Scope of work:

1. Hypersensitivity

2. Blood Group substances

3.Transplantation immunology

MY POLICY

- Punctuality to lectures:
- Can’t come in once lectures have started
- Class participation; interactive lectures
- Questions for either biochemists or biologists at any time
- Help me know you by name etc.
- Phone call? Receive outside, don’t come back!!

Hypersensitivity

- Definition
- It is an altered state produced by an Ag in which pathological reactions can be subsequently elicited by a second encounter with that same Ag or structurally similar substance

Definition contd.

- It is an example of immune system dysfunction giving rise to pathological reactions/diseases i.e. are immune reactions that lead to tissue damage or even death in some cases

Comparison with Immunity:

- Immunization results when a person acquires an increased resistance to a substance he has been previously exposed to. The substance is called an Immunogen. The individual is called an immunized person. He has protection from the Ag/Immunogen

Contd.

- Sensitization or Hypersensitivity results when a person acquires an increased susceptibility to a substance previously exposed to. The substance is normally called a sensitizer or allergen. Individual called sensitive or allergic person. The Antigen (allergen) causes destruction i.e. disease.

Sites affected and symptoms

- Allergic responses typically elicit symptoms in the respiratory and gastrointestinal tracts and the skin. These are the most common points of contact with allergens.

- Symptoms mainly include sneezing, wheezing, nasal discharge, vomiting, diarrhea, itching, rashes and even death.

Types of Hypersensitivity reactions

- **Classification based on type of response and effector mechanism responsible for cell and tissue damage and time for reaction to occur.**
- **a) Immediate – Type I**
- **b) Antibody-mediated – Type II**
- **c) Immune complex-mediated – Type III**
- **d) Cell-mediated – Type IV or Delayed**

- **b and c called intermediate**

TYPE I HYPERSENSITIVITY

- **Also called Immediate; occurs within seconds to min of Ag exposure, e.g. itching upon mosquito bite.(Gerald,p599)**

- **Many types of allergens involved:**

Proteins, plant pollens, food, drugs, insect products and mold spores, etc.

Sub-types of immediate hypersensitivity

1. **Anaphylaxis/systemic/generalized:**

Ag introduced into system intravenously e.g. dogs given second injection of inactivated jellyfish or guinea pigs given egg albumin a second time: give prompt response;

i.e. acute shortness of breath and die within minutes. Called Anaphylactic shock

Some individuals respond to bee sting or Penicillin similarly

Type 1 Contd.

2 Cutaneous:

Ag introduced into the skin; get reaction immediately at local site, e.g. mosquito bites cause itching reaction. Effect lasts for a maximum of 2hrs when stimulus removed.

See typical wheal and erythema reaction. at site

Type 1 contd.

3. Atopic:

Caused by spontaneous reaction with no deliberate Ag introduction to many environmental or unknown allergens e.g. pollen, fungal spores, animal fur, animal dander, house dust and some foods

- **Appears to be heritable**

Examples of Atopic allergy

Asthma:

Characterized by obstruction of the Bronchioles (airwaves) leading to difficulty in breathing, hence wheezing effect and coughing.

Specific genes up-regulate their IgE levels

Hay fever: Itching and watery discharge from eyes and nose. Reddening and swelling of the eye may result

Atopic cont.

- **Food allergy:**

Swelling of lips, tingling and itching in throat, nausea, vomiting, abdominal cramps, diarrhea.

Mainly from nuts, shellfish, eggs, cow or soybean milk etc.

Breast feeding of newborns encouraged.

Mechanism of type I Reactions

- **Involves IgE Abs. bound to mast cells.**
- **Aggregation of IgEs on mast cell by allergen.**
- **Mast cell degranulation with mediator release. These are primary mediators**

- **Mediators are vaso-active amines (histamine, serotonin), proteases, heparin, eosinophil chemotactic factor (ECF) which are all present in mast cells.**
- **They trigger allergic responses.**

Mechanism contd.

- Other mediators:
- **Leukotrienes (arachidonic acid derivatives), prostaglandins, bradykinins, cytokines etc.**
- **Are secondary mediators.**
- **Synthesized after target cell activation**
- **Bradykinin - plasma hormone; causes blood vessel dilation and smooth muscle contraction**

Effects of Histamine

- Histamine dilates blood vessels with drop in blood pressure. Can be lethal; called anaphylactic shock.
- Contracts bronchial smooth muscles (wheezing)
- Increases capillary permeability and mucus secretion of goblet cells (watery eye, nose discharges) as in hay fever

Mechanism contd.

- **Leukotrienes/prostaglandins cause bronchoconstriction.**
- **Leukotrienes (Slow reactive substance of anaphylaxis SRS – A) also cause vascular permeability and mucus production.**
- **Are responsible for prolonged bronchospasm in asthmatics. Their effects are longer lasting than those of histamine. More important in asthma than histamines**

Mechanisms contd.

- **Cytokines also produced and recruit inflammatory cells to site of allergic reaction.**
- **(Read on CYTOKINES)**

Asthma

- **Attack initiated when sensitized person is exposed to allergen that binds to mast cells.**
- **Bronchoconstriction response begins within minutes of exposure due to mediator release.**
- **Mediators bind to smooth muscle cells surrounding the airwaves. Contraction results, narrowing the airwaves**

Therapeutic Principles to cure Type I hypersensitivity

- **Immunological de-sensitization – 2 ways:**

a. Give small increasing doses of Ag till all mast cells degranulated - temporary

b. Formation of blocking Abs (IgG_2)

- **Administration of humanized monoclonal anti – IgE antibodies**

Contd.

- Administration of anti – histamines (not effective in asthma treatment). Bind to histamine receptors – blocks histamine binding
- Vasoconstrictors; reverse histamine effect. Blood vessel constriction with increase in pressure

Therapeutics contd.

- Epinephrine (Adrenalin): stimulates cAMP production by binding to B-adrenergic receptors on mast cells

- Cortisones – reduce histamine levels by blocking conversion of histidine to histamine

Therapeutic principles contd.

- Bronchodilators: methylated xanthines (caffeine, theophylline, theobromine) they stabilize cAMP levels
- Enzymes, e.g. monoamine oxidase: removes mediators
- Sodium cromoglycate: prevents Ca++ influx into cells, hence mediator release inhibited

Modulation of anaphylaxis

- Interplay between cAMP and cGMP levels in cell.
- High cGMP: increased release of mediators, hence severity of reaction
- High cAMP: decreased release of mediators, protection from reaction.
- Drugs to stimulate adenyl cyclase synthesis or inhibit phosphodiesterase synthesis used often as therapy.

Prausnitz – Kustner (PK) reaction

- **Used to passively sensitize normal individuals to give atopic allergic response**
- **Inject sera of atopic person into skin of normal individual**
- **Challenge after 1 day or up to 6 weeks with allergen at same skin site**
- **See wheal and erythema response**

Application of P – K test

- **To identify etiology of many unknown environmental allergens:**
- **To avoid direct skin test on young children**
- **Sera of atopic persons do not give noticeable reaction in test tube, but with passive transfer the wheal and erythema reaction readily seen.**

Serum IgE

- Type I allergic persons have higher than normal serum IgE levels
- Levels can be measured as diagnostic tool
- Level higher than in normal people
- RIST (Radio ImmunoSorbent Test) measures total IgE in serum
- RAST (Radio Allergo Sorbent Test) measures IgE conc. to a specific allergen

RIST

- Total IgE Measurement:
- **Involves competitive binding radio-immuno assay**
- **Uses patient's serum as test IgE**
- **Competes with ^{125}I labeled IgE to bind to anti – IgE on inert adsorbent**
- **Radioactivity decreases as test IgE level increases**

RAST

- Specific IgE measurement:
- Specific IgE to a specific allergen measured
- Uses radio-labeled immuno assay.
- Bound radioactivity increases with level of specific IgE bound
- Can detect few nanograms of IgE present

Detection of type 1 hypersensitivity

- Normally by skin test.
- Introduce potential allergen to skin site by scratching or intradermally e.g. forearm
- See wheal and erythema response within 30 min
- Drawback: allergen may sensitize individual

TYPE II REACTIONS

- **Involves Ag – Ab reaction initiated by IgG. No IgE involvement, no release of vasoactive amines required.**
- **Cell damage caused by neutrophils and natural killer (NK) cells.**
- **Occurs within 2 – 8 hrs. Called cytotoxic hypersensitivity**

Type II contd.

- Mechanism:
- **Abs directed against cell – surface Ags mediate cell destruction via:**
- **i. Release of cytotoxic killer (K) cells**
- **ii. Cell lysis thro' complement activation**
- **2 types; isoimmune and autoimmune reactions**

Type II contd.

- **Isoimmune:**
- **Reaction against individual's own body. e.g. Blood transfusion reactions; recipient reacts to the transfused blood. Gives isoimmune hemolytic anemia**
- **The CHO on RBC transfused behaves as iso Ag**
- **Erythroblastosis fetalis: (blue baby disease) due to Rh incompatibility between baby's dad and mom.**

Type II examples

- Autoimmune:
- **Produces Ab against its own body components, e.g. autoimmune hemolytic anemia. Ab directed against own red cells as Ag. Causes autoimmune diseases**

TYPE III REACTIONS

- **Due to immune complexes. Occurs within 2 – 8 hr of allergen introduction**
- **Mechanism:**
- **Ag – Ab complexes deposited in various tissues induce complement activation reaction.**
- **Inflammatory responses result as a consequence**
- **Two types: localized and generalized; viz.**

  Localized: Arthur's reaction. Occurs in Ab excess, Ag_2Ab_3 etc

Examples of Arthur's reaction

- **Pneumonitis; Reaction occurs in lungs due to inhalation of bacterial or fungal spores, dried faeces in air**
- **Farmer's lung: inhalation of thermophilic actinomyces from moldy hay**
- **Pigeon fancier's disease: from inhalation of a protein in dried pigeon faeces.**
- **All are localized reactions in the lungs.**

Type III contd.

- **Generalized reactions:**
- **Occur in Ag excess; Ag_3Ab_2, Ag_4Ab_3 etc.**
- **Involves deposition of soluble complexes in vicinity of reaction. e.g.**
- **Complement fixation involved**
- **Serum sickness – fever, rashes, malaise**
- **Rheumatoid arthritis – complexes normally in joints**
- **Causes inflammation and pain in joints**

Type IV (Delayed)

- **Cell – mediated. Occurs within 24 – 72 hrs**
- Mechanism:
- **Sensitized T cells (DTH) cells**

undergo blast transformation→ lymphoblasts

Killer cells and cytokines produced by blasts

- **Cytokines activate macrophages or Tc cells which cause direct cell damage.**
- **e.g. Contact dermatitis; graft rejection**
- **Reactions to M. tuberculosis**

Delayed reactions contd.

- **Tuberculin skin test - causes necrosis**
- BCG vaccine gives increased resistance to tuberculosis, but does not give complete protection.
- Individuals are sensitive by delayed reaction which can cause necrosis if severe enough. (Infant BCG vaccination)
- BCG: Bacille Calmette Guerin

Delayed contd.

- **Penicillin allergy: very common allergic reaction. Mainly in bulk penicillin handlers;**
- **nurses, doctors, workers in pharmaceutical industries, etc.**
- **Patch test used to differentiate type I penicillin allergy from delayed type**
- **Delayed penicillin allergy more common**

Delayed

- Examples:
- **Contact dermatitis: By mere contact of skin by allergen.**
- **Responsible for many allergic skin diseases**
- **Cosmetics, antibiotics, metals (jewelry), catechols from the poison ivy plant etc. are potential allergens**

AUTOIMMUNITY & DISEASES

- **Arises when immune system produces Abs against own body components, i.e. some of self Ags.**
- **Response gives rise to diseases called autoimmune diseases.**
- **Organ – specific or systemic diseases.**
- Organ-specific: **reaction confined to a specific organ**
- Systemic: **directed to many organs**

Why Autoimmunity?

- **1. Change in distribution of self Ag, allowing access to lymphoid cells that have never encountered them before**

- **2.Change in structure of self Ag. giving rise to Ab formation against new structure.**
- **cross-reacts with native component**

- **3. Emergence of abnormal or forbidden clones**

Some examples

- **Myasthenia gravis**
- **Systemic Lupus Erythematosus (SLE)**
- **Insulin Dependent Diabetic Mellitus (IDDM)**
- **Multiple Sclerosis**
- **Grave's disease**
- **Male infertility**
- **Addison's disease**
- **Hashimoto thyroiditis, etc.**
- **Have both genetic and environmental basis**
- **See Handout**

Hashimoto disease

- **Auto Abs and sensitized T_{DTH} against thyroid Ags e.g. thyroglobulin & thyroid peroxidase**
- **Ab binding to above Ags. Prevents I_2 uptake by both**
- **Leads to decreased production of thyroid hormones, i.e. hypothyroidism**
- **Cellular infiltration of thyroid gland causes gland enlargement called goitre. Common in middle –aged women**

Pernicious Anemia – from B_{12} deficiency

- **An autoimmune disease. Auto Abs to Intrinsic Factor (IF).**
- **IF facilitates B_{12} uptake from small intestine**
- **Binding of auto Ab to IF blocks B_{12} absorption**
- **Lack of B_{12} prevents proper hematopoiesis with low levels of functional mature RBC**

ALLOANTIGENS ON CELL SURFACE

- Are of 2 types:
- Blood Group substances: involved in blood transfusion

- Histocompatibility substances: involved in tissue/organ transplantations

Definition

- Allo Ags: Substances from certain individuals that are immunogenic in other members of same species, but not in the donor.
- Are Ags that segregate genetically within a species such that they are present in some but absent in others.
- Allo: Greek = other
- First identified on erythrocytes

Blood Groups

- Most important are ABO and Rh for blood transfusion
- All humans classified into 4 groups based on red cell agglutination by normal human serum (see table provided).
- An individual's blood group based on type of red cell allo Ag
- E.g. A with allo Ag A on red cell etc.

Blood groups contd

- Group O: no distinct allo Ag found on red cell surface. Obligatory precursor for A, B
- Group AB has both allo Ag A & B on surface
- Group A are of 2 types: A_1 & A_2 (80% A_1, 20% A_2)
- **Distribution**
- Saliva, gastric juice, sweat, pancreatic secretions, ovarian cyst etc. in addition to red cell membranes

Genotype vs. Phenotype

Genotype	Phenotype
AA, AO	Group A
BB, BO	Group B
AB	Group AB
OO	Group O

Allo-antibodies

- Allo Abs: anti-A in B group, anti-B in A group and both anti-A and anti-B in O group. None in AB group.

- ABO allo Abs are of IgM class. Arose from immunization from gut flora which can cross react with red blood cells. Called hemagglutinins

Chemistry of ABO allo Ags

- Are glycoproteins of overall basic structure:
- 15 amino acid polypeptide + 5 sugar residues common to all. Called the H Ag
- Specificity associated with carbohydrate portion
- Composition: 75 – 80% CHO; 20 – 25 protein

Chemistry contd.

- Group A possess glycosyltransferase enzyme that adds N –acetylgalactosamine moiety to the H Ag to give A
- B group glycosyltransferase adds a galactose moiety to the H Ag to give B allo Ag (difference bet. A & B??)
- AB group has both A and B allo Ags. Add appropriate sugar to some of their H Ags

Minor Group allo Ags

- MNS system
- Lewis System
- Duffy Group
- Kell Group

- All may sometimes be involved in transfusion reactions

Rh Antigen

- Discovered alongside the ABO blood group Ags. Its discovery paved way for solving erythroblastosis fetalis problem.
- The second most important in blood transfusion.
- Named after monkey species in which originally identified

Classification of Rh Ag

- By 2 schemes:
- Weiner (uses Rh or H): Hence Rh^+ or Rh^-
- Fischer –Race uses combination letters Cc Dd Ee, based on type of Ag found on red cell surface.
- Individuals that possess the D Ag are Rh^+; those who lack it are Rh^-
- The D is most potent to cause transfusion reactions.

Rh Antibodies

- Anti- Rh Abs are of IgG class and arise from isoimmunization; e.g. through blood transfusion or pregnancy.
- Erythroblastosis fetalis: result of Rh incompatibility bet. father (Rh+) and mother (Rh-).
- Mother sensitized to paternal Rh Ags

Use of Rhogam

Blood Typing

- Involves identification of red cell allo Ags.
- Uses:
- Blood transfusion
- Genetic analysis, e.g. disputed paternity
- Anthropological survey of human populations; archeological work;
- Forensic medicine to distinguish human blood from animal blood, different human blood types in blood stains; semen (rape) saliva
- meat adulteration etc. All now **Superseded by DNA analysis**

Typing Method

- By agglutination reaction
- Unknown red cells typed with known antisera and vice versa
- Reaction carried out in physiological saline
- Both major and minor cross match required.

- Major cross match:

- Donor red cells + recipient's serum in presence and absence of Coomb's serum

- Tests for Abs in recipient's serum against donor cells;
- Major cause of severe hemolytic reactions

- Minor cross match:

- Recipient's red cells +Donor serum in presence and absence of Coomb's serum

- Tests for allo Abs in donor serum that attack recipient's red cells.
- Often very rare Since donors must have low Allo Ab titre to qualify as donor.

Coomb's Serum

- Consists of rabbit anti-serum to human γ globulins
- Used 'cos some patient's Abs do not agglutinate red cells directly *in vitro*,

- Coomb's serum: agglutination readily occurs

Transfusion Reactions

- Blood considered for transfusion must have the same major red cell allo Ags as those of recipient.
- Major and minor cross matches must be routinely carried out.
- Rh incompatibility also checked.
- These precautions avoid transfusion reactions most times

Reactions contd.

- Transfusion reactions rarely occur due to ff:
- Most red cell allo Ags only weakly immunogenic; do not mount serious immune responses
- Transfused red cells survive for limited time (3 – 4 weeks averagely)
- Most die before reasonable Abs formed to react with donor cells

How safe is transfusion?

- Statistics of success rate: (US, 1980s)
- a) 2 rxns per 100 transfusions
- b) 1 death per 1000 transfusions (13,000)**
- c) 4.5 x10^6 transfusions per year (24x10^6)
- ** 2001 data
- Hence success rate very high – pretty safe

Transfusion reactions contd.

- Reactions occasionally do occur.
- Serious and sometimes fatal due to massive intravascular clumping and hemolysis of red cells in recipient.

- This due to attack on donor's red cells by recipient's allo Abs

Transfusion reactions contd.

- The reverse, attack on recipient's red cells by donor allo Abs rarely serious due to ff:
- Allo Ab titre in all donors often very low (a requirement as donor)
- Dilution of donor blood in recipient (10 – fold) further lowers donor Ab titre.
- Prevents any serious reaction
- Reactions sometimes due to minor groups

Types of Transfusion reactions

- 1. Hemolytic rxn from ABO or Rh incompatibility. Why?

- Clerical errors, improper typing???
- Kell and Duffy group allo Ags similar rxn as ABO
- Outdated blood hemolysed

Hemolytic rxn contd.

- Chills with shaking, muscle ache, back pain, head ache, fever, chest pressure.
- Stop transfusion to prevent shock or renal failure from blood clots in vessels
- Severe rxns sometimes fatal

Reactions

- 2. Febrile reaction:
- Caused by leukocyte and platelet differences. Are histocompatibility Ags on nucleated cells
- Allergic type II isoimmune reactions
- Hemolytic disease of newborn due to Rh mismatch

Actions to take

- When hemolytic transfusion rxn occurs: stop transfusion immediately and do ff:

1. Compare blood from patient with pre-transfusion specimen

Presence of free Hb or increase in serum bilirubin confirms hemolytic rxn

2. Analyse patient's urine for Hb and albumin

Investigations contd.

- 3. Review typing procedures and repeat process to determine cause of rxn
- 4. If used Universal donor blood, repeat cross match to see if rxn due to minor side incompatibility
- 5. Examine remaining blood records;

date of collection, bacterial contamination etc.

6. Review blood bank records

Universal donors and Recipients

- When to use them?
- Only under emergency conditions

Universal Donors: Are type O; their red cells cannot react with anti-A or anti-B allo Abs in recipient

2 types of UDs

Based on recipient. For young female recipients, UDs must be type O Rh^-

UDs and URs

- **For old females past monopause, UDs can be type O Rh+**
- **Note: Universal donor blood must never be used if ample time for cross –matching**
- **Universal Recipients are type AB since they lack anti A and anti B allo Abs in their serum**