

# Student to student Immunology review 3/25/1999

## 1<sup>st</sup> line of defense

### Skin

- ◆ Only staphylococcus Aureus penetrates the skin
- ◆ pH 5
- ◆ Anti-bacterial secretions

### Mucosa

- ◆ Prevents attachment
- ◆ High amount of desquamation

### Urine, tears, cilia(movement), bile

- ◆ Contain lysozymes

### Stomach pH of 2.0

- ◆ Vibrio Cholera can pass through this pH
- ◆ Bile, digestive enzymes, normal flora

## PATHOGENIC CYCLE

### Encounter

- ◆ Amount of pathogen is important
- ◆ Contact is important

### Entry

- ◆ How does entry occur

### Spread

- ◆ Multiplication within the cell
- ◆ Is there damage to the host cell

### Damage

- ◆ What is the tissue damage
- ◆ What is the host response

### Outcome

- ◆ The race between the immune system and the bacteria or virus

Remember the difference between endotoxins and exotoxins.

Endotoxins are normally lipopolysaccharides, heat stable, it normally comes from gram negative bacteria. These toxins cause fever, leukocytosis and generalized immune response. High levels of endotoxins produce disseminated intravascular coagulation which leads to vessel contraction, shock, death.

Exotoxins are protein and heat labile

## How do bacteria and viruses evade the immune system?

- ◆ **Toxins inhibit recruitment of immune cells**
- ◆ **Kill macrophages and T-cells**
- ◆ **Block antibody binding**
- ◆ **Proteolysis of Ab**
- ◆ **Survive within phagocytes and mucosal cells**
- ◆ **Inhibit cell killing**
- ◆ **Ag variation**

## Acquired immunity

- ◆ That immunity which adapts to recognize specific antigens
- ◆ B cells and T cells develop clonally.
  - ◆ Their antigen receptors differ.
  - ◆ B cells have surface antibody
  - ◆ T cells have T cell receptors

## Antigen definitions

An antigen is capable of reacting with an Ag receptor e.g. secreted Ab, cell surface Ab, T cell receptor.

**Immunogen-** is an antigen which elicits a response antibody or cell mediated response

**Allergen-** is an antigen which elicits an allergic response.

**Tolerogen-** elicits a negative response. This has more to do with the method of administration than the actual substance which is being administered.

**Hapten-** is an antigen which is incapable of eliciting a response by itself it must be conjugated to something else e.g. single ring structure like Dinitrophenol (DNP).

**Epitope-** the portion of antigen in direct contact w/ the antibody.

**Paratope-** is the portion of the antibody which is in contact with the epitope

**Valence-** is the number of epitopes on an antigenic molecule (this number can be large) or it is the number of binding sites of an Ab (this will most likely be an even number between 2 and 10).

## **Epitopes**

Physical features:

Continuous- a poly peptide in which all the amino acids are in a row.

Discontinuous- an epitope which is a structural feature of the protein, in other words, due to the 3 dimensional conformation of the protein two areas of amino acids are brought together even though when the protein is denatured these areas would be found far apart.

- ◆ Discontinuous epitopes may be bad epitopes if the conformation is not stable (if its “floppy”)

There are immunodominant epitopes- they are epitopes that are more readily bound or more commonly bound.

Cross reaction of Antibodies on different Ags can also occur.

- ◆ This can be due to identical epitopes on different antigens
- ◆ Similar but not identical epitopes
  - ◆ Hypothesized as one reason for autoimmunity

## **Chemical and physical properties of Immunogens**

\*\*\*The more foreign the antigen the more immunogenic it will be. For example, Mouse albumin will be more immunogenic in a human than in a rat.

Chemicals which act as immunogens from best to worst:

**\*\*\*Protein> Carbohydrates>composite substance>nucleic acids>lipids>metals**

-there are caveats and overlaps in this definition.

Definition of terms used when discussing antigens

**Autologous** – the antigen comes from self (except the lens)

**Syngeneic** – The antigen comes from a twin or an inbred strain of animal.

**Allogeneic** - The antigen comes from the same species but has individual allelic differences.

**Xenogeneic**- The antigen comes from a different species.

## What makes a good antigen?

- 1) Size
  - ◆ Bigger is better
  - ◆ Aggregates are better than soluble proteins.
  - ◆ A molecular weight of less than 10,000 is a poor antigen.
- 2) Rigidity or complexity
  - ◆ More complex = more rigid = better antigen
- 1) Accessibility
  - ◆ Surface epitopes are better than epitopes buried inside the protein
- 1) Digestibility
  - ◆ This is mainly for T-cell antigens
  - ◆ Proteins are more digestible than polysaccharides

## Classification of Antigens

### T cell independent antigens-

- ◆ There is no need for T cell help
  - ◆ Type I antigens
    - ◆ Bind 2 different binding sites for 2 different receptors
- ◆ Type II Antigens
  - ◆ These have repeating epitopes which cross link surface antibody
  - ◆ Dextran or polysaccharides.

### T dependent antigens-

- ◆ Will be proteins (hydrophilic)
- ◆ The B-cell gets 2 signals
  - ◆ 1 from antigen binding to Ig and the second from the T helper cell.

### What makes a T cell antigen

- ◆ **Must be protein or peptide!**
- ◆ Should be foreign
- ◆ Will be hydrophobic or mixed (-phobic and -philic)
- ◆ Will be 8-25 AA's long and binds to MHC to be presented on the surface to T cells.

### Super antigens

- ◆ T-cells only
- ◆ The peptide form is not needed
- ◆ Connects TCR to MHC and activates the T cell without peptide.
  - ◆ Staph A toxin and TSST are examples of super antigens.

## Antibodies

### Structure

- ◆ Bilateral symmetry
- ◆ Heavy chain with 4-5 domains
  - ◆  $V_H$  and 3-4  $C_H$
  - ◆  $C_H$  has the effector function
  - ◆ All Ig's have ~30% identity at the  $C_H$  region
- ◆ Light chain with 2 domains
  - ◆  $V_L$  and  $C_L$
  - ◆  $C_L$  binds the light chain to the heavy chain.

Ab are found in serum, on the B cell surface, on mast cells, intracellularly

### Light chains

- ◆  $\kappa$  or  $\lambda$
- ◆ Any light chain can go with any heavy chain

**Papain**- cleaves the antibody to give 2 Fab portions and 1 Fc portions

**Pepsin**- cleaves the Ab to give  $(Fab')_2$  and Fc

**You must know what part of an Ab can be immunogenic.**

### Isotypic-

- ◆ Class or subclass of the H chain
- ◆ Product of one C-region locus

### Allotypic-

- ◆ Allelic differences at a given locus
- ◆ Genetic variants within a species

### Idiotypic-

- ◆ Differences in the hypervariable region either in the heavy or light chain.

## Monoclonal Ab

- ◆ A population of Ab which all recognize 1 specific epitope

The V region of Ab

Hypervariable regions

- ◆ Ag binding

Framework regions

- ◆ Have some variability but are not involved in binding.
- ◆ Act as the base for the hypervariable region

## Ab classes

### IgM

- ◆ Primary response for all Ag administered parenterally
- ◆ Major class elicited by a T-independent Ag for both 1<sup>st</sup> and 2<sup>nd</sup> exposure
- ◆ The secreted form has a pentameric structure w/ J-chain
  - ◆ Membrane form is monomeric
- ◆ Stays in the vasculature
- ◆ 5-10 day  $T_{1/2}$
- ◆ doesn't cross the placenta
- ◆ Activates complement
- ◆ Acts as an opsonin via the complement receptor **NOT the Fc receptor**

### IgG

- ◆ Secondary response for most T dependent Ag
- ◆ 4 subclasses
- ◆ 21-23 day  $T_{1/2}$ , IgG<sub>3a</sub> is shorter
- ◆ Crosses the placenta
- ◆ Each subclass has variable complement activation ability
- ◆ Acts as an opsonin via the Fc $\gamma$ R

### IgA

- ◆ 2 subclasses in humans
- ◆ dimeric structure in the secreted form
- ◆ Found in mucosal secretions and the serum (unknown function in the serum).
- ◆ Has a J chain and secretory component
- ◆ 2-6 day  $T_{1/2}$
- ◆ does not cross the placenta
- ◆ Fixes complement in the Alternative pathway **NOT the classical pathway.**
- ◆ Acts as an opsonin for neutrophils

## **IgD**

- ◆ Not found in the plasma
- ◆ membrane bound defines the mature B cell
- ◆ 3 day  $T_{1/2}$
- ◆ doesn't cross the placenta

## **IgE**

- ◆ involved in allergic reactions via binding to mast cells
- ◆ monomeric
- ◆ 2-3 day  $T_{1/2}$
- ◆ doesn't cross the placenta

## **Immunoglobulin as Antigen**

### **Isotype-**

- ◆ Difference in light or heavy chain type in the constant region between different species
- ◆ The greater the difference phylogenetically between the recipient and donor the more epitopes there will be

### **Allotype-**

- ◆ reflects the variation within a species (allelic differences)

### **Idiotype-**

- ◆ variable region
  - ◆ recognize many idiotypes
  - ◆ polyclonal
- ◆ "anti"-anti idiotype
  - ◆ is an internal image
  - ◆ Form an antibody (b) to another antibody's (a) variable region
  - ◆ This is one theory as to how the immune system regulates it's response.

## Antibody and T cell receptor genetics

Ab's are extremely heterogeneous. If we were to follow the one gene one protein rule, there is not enough genetic material in a cell to produce all the diversity found in Ab's. There must be a special system to create this diversity.

### **Ab heterogeneity**

- ◆ **Constant region**
  - ◆ multiple C<sub>H</sub> and C<sub>L</sub> isotypes and allotypes
  - ◆ alternate terminal ends (RNA level) of H chains (transmembrane vs. Secreted).
- ◆ **Variable region**
  - ◆ Multi gene families
  - ◆ Somatic cell recombination of DNA
  - ◆ Somatic cell hypermutation of V-region genes

### **Strategies used for the diversification of the Ab response.**

#### Segmentation

- ◆ V<sub>L</sub> has V<sub>κ</sub> and J<sub>κ</sub> or V<sub>λ</sub> and J<sub>λ</sub>
- ◆ V<sub>H</sub> has V<sub>H</sub>, D<sub>H</sub>, J<sub>H</sub>
- ◆ There are multiple gene segments within each category e.g. V<sub>H</sub> has 200-300 segments D<sub>H</sub> has 12-20 segments etc.

#### Recombination

- ◆ Antibodies are made up of one of each segment (combinatorial joining).
  - ◆  $H = 1V + 1D + 1J$
  - ◆  $L = 1V + 1J$

#### Junctional diversity

- ◆ imprecise joining
- ◆ addition of N segments

## Somatic cell recombination

- ◆ There are signal sequences which identify edges of gene segments for recombinase recognition.
  - ◆ sequences look like this 7' mer---spacer---9' mer
    - ◆ conserved sequences for 7 and 9' mers
    - ◆ The spacer is either 12 or 23 nts long
  - ◆ one 7' mer can pair with another 7' mer via DNA hybridization(9' mers can also) bringing two gene segments together

- ◆ **There are rules:**
  - ◆ **12/23 rule**
    - ◆ There must be one 12 and One 23 for recombinase to work. This makes it so that in the heavy chain V can only recombine with D and D with J not V to V or V to J.
  - ◆ **cis joining**
    - ◆ there is no joining across chromosomes
  - ◆ **Deletion of intervening DNA**
    - ◆ When DNA recombines the intervening DNA is removed

**The order in which V region gene segments join:**

**Allelic exclusion:**

- ◆ Only one functional VDJ rearrangement for  $V_H$  and one VJ for  $V_L$  can be produced in one cell. Once this VDJ exists it stops further  $V_H$  rearrangement and  $V_L$  begins rearrangement.

**Junctional diversity**

- ◆ Imprecise joining
  - ◆ V can contribute 1 or more nt and then J contributes 1 less.
  - ◆ Must be in frame to be functional
  - ◆ Occurs in both  $V_H$  and  $V_L$
- ◆ N-segment addition
  - ◆ terminal deoxynucleotidyl transferase adds nucleotides to the 3' ends of cut DNA before the ends are joined.
  - ◆ Only occurs in  $V_H$  not  $V_L$

**Somatic Hypermutation**

- ◆ This occurs in functionally rearranged  $V_H$  and  $V_L$
- ◆ A high rate of mutation occurs during rapid proliferation of the B cell.
- ◆ It is the mechanism for "affinity maturation"

## T cell receptor diversity

Similar to B cells it has:

- ◆ combinatorial joining
- ◆ junctional diversity
- ◆ N- segment addition in this case for both  $\alpha$  and  $\beta$  chains

In addition TCR diversity has:

- ◆ D to D joining
- ◆ D segments read in all three reading frames

**\*\*\*But there is NO SOMATIC HYPERMUTATION\*\*\***

Illegitimate rearrangement can be the cause of malignancies.

Once rearrangement has occurred transcription is enhanced and goes into the constant region going through at least  $C\mu$ .

## **Switch recombination**

- ◆ The second type of gene rearrangement in Ig's
  - ◆ there are switch regions which occur in front of each  $C_H$  gene except  $C\delta$
- ◆ Switch recomb is cut and paste of the constant region to define the effector function of the antibody being made.
  - ◆ it is usually cis recombination
  - ◆ recomb. goes down stream.

How is the selection of the proper isotype done?

- ◆ B cell CD40 binds T cell CD40L initiates isotype switching
- ◆ cytokines cause specific switches
  - ◆ they induce the expression of proteins which bind specific DNA sites around particular switch regions and opens up the DNA.

## **Isotype exclusion**

only on H chain isotype and one L chain isotype is expressed in any given B cell.

## **Monoclonal Ab**

- ◆ produced by a single clone of B-cell/plasma cell such that all Ab's are identical

**Affinity( $K_a$ )** measure of the strength of binding between an epitope and a paratope.

$K_a = \frac{[Ag-Ab]}{[Ag][Ab]}$  = association constant, the dissociation constant =  $K_d = 1/K_a$

**High affinity  $K_a = 10^{10} - 10^{12} M^{-1}$  or  $K_d = 10^{-10} - 10^{-12} M$**

**Avidity**- the measure of strength of binding of a whole Ab to a whole Ag.

- ◆ multivalency must be taken into account as well as strength of binding
- ◆ In addition spatial arrangement of the epitopes must be taken into account

# Immunoassays

Sensitivities of immunoassays

- 1) Precipitation.....Poor
- 2) Agglutination.....Good
- 3) Liquid phase radioimmunoassays(RIA).....Very good
- 4) Solid phase RIAs and EIAs(enzymeimmunoassays).....Excellent

Precipitation Rxn

**\*\*\*\* Must know that you need at least bivalent Ab and bivalent Ag to get enough cross linking to form large complexes\*\*\*\***

## Secretory Immune System

**IgA is the predominant class of Ig in external secretions**

- ◆ A high density of IgA secreting cells exist in the gut lamina propria and in the mammary gland
- ◆ A low density exists in the oral cavity, G.U. tract

The secretory form of IgA has an extra 19 AA's this portion binds to the J chain.

- ◆ binds secretory component synthesized by epithelial cells
- ◆ IgA is transported transcellularly through epithelial cells (after binding to pIgR)
- ◆ there are 2 subclasses of IgA
- ◆ IgA<sub>1</sub> predominant in T dependent response
- ◆ IgA<sub>2</sub> in T-independent responses

**Where are the IgA secreting plasma cells found?**

- ◆ diffuse lymphoid tissue
  - ◆ IgA plasma cells in the gut outnumber all other plasma in the body.
- ◆ Aggregated lymphoid tissue
  - ◆ G-, B-, N-, M- (Gut, Bronchus, Nasal, Mucosal) associated lymphoid tissue(ALT)
    - ◆ GALT includes Peyers patches and the appendix.
      - ◆ Follicle of the Peyers patch contains many surface IgA containing B cells no plasma cells.
      - ◆ M cells over Peyers patch areas assist the B cells by transporting antigen into the follicle.

### **IgA mechanism for host protection.**

- ◆ Functions as a barrier
  - ◆ prevents attachment, or entry
- ◆ Protease resistant
  - ◆ must be to work in the gut
  - ◆ IgA proteases are used by bacteria as a pathogenic mechanism.
- ◆ Provide some intracellular protection in mucosal cells of the gut.
- ◆ Modulates an inflammatory responses mediated by other Ig classes
  - ◆ Secretory component = gravidin which inhibits the arachadonic acid pathway for prostaglandin and leukotriene production, therefore it inhibits inflammation.

### **IgA secreted by mammary gland.**

- ◆ IgA produced at this site are destined for the lumen of the gut of a neonate. This IgA Ab protects the GI tract of the neonate.
- ◆ GALT is the major source of IgA precursor cells that end up in the mammary gland.

### **Immunization to elicit secretory IgA**

- ◆ **Route of immunization**
  - ◆ oral -Sabin polio vaccine
  - ◆ Bronchial
  - ◆ Nasal
- ◆ Dosage and form of immunogen
  - ◆ live attenuated virus is more effective than killed microbes
- ◆ Memory in IgA is not well developed.

## Phagocytes

- ◆ Ingest microorganisms, particles or other cells with the purpose of killing and causing an inflammatory response.

### Neutrophils

- ◆ targets extracellular bacteria to ingest and kill them quickly
- ◆ Found in the tissues
- ◆ produce G-CSF, GM-CSF, IL-1, TNF, IL-8
- ◆ The source of Neutrophils is bone marrow (BM)
- ◆ Maturation take 14 days
- ◆ Can be stored in the BM, IL-1 can cause release.
- ◆ Marginal pool exists through out the body, can be release by epinephrine
- ◆ Spleen clears neutrophils
- ◆ Function
  - ◆ Adherence
  - ◆ Movement
  - ◆ Phagocytosis and degranulation
  - ◆ Killing
- ◆ Chemotactic factors which neutrophils have receptors for:
  - ◆ C5a
  - ◆ FMLP
  - ◆ Chemokines
- ◆ Opsonins
  - ◆ IgG
  - ◆ C3b
- ◆ Killing
  - ◆ Non-oxidative method
    - ◆ involves degranulation
      - ◆ Primary granules
        - ◆ defensins
        - ◆ Bacterial permeability increasing protein
      - ◆ Secondary granules
        - ◆ Lysozyme
        - ◆ Lactoferrin
        - ◆ Nitric oxide
  - ◆ Oxidative killing (toxic substances used to kill bacteria)
    - ◆ Superoxide production
    - ◆ H<sub>2</sub>O<sub>2</sub>
    - ◆ Hydroxyl radicals
    - ◆ H<sub>2</sub>O<sub>2</sub>, myeloperoxidase and Cl<sup>-</sup> produce HOCl (hypochlorous acid) which react with Amino acids to produce chloramines
      - ◆ Bad thing is that HOCl can destroy protease inhibitors which allows proteases in the granules to cause tissue damage.

## Monocytes and Macrophages

- ◆ Produce many cytokines
- ◆ derived from bone marrow
- ◆ monocyte is an immature macrophage
- ◆ Macrophage is the functional cell
- ◆ **Macrophages**
  - ◆ fixed
    - ◆ resident in tissues with differences in function e.g. Kupfer cells Alveolar Macrophages.
    - ◆ function in clearance of unwanted particles from the blood stream
      - ◆ neutrophil opsonins function for macrophages as well
  - ◆ Mobile
    - ◆ Monocytes circulate to sites of infection and inflammation and mature to macrophages
    - ◆ Macrophages function to kill a variety of organisms (especially intracellular organisms).
- ◆ **Activation of macrophages**
  - ◆ Th1 cells secrete IL-2 and IFN- $\gamma$  which leads to Macrophage activation and cell mediated immunity.
  - ◆ IL-4 and IL-10 lead to macrophage suppression.

## Complement

Complement acts to:

- ◆ opsonize
- ◆ activate inflammation
- ◆ lyse pathogens

### Classical Complement pathway

- ◆ Antibody dependent
- ◆ C1q binds to Fc portion of an Ab, C1r binds and cleaves C1s which in turn cleaves C4 and C2. C4 becomes C4a (acts as a chemoattractant) and C4b (reacts with the pathogen surface) and bind C2a (C2b does nothing). C4bC2a is called the C3 convertase and this converts C3 into C3a ( an anaphylatoxin) and C3b.

### C3 convertase

- ◆ produces C3b which acts as an opsonin.
- ◆ produces C3a a potent anaphylotoxin.
- ◆ is an amplification point of the complement cascade
- ◆ It is regulated by Decay accelerating factor (DAF) promotes the inactivation of C3 convertase

C3b bind C4bC2a to form C4bC2aC3b which converts C5 into C5a (important chemoattractant factor and Anaphylotoxin) and C5b. C5b bind to the pathogen surface and bind C6, C7...C9. This forms the membrane attack complex which causes osmotic lysis in gram negative bacteria.

### **Alternative pathway of the complement cascade**

- ◆ The classical pathway needs Ab but to produce antibodies it takes time. This is why we have the alternative pathway.
- ◆ C3 can be activated spontaneously in solution.
- ◆ Activated C3 (C3i) complexes with factor B (C3iB) which is cleaved by factor D(C3iBb) which is a C3 convertase when stabilized by factor P (C3bBb). C3bBb converts C3 to C3b which reacts with surface carbohydrates on the pathogen.
- ◆ Bound C3b can also bind factor B etc. and become a C3 convertase and thus amplifying the response (cross talk between Classical and alter. pathways)
- ◆ C3bBb can complex with C3b to form a C5 convertase to continue the pathway
- ◆ **How is it inhibited?**
  - ◆ Complement control proteins
    - ◆ C1 inhibitor
    - ◆ DAF
    - ◆ C3bBb and C3iBb are antagonized by factor H
      - ◆ C3b complexed with factor H is a substrate for factor I which cleaves C3b
      - ◆ C4 Bp does the same that factor I does, except in the classical complement pathway
- ◆ Host cells are resistant to the membrane attack complex.

### **Receptors for complement components:**

**CR1**-B receptor for C3 opsonized pathogens

**CR1 and CR2**- expressed on B-cells function in co-stimulation of B cells

Something else to be sure and know about complement

**\*\*\*\* C5a, C3a and C4a are all anaphylotoxins and C5a is also a chemotactic factor\*\*\*\***