Pulmonary Host Defence

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Introduction

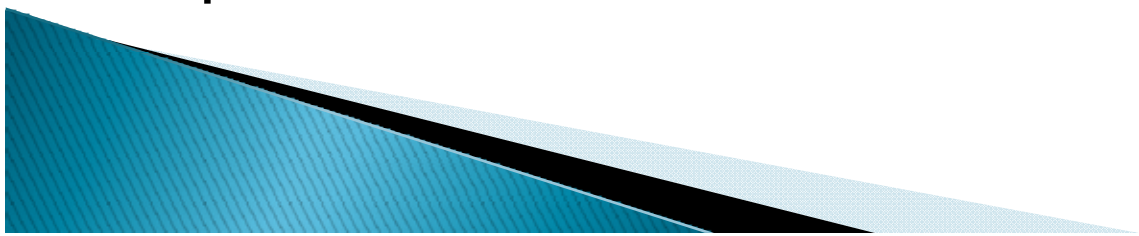
- major function of the respiratory system is to procure O_2 & to eliminate CO_2 from the body at rates required by tissue metabolism
- Ambient inspired air along with O_2 contains noxious gases and a multitude of particulates including viable microorganisms
- Toxic substances in air are derived from many sources

- naturally occurring processes can give rise to large amounts of aerosolized particles including
  - resuspension of soil
  - emissions from volcanic activity
  - forest fires, photochemical reactions etc.

- Airborne particles also include biologically derived viruses, bacteria, fungi, algae, spores, and pollens

- Industrial and occupational sources add SO_2, CO,NO_2, NH_4,O_3, hydrocarbons & inorganic and organic particles to the inspired air

- Upper and lower airways represent the largest epithelial surface exposed to the outside environment
- Size of alveolar surface is that of a tennis court
- To allow gas exchange foreign substances and microorganisms must be stopped and removed without inducing inflammation
- variety of defences exists to protect host from the harmful effect of microorganisms & air pollutants



Major constituents of lung defences

Airways and their mucosa

- Luminal defence mechanisms
  - Anatomical barrier
  - Cough
  - Mucociliary clearance
  - Secretory IgA
  - Lysozymes, lactoferrins
  - Defensins
- Epithelial cells
  - Epithelial barrier
  - Mucin release
  - Antimicrobial peptides
  - Bacterial receptors
  - Chemotactic factors
  - Growth factors; cytokines
- Blood derived cells of the mucosa
  - Dendritic cells
  - Lymphocytes (T-cells; cd; NK cells)
  - B lymphocytes
  - Eosinophils; mast cells; basophils

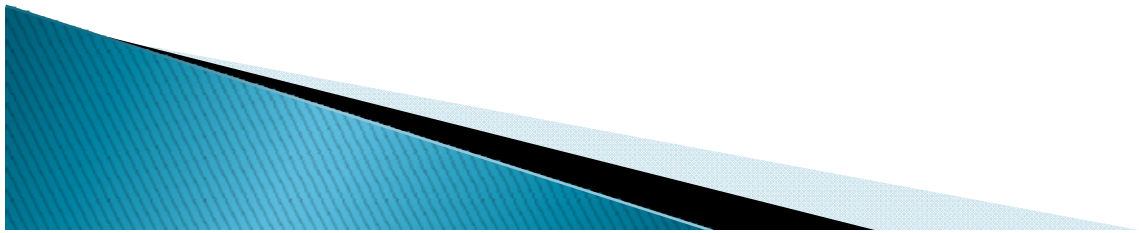
Alveolar spaces

- Pneumocyte types I and II
- Alveolar macrophages
- Lymphocytes
- Neutrophils
- IgG and opsonins
- Surfactant

Luminal defence mechanisms

Anatomical barrier

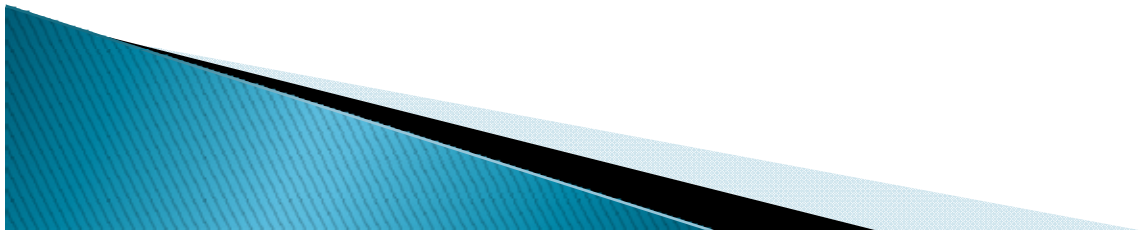
- nose is efficient upper airway defence mechanism that removes most airborne particles and water-soluble gases from inspired air
- Air entrained through the nasopharynx is subject to filtration through tortuous epithelial passages
- foreign particles of $\geq$ 10 µm effective diameter are efficiently removed & nasal hairs assist in trapping larger inhalants
- sneezing is an effective means of expelling some of airborne contaminants



- Microbes deposited into & attempting to colonize the nasopharynx has to compete against a dense flora of nonpathogenic endogenous bacteria
- Ciliated mucosal lining of the posterior nasopharynx propels mucus and trapped foreign material into the oropharynx
- bacteria become mixed with saliva and are then swallowed or expectorated
- Anatomic barriers of the epiglottis and vocal cords make aspiration into the trachea physically difficult

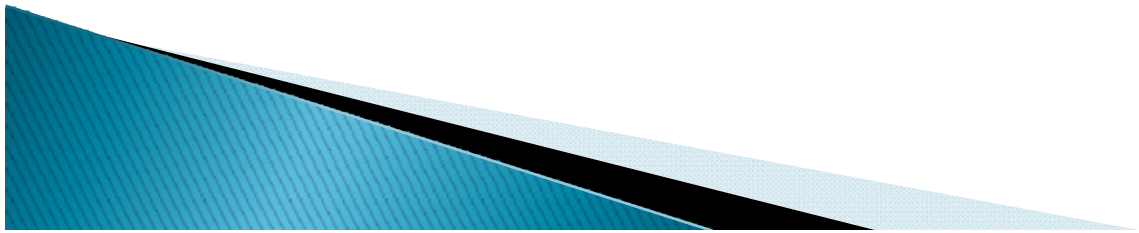
Cough & Mucociliary clearance

- glottis and upper airways have strong sensory innervation pathways & initiate cough reflex to unwanted aspirants
- mucociliary escalator removes particles of 2 to 10 µm in size that pass into the lower respiratory tract & are trapped in the tracheobronchial tree
- Orchestrated motion of cilia by pseudostratified respiratory epithelium effectivly moves infectious material into the central airways → swallowed or removed by cough

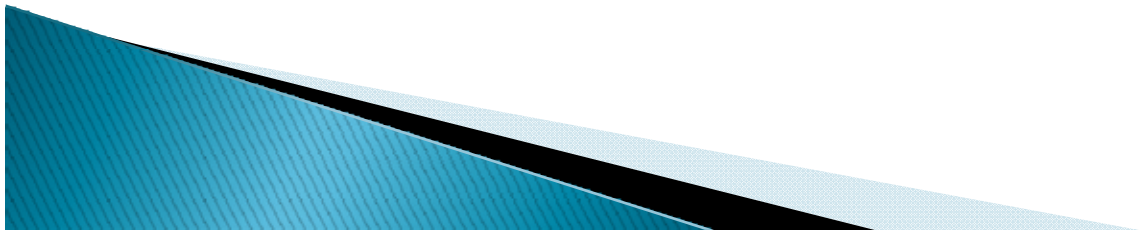


- Cilia beat at a frequency of between 1,000 to 1,500 cycles per minute propelling the overlying mucus at progressively increasing rates up the airways
- Linear velocities of mucus transport range from O.5 to 1 mm /minute in small airways to 5 to 20 mm /minute in the trachea and main bronchi
- Particles deposited in the trachea or proximal bronchial divisions are cleared with a half-time of about 30 minutes while distal ones are removed with half-times approaching several hours
- Material deposited on ciliated epithelium is vertually removed within 24 hours

- The airway mucus is composed of
  - sole phase -5-10 mm deep periciliary liquid allowing the cilia to beat
  - gel phase of 2-20 mm thickness on the surface of the cilia
- In purulent bronchitis decreased elasticity and increased viscosity of the mucus lining layer results in decreased mucus & loss of mucociliary clearance
- Disturbed mucociliary transport is also observed in patients with cystic fibrosis
- Cigarette smoke irritates the ciliated epithelium and is associated with ciliostasis and decreased particle transport



- Atmospheric pollutants – SO_2, NO_2, O_3 irritate the respiratory mucosa and depress mucociliary function
- General anesthetics, alcohol, and viral infections may decrease mucociliary clearance
- Pharmacologic agents may improve transport by changing the volume and physical properties of fluid and mucus or by improving ciliary activity
- Adrenergic agents, cholinergic agents, biologically active amines, and methylxanthines may be therapeutic in diseases associated with impairment of mucociliary transport

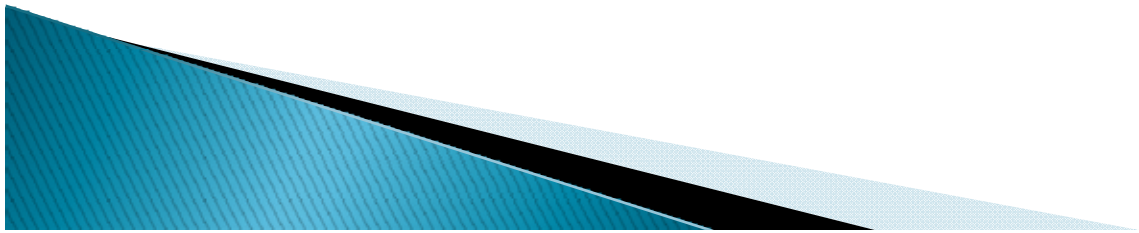


Secretory IgA

- released by the epithelial cells as dimeric molecules, associated with a single J chain of 23,000 daltons
- IgA neutralise toxins and viruses and block the entry of bacteria across the epithelium
- IgA are poor activators of the classical pathway of complement
- activate the alternate pathway for better opsonisation of bacteria

Annu Rev Immunol 1986; 4: 389–417

- Number of circumstances can alter protective barriers
  - Malnutrition – affects the integrity of mucosal epithelial cells and enhances bacterial adherence
  - Cigarette smoke and noxious fumes – disrupt the anatomy of epithelial junctions and enhance the passage of airway substances into inaccessible area
  - Bacteria – elaborate proteolytic enzymes
    - break down IgA
    - Promot selective colonization
  - persistence in matrix-enclosed biofilms help avoid innate immunity and create chronic infections



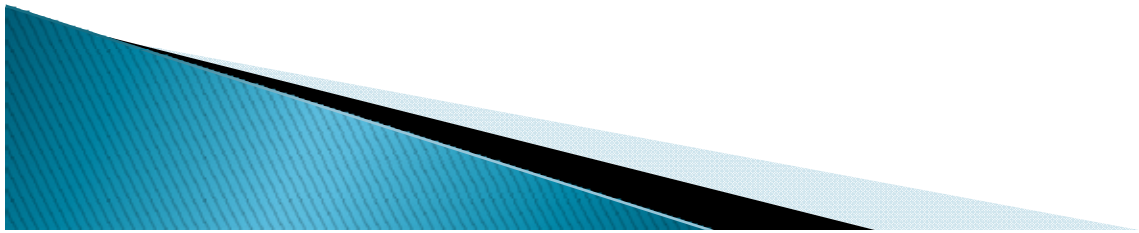
Lysozyme

- Secreted by glandular serous cells, surface epithelial cells and macrophages & represents an important antimicrobial defence -against Gram-positive bacteria

J Immunol 2000; 165: 5760-6

- Help in eliminating & decrease in systemic dissemination of pulmonary pseudomonas infection and group B streptococci

- clinical study of susceptibility and resistance to acute bronchitis showed a correlation of protection with levels of macrophage-derived lysozyme

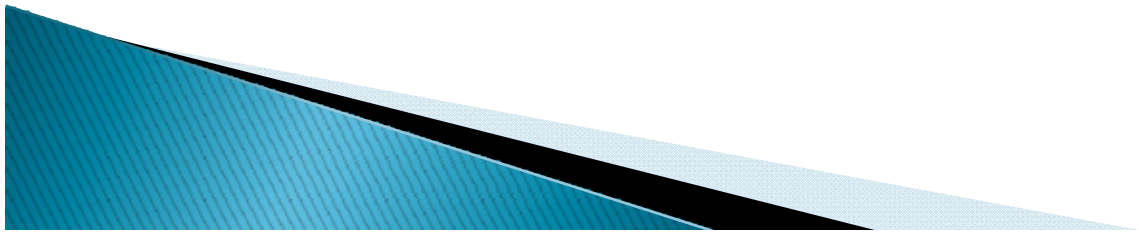


Lactoferrin

- iron-binding protein that reduces the availability of elemental iron needed for bacterial replication
- Lactoferrin are bactericidal by binding to endotoxin

J Clin Invest 1991; 88: 1080-1091

- Able to kill & agglutinate bacteria by recognising on the basis of carbohydrate motifs & stimulating superoxide production by neutrophils
- Concentration of lactoferrin is increased in the lower respiratory tract in chronic bronchitis



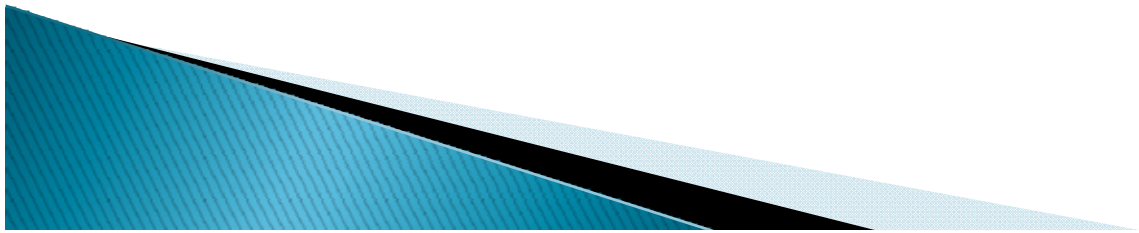
Defensins

- Antimicrobial proteins composed of small, single-chained cationic peptides & classified as α or β
- Cause pores in microbial membranes resulting in death and are active against a broad spectrum of pathogens→ gram + ve & – ve bacteria/ mycobacteria/ fungi & viruses
- Both classes of defensins are capable of
  - complement activation
  - chemokine stimulation
  - CD4+ T cell proliferation

- Act by increasing permeability & are up-regulated in the lung in response to IL-1

Proc Natl Acad Sci USA 1998; 95: 14961-6

- Alternative complement cascade is triggering by binding to complement component
- Function is highly salt concentration dependent & is impaired in CF
- Tracheal antimicrobial peptide is best characterised members of the β-defensin family → highly expressed in the ciliated airway epithelium & is up-regulated in response to bacterial LPS_1



Epithelial cells

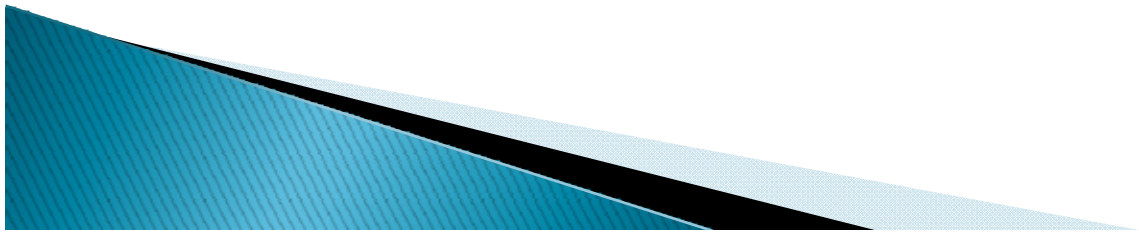
- Epithelial lining of luminal surface provide a mucosal barrier and contribute to the mucociliary clearance function
- Attached to neighbouring cells by – tight junctions, intermediate junctions, gap junctions and desmosomes
- Desmosomes mediate mechanical adhesion of cells to their neighbours and tight junctions completely obliterate the intercellular spaces just below the luminal surface

- Organisation of epithelial cells creates mechanical barrier & an ionic gradient allow bidirectional secretion of substances including antioxidents

Cytometry 1993; 14: 747–756

- Epithelial cells recruit inflammatory cells by releasing chemokines in response to bacterial products /viral infections /cigarette smoke

- Upregulate adhesion molecules in response to inflammatory stimuli → adhesion of neutrophils & mononuclear cells to inflamed area



- On exposure to IFN-γ epithelial cells can express MHC I & II
- limited capacity for presenting antigens to lymphocytes or to amplify an antigen-driven lymphocyte response
- By secreting antimicrobial peptides (β-defensins / lactoferrins) directly contribute to host defence

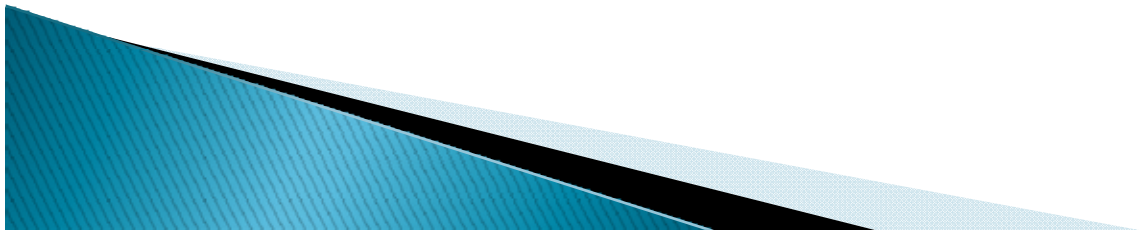
Proc Natl Acad Sci USA 1998; 95: 14961-14966

Blood derived cells of the mucosa

Dendritic cells

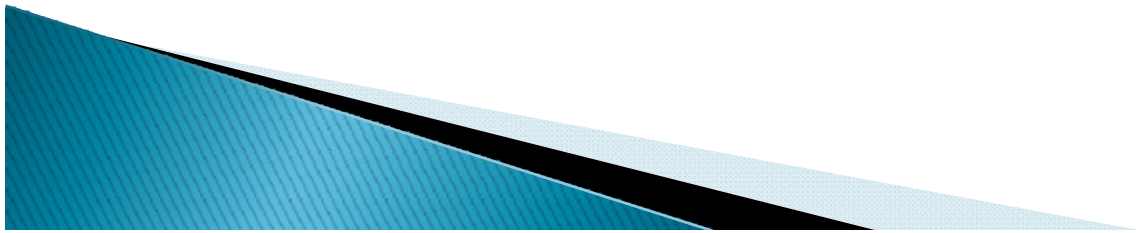
- Dendritic cells (DCs) are bone marrow-derived cells present from nasal mucosa to the lung pleura
- DCs lie above and below the basement membrane in a resting or immature state & extend their dendrites between the epithelial cells
- Form a network optimally situated to sample inhaled antigens
- Human lung DCs characterised by a high endocytic activity

- DCs involved in both innate & adaptive immune responses
- Respond immediately by stimulating NK cells to produce cytokines and kill targets in response to microbes invasion
- Maturation is process which transforms DCs into efficient APCs – activated
  - Directly – pathogen recognition receptors
  - Indirectly – inflammatory cytokines
- Generate effector B cells in the lymph nodes draining the lung



T cells

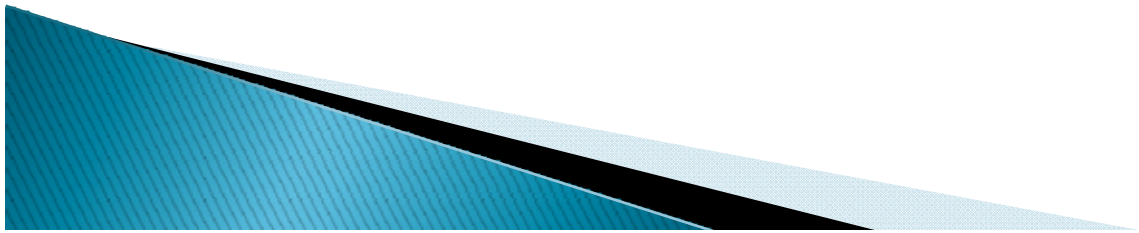
- Originate in the bone marrow from hematopoietic stem cells and then migrate to the thymus to undergo maturation
- Mature naive T cells enter the bloodstream and continuously circulate through the peripheral lymphoid organs
- In pulmonary infection antigens are carried from the lungs to the lung-associated lymph nodes by APCs
- In lymph nodes naive T cells encounter APCs & process of T cell activation, proliferation, and differentiation begins



- Development of adaptive immune response is induced when antigen from the site of infection is presented to a naive T cell bearing the antigen-specific T cell receptor

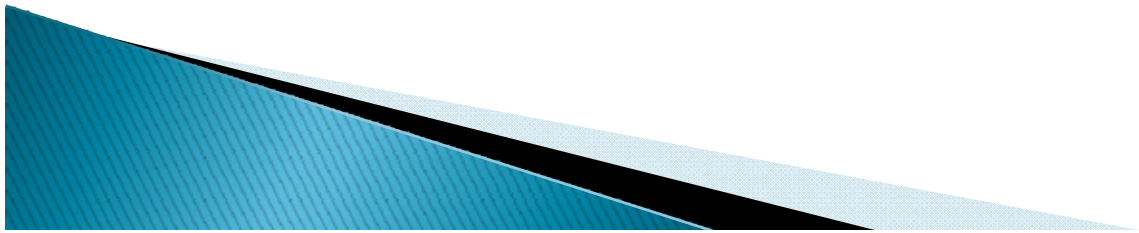
- BALT is a loosely organized group of lymphocytes that expands in response to pulmonary infections

- mucosal lymphoid tissue shares structural similarities with lymph nodes & serves as an additional site of T cell proliferation during chronic immune activation



- Each circulating naive T cell bears a TCR for a single antigen
- During infection only antigen-specific T cells will proliferate and differentiate into effector cells
- This clonal expansion of antigen-specific T cells occurs in the draining lymph nodes
- Activated T cells exit the lymph nodes and migrate back to the site of infection along a chemotactic gradient in the tissue

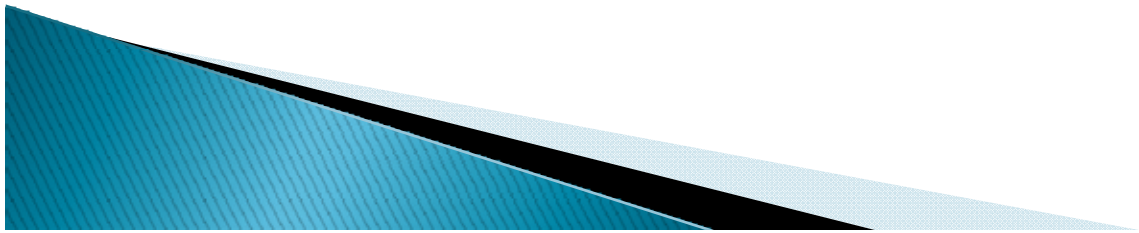
- T cell subsets are distinguished based on cellular surface markers and function
- CD4+ T cells polarize into T helper 1 (Th1) or Th2 cells
- Polarization of CD4+ cells is determined by the type of APC and the inflammatory milieu
- Th1 cells secrete IFN-γ which activates macrophages and facilitates clearance of intracellular pathogens



- Th2 cells secrete IL-4, IL-5, IL-6,IL-10, & IL-13 which are responsible for inducing T-dependent humoral immunity
- Th2 cytokines induce isotype switching of immunoglobulins from IgM to IgG, IgA, and IgE production

Eur Respir J 2001; 18:846-856

- Cytotoxic CD8+ T cells are responsible for the recognition and elimination of host cells infected with viruses and intracellular bacteria
- Overall T cells mediate a diverse set of functions including phagocyte activation, cellular killing, and immune modulation



NK cells

- derive from the same haematopoietic lineage as T lymphocytes but do not have to mature in the thymus & do not express re-arranged antigen receptors

- NK cells survey the body -- looking for cells that have altered expression of HLA class I tissue antigens due to viral infection or transformation

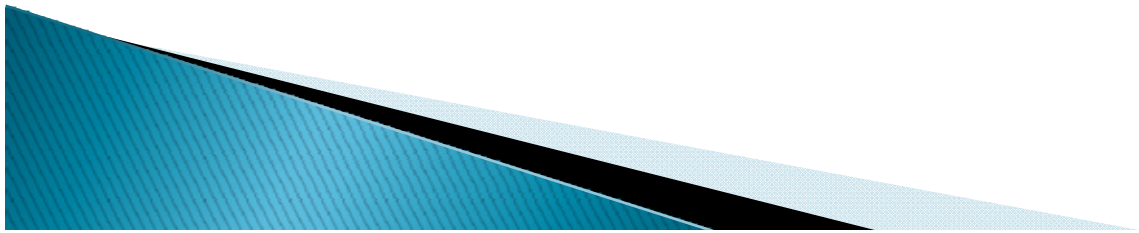
- fail to receive a cellular signal of normal HLA class I it enter a programme of activation leading to lysis of the infected cell and release of IFN-γ

- Local release of IL-12 is an important early event leading to stimulation of NK cells for rapid anti-viral responses in lung

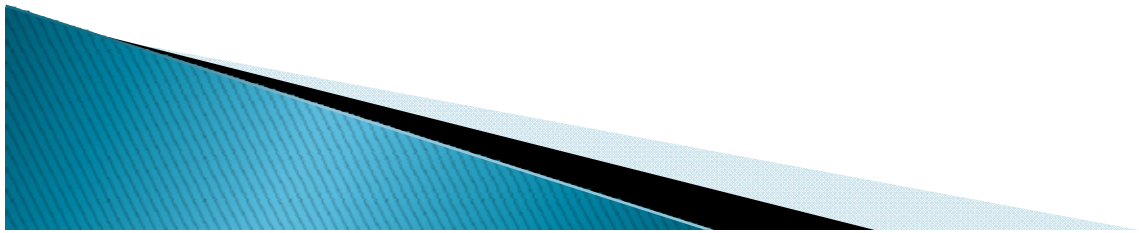
J Virol 1998; 72: 4825-31

- B lymphocytes are scattered in the airways

- After recurrent infections lymphocytes are found in follicles around the airways called bronchus-associated lymphoid tissues



- After initial exposure B-cell memory is established with a ability to mount a very rapid IgG antibody response on any subsequent re-exposure
- B lymphocyte-derived IgG response is unique in the immune response in showing affinity maturation
- ability to introduce small numbers of random mutations into the genes for the antibody, somatically mutating the sequence so that receptors with better affinity for the epitope are selected at each generation of cell division



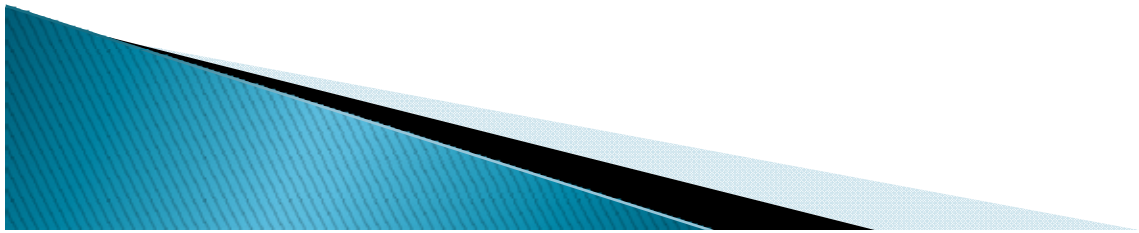
Eosinophils, mast cells and basophils

- Effector cells of immediate hypersensitivity reactions and allergic diseases
- Mature mast cells are found throughout the body predominantly located near blood vessels, nerves and beneath epithelia
- Usually not present in tissues, basophils are recruited to inflammatory sites along with eosinophils

- Eosinophils are seen in infiltrates of late phase reactions and contribute to pathological processes in allergic diseases

- Eosinophilic recruitment and activation is promoted by cytokines produced by Th2 cells

- Eosinophils release mediators that are toxic to parasitic organisms and may injure normal tissues



IMMUNE RESPONSE IN THE ALVEOLAR SPACES

Alveolar epithelial cells

- Pneumocytes are the major source of surfactant proteins
- SP A and SP D are members of the collectin family of mamalian lectins that contribute to pulmonary host defences
- SPs enhance the phagocytosis and killing of microbes

J Clin Invest 2002; 109: 707–712

Alveolar macrophages

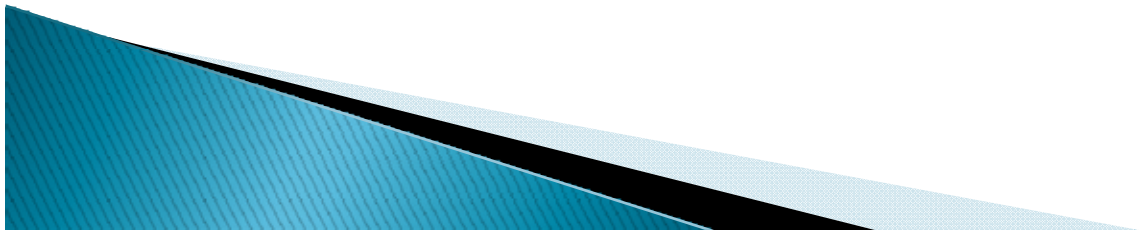
- Resident mononuclear phagocytes of the lung & provide the first line of defence against organisms or particles reaching the lower airways

- neutralise the invading pathogens or recruit neutrophils and other mononuclear cells

- Ability to interact with pathogens is mediated by surface receptors capable of binding to specific ligands→ including toxins, polysaccharides, lipopolysaccharides, complement proteins and Igs

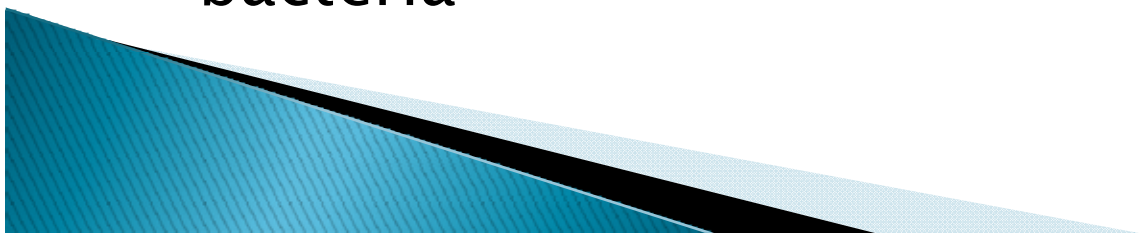
- AM initiate lung inflammation by the release of IL-1α & IL-1β or TNF -α, leading to inflammatory cascades in the alveolar milieu such as
  - appearance of adhesion molecules on endothelial cells or epithelial cells
  - release of chemokines and growth factors
- Important part of innate immunity this leads to activation of neighbouring cells and attract neutrophils
- Control inflammation by the release of inhibitors of IL- 1 or TNF-α in the form of IL-1 receptor antagonists or TNF soluble receptors or by release of IL-10

Am J Respir Cell Mol Biol 1995; 13: 83-90



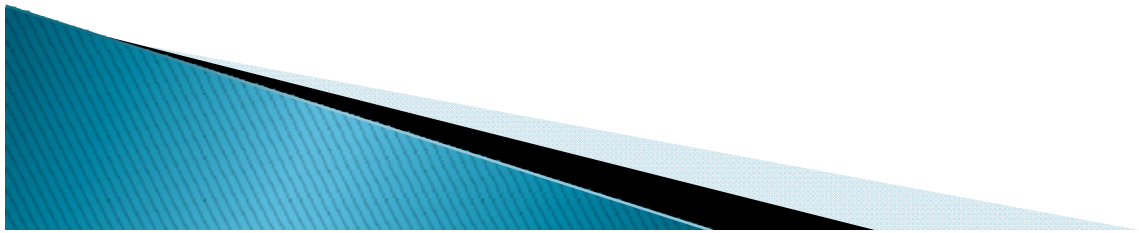
- AM have bactericidal activities realised by the production
  - lysozymes or defensins
  - cationic proteins capable of killing a wide variety of bacteria, including mycobacteria or fungi
  - Reactive oxygen intermediates (superoxide anion, hydrogen peroxide, hydroxyl radicals/or reactive nitrogen)

- Produce several components of complement which promotes the clearance of immune complex required for eliminating antibody coated bacteria



- AMs can acquire characteristics of DCs and may be able to activate T-cells
- Under the influence of innate and adaptive immune mechanisms may change their antigen capacity and/or cytokine production
- Can produce IL-12 when
  - stimulated by bacterial lipopolysaccharides and IFN-γ
  - during the interaction of CD40 – CD40L on T cells & macrophages

Am J Respir Cell Mol Biol 1999; 20: 270–278

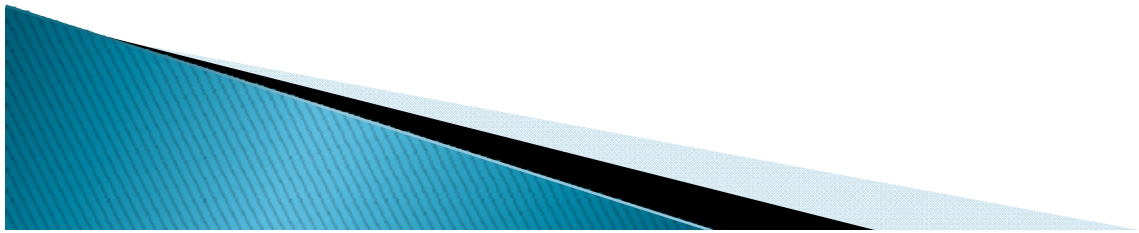


Lymphocytes

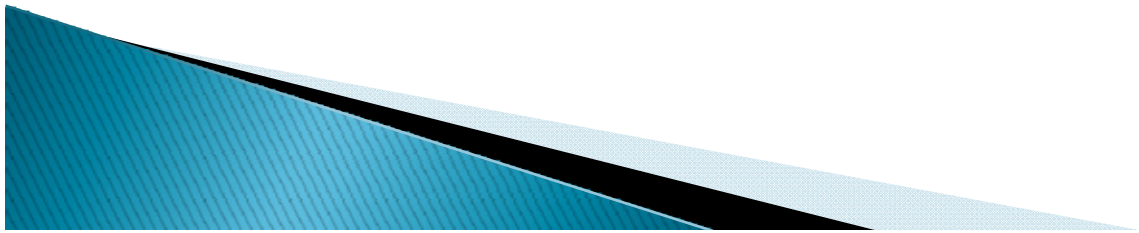
- Alveoli contain 10% lymphocytes of which
  - 50% are CD4
  - 30% are CD8
  - 10–15% are killer or NK cells
  - 5% B lymphocytes

- CD4/CD8 ratio is 1.5, similar to that of peripheral blood

- lymphocytes have altered phenotype and function in alveolar milieu → NK cells in the alveoli have a reduced cytotoxicity compared with interstitial NK cells

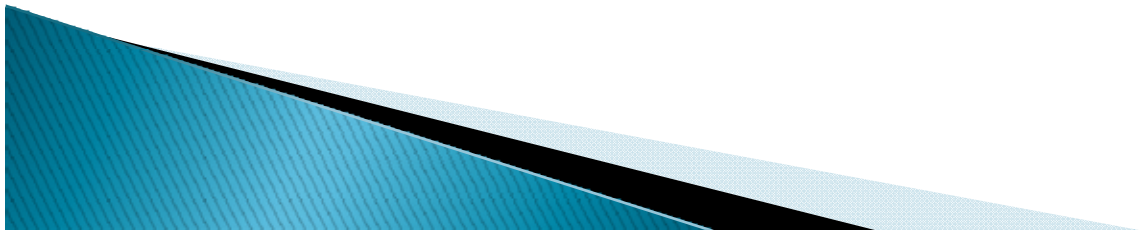


- B lymphocytes, CD4 and CD8 T-cells are major components of the adaptive immune response and most T-cells have a memory phenotype
- Once they are primed, T lymphocytes may be reactivated by DCs around the airways and vessels
- In vitro studies have shown endothelial cells potentially the most efficient antigen presenting cell
- CD4 and CD8 cells are key elements for the defence against viruses and bacterial clearance



Neutrophils

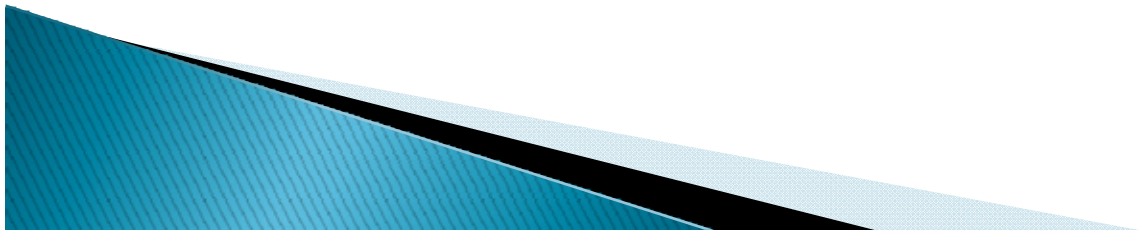
- Neutrophil recruitment is a major component of the protective host response to bacterial infections in the acute setting
- BAL normally represent 2% of the cells
- Massive flux of neutrophils occurs if AMs in the alveoli are unable to control infectious agents
- Chemokines are also small polypeptides critically involved in neutrophil recruitment



- Activated neutrophils eliminate microorganisms by
  - Phagocytosis
  - release of oxygen radicals
  - production of cytotoxic peptides or proteins

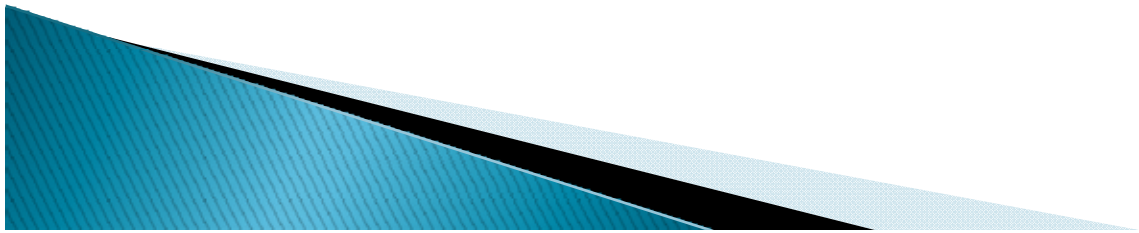
- Bacterial carbohydrate residues are attacked by enzymes, such as sialidase, x-mannosidase, β-glucuronidase, N-acetyl- β -glucosoaminidase and lysozyme

- Cytotoxic protein, such as neutrophil defensins and serine proteinases, damage bacterial membranes



Immunoglobulins and opsonins

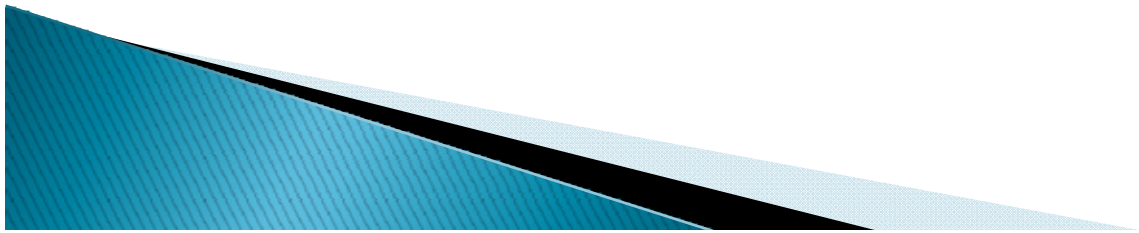
- Surfactant, fibronectin and C-reactive protein all have opsonic activities
- IgG constitutes 5% of the total protein content of BAL is the predominant Ig in the alveoli
- IgG_1 and IgG_2 are present in greatest concentration (65% and 28%, respectively)
- IgG_1 and IgG_3 are important as these two antibodies can fix complement



- IgG_2 is a type-specific antibody against pathogens such as Streptococcus pneumoniae or Haemophilus influenzae

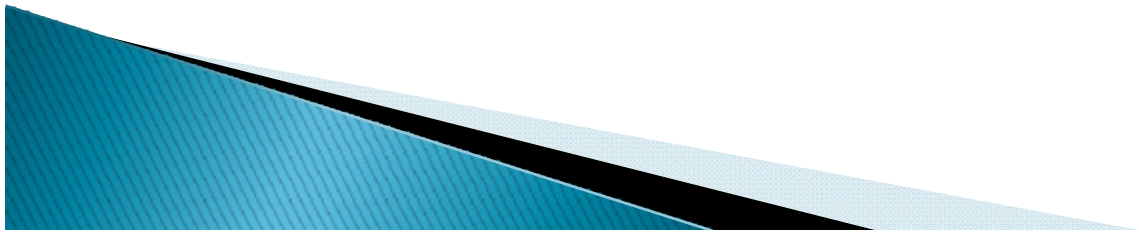
- IgG_4 acts as a reaginic antibody in allergic diseases & its increased levels lead to hypersensitivity pneumonitis

- Absence of IgG_4 leads to a predisposition to sinopulmonary infections and bronchiectasis



Summary

- Lung is challenged by the greatest onslaught of microbial pathogens most of which are capable of causing lethal infections if unopposed
- Immune response to respiratory infection must be rapid and efficient
- First line of defence comes from barriers such as mucus and cilia
- Followed by a battery of mediators that constitute the innate response including lactoferrin, lysozyme, collectins and defensins
- Activation can lead directly to lysis of pathogens, or to destruction through opsonisation or the recruitment of inflammatory cells



- The adaptive immune response includes the production of neutralising antibodies and the responses of T lymphocytes
- Different populations of T lymphocytes dramatically alter the balance between clearance of the pathogen and induction of tissue damage depending on the cytokines they secrete
- Dendritic cells bridge innate immunity with adaptive immunity
- Knowledge of these mechanisms is key when modulating immunity to increase defence mechanisms or decrease allergic phenomena