Stanford Microbiology and Immunology 104/204: Innate Immunology,

Lecture 5: Barrier epithelia and antimicrobial peptides

Structure of a lung

Trachea lead to bronchi to bronchioles to alveoli
It is a dead-end structure that must deal with inhaled particles which would otherwise accumulate in the alveolae

Mucus escalator

In the bronchioles the mucus escalator moves material up to the throat.
This escalator functions in the eustachian tubes, nose and lungs
Think of the escalator as moving flypaper that traps particles and moves them out of the lung.
The bronchiolar epithelia are composed of epithelia cells with many cilia, goblet cells that secrete mucus and mucus glands
In an uninfected state the mucus glands produce most of the mucus but during an infection the goblet cells are induced to produce large amounts of mucus resulting in the production of phlegm.

Alveoli

This is the location where oxygen and carbon dioxide are exchanged.
A thin epithelium is in close contact with capillaries, allowing rapid gas exchange.
Alveolar lumen contains
- Macrophages
- Complement
- Antibodies- IgA
- Surfactant – also an opsonizing lectin

Alveolus is a dead end – it contains no cilia and cannot cough things out;
Particles must be removed by macrophages

Infection and biowarfare considerations

Particles above a certain size (~10 um) will contact the mucus-lined walls of the bronchioles and be swept from the lungs.
Inhaled pathogens that survive in the alveolae must be smaller than this – in the 1-5 μm range we remember from the anthrax mail case.

How do you defeat the innate immune system in the lungs?

Streptococcus pneumoniae
- Colonizes nasopharynx
- Cilliary paralysis following influenza (dependent upon the influenza virus) allows the bacteria to enter the lung
- Capsule prevents phagocytosis by macrophages
- Virulence factor – pneumolysin may inhibit ciliated cells

Other bugs that do similar things
- Mycoplasma pneumonaie – enter epithelia

Bordetella pertussis – bind microvilli
Pseudomonas aeruginosa- inhibit cilia beating
Chlamydia pneumoniae –inhibit cilia beating
Neisseria meningitides – bind microvilli

Anthrax

Not a common inhaled pathogen but worth discussing because of the public discussion of particle size.
Spore clusters must be in smaller than 5 microns to make it into the alveoli without being caught in the escalator.

The spores are taken up by macrophages in alveoli and germinate within these cells. The germinated cell does not appear to divide during its life within the macrophage. The germinated cell lyses the lysosomal membrane and lives within the cytoplasm. The macrophage moves a lung lymph node where the infection proceeds.

Upon release from the macrophage, vegetative anthrax cells develop a capsule and are no longer phagocytosed.

Tuberculosis

Mycobacterium tuberculosis is infectious through the air
This microorganism has evolved methods of surviving for long periods in infected macrophages – we've seen this approach before with microorganisms like Salmonella, which can survive an inflammatory event by living within a macrophage.

Infections of insect tracheoles:

Will show below that they react by producing antimicrobial peptides.
It is difficult for them to remove particles – there are no macrophages. Perhaps as a result of this, parasitic mites can live within the trachea of honey bees.

Insects do not use a mucus layer in the same manner as vertebrates do.

It is important from a public health point of view as microbes vectored by insects must cross this barrier.
Insect guts are protected by a peritrophic matrix - a highly crosslinked chitin and protein containing semi-permeable membrane.

There are at least three types of peritrophic matrix
1. no matrix
2. Type I matrix synthesized post meal
3. Type II matrix, continuously synthesized

Malaria parasite uses chitinase to cut through this layer after it has formed.
Baculoviruses carry a virulence protein that degrades mucus.

Secreted antimicrobial peptides

Humans continuously secrete antimicrobial peptides and proteins and can deliver them upon activation by epithelia and immune cells.
Nasal fluid is a good example of a fluid containing antimicrobial peptides
Some antimicrobials are:
Lysozyme: cleaves peptidoglycan – recent discussions suggest that it might play an important role in clearing bacterial products to limit inflammation.
Lactoferrin: sequesters iron, blocks biofilm development of pseudomonas auriginosa
Alpha and β Defensins: antimicrobial peptides

Can be antibacterial, fungal, viral or in plants – anti-insect

The list of antimicrobial peptides is growing and it is difficult to state exactly how many families of peptides exist.

In humans there are:

α-defensins:	HNP1-4 granulocytes
	HNP5 paneth cells of gut, genital tract
β-defensins	hBD-1,2 skin lung gut (epithelia)
	hBD-3 skin lung tonsils
	hBD-4 testis, Gastric antrum
Cathelicidin	LL-37 granulocytes, lung, skin testis, gut, l lymphocytes

In humans alpha and beta defensins differ by where they are expressed (granules vs epithelia) but this is not the case in other creatures. They can be distinguished by the order of their 3 disulfide bonds.

In flies there are arguably 4 groups of peptides in addition lysozymes:

α-helical/ linear	cecropin
open ended cyclic	defensin
	drosomycin – anti fungal
Proline-rich	drosocin
	Metchikowin
Glycine-rich	diptericin
	attacin

In addition there are newly discovered families of cyclical peptides (theta defensins and others).

These small peptides were identified first in the cecropia moth and rabbit neutrophils and were not regarded as being of general interest until their discovery in frogs (of all things).

Cecropia were a useful tool as the pupae overwinter and shut down most of their metabolism in a refrigerator but still raise a rapid immune response and they are relatively large insects. The immune response is easy to spot as new protein synthesis.

Synthesis of peptides

All are secreted proteins, made with at least a signal sequence

α-defensins are synthesized as pre-pro-proteins and the pro-form is stored in granules. In humans, these are secreted into the lysosome of neutrophils.

β-defensins contain only a pro-sequence and are secreted directly into the extracellular milieu.

Cathelicidins are broadly found throughout mammals. These are unusual in that their pro-region (cathelin) is highly conserved and was identified first. The actual antimicrobial peptides are highly variable and are cleaved from cathelin for activation.

Buforin: a frog skin peptide derived from the proteolytic degradation of histones.

Mode of action

The majority of papers describe these peptides as pore formers but recent papers suggest that several of the peptides may act intracellularly and have effects on microbes even at sub-killing concentrations.

The lytic mechanisms for the alpha-helical/linear/cationic peptides are the best resolved but are still far from complete.

The alpha-helical proteins are amphipathic and their action depends upon this.

Non-cell specific peptides – like mellitin – a component of bee venom form barrel stave pores in membranes. In this model, the peptides must insert themselves across the membrane and contact both the phospholipid heads and hydrophobic cores of the lipid molecules

Cell specific peptides seem to work using a different mechanism

The principle model here is the torroidal pore or carpet model. In this model, the amphipathic helices insert into the outer surface of the membrane. A pore is formed in which the peptide remains in contact with the phospholipid heads of the lipids and the outer and inner leaflets of the membrane are fused. This can proceed to the carpet model in which the peptides completely cover both sides of the membrane and break it up, acting like a detergent.

Buforin is a histone H2A proteolytic product that does not lyse cells. It may enter cells and bind DNA.

Other methods

A pig cecropin PR-39 kills cells by rapidly stopping protein and DNA synthesis before the cells lyse. This is different from what was seen in cecropin controls.

A recent publication looking at the human cathelicidin LL37 showed that this peptide could enter cells. They propose an interesting model in which the peptide binds to DNA and induces its uptake and expression in human cells, leaving open the possibility of an entirely new class of virulence factors.

Bacterial transcriptional responses to antimicrobial peptides suggest that at least at low concentrations the bacteria are not acting as if they are suffering from common stress reactions that would be anticipated if the cells were being lysed.

Antimicrobial peptides can have functions beyond the antimicrobial

DNA entry LL37
Angiogenic:
Signaling
Mitogenic: defensins
Chemoattractant: defensins

Helicobacter uses an antimicrobial peptide as a virulence factor:

Helicobacter secretes a cecropin
This may help it defend its niche against other microbes
The cecropin also induces inflammation and attracts macrophages and monocytes.

Application –many want to use these peptides for medicinal purposes and argue that microbes can't develop resistance to these peptides. The argument goes something like: since the peptides are so different from each other they must work by a general mechanism as there is no specific target that can be mutated. Dream on.

Methods of Evading AMP function

Pseudomonas and Xenorhabditis

Both of these microbes secrete proteases that cleave antimicrobial peptides, rendering them ineffective. Clinical isolates of Pseudomonas have already been isolated that have altered sensitivity to cationic peptides.

Treatment of Salmonella with cationic peptides induces changes in transcription that should lead to alteration of LPS so that it is less charged and less likely to interact with cationic peptides.