
<td>Many fungi, <em>Leishmania</em> spp., trypanosomes</td>
</tr>
<tr>
<td><strong>Macromolecules</strong></td>
<td>Dextran sulphate, erythrocytes from heterologous species</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates, for example agarose</td>
</tr>
</tbody>
</table>
The major complement components with biological activity are summarised here. C3 is a central component which is cleaved into C3a and C3b. C5 is acted on by C3 convertase and C3b to produce C5a and C5b. C3b has several different activities, the major one being as an opsonin. C3a and C5a are anaphylotoxins which trigger mast cells to release inflammatory mediators. C5a is a chemotactic agent which recruits professional phagocytes to a site of inflammation. C5b to C9 interact to form the membrane attack complex (MAC) which inserts into membranes and results in cell lysis.
The function of complement system:

The complement displays a wide range of biological activities when activated:

1. Mediated Antigen-Antibody Reaction
2. Mediated inflammatory response
3. Facilitate phagocytosis.
4. Facilitate blood coagulation
5. Neutralize viruses effect & the effect of bacterial LPS
6. Play a role in the non-specific resistance to microbial infection
7. Associated with immune distraction - of blood cellular component
• Complement fixation test:
• This is very sensitive and is capable of detecting Antigen & Antibody. It is used for serological diagnosis with the principle:
• The ability of Antigen–Antibody complex to fix complement is used for diagnosing disease.
• 1. Spirochaetal disease e.g. Syphilis (Wasserman reaction)
• 2. Rickettsial disease e.g. Typhus fever.
• 3. Viral disease like lymphogranuloma venerum.
• 4. Parasitic disease e.g. Kala-azar, hydatid cyst, amoebiasis.
• THE
• END