Antibiotic Molecules: Intracellular

Secondary article

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Intracellular antibiotic molecules are the essential components of innate, i.e. nonadaptive, immunity. Some are stored proteins with highly specific antibiotic activity, others are stored peptides that have a broad antibacterial spectrum, yet others are short-lived molecular reagents that are generated by specific activation of phagocytes, the cells equipped especially for microbicidal activity.

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Introduction

Antibiotic molecules are compounds generated by one living organism that are able to kill other living organisms. Although not strictly part of the definition, it is understood that antibiotics are active against microorganisms. Not only was Alexander Fleming the first to discover antibiotic molecules generated by microorganisms (penicillin), he also discovered lysozyme, the first antibiotic molecule known to be generated by mammalian cells.

All living organisms are surrounded by microorganisms and have developed ways to protect themselves against these. Although the defence against microorganisms in general is effective, each cell in our body carries the testimony of a successful intrusion of microorganisms, millions of years ago – the mitochondria which are indispensable for proper energy metabolism of our cells. Advantageous as this has been, the degree of specialization of cells that make organs and bodies work would probably not survive a similar assimilation of microorganisms and we would rapidly succumb to infections without an effective defence against microorganisms. Several lines of defence have developed. Microorganisms in general make first contact with our body at mucosal surfaces. The sweeping flow of mucus transports microorganisms centripetally, to be sneezed out or swallowed. In addition to this physical defence, two chemical systems of basic or innate defence exist at possible ports of entry of microorganisms: the mobile phagocytes, which carry a ready-to-go armory of preformed or easily assembled antibacterial molecules to be delivered where microorganisms enter; and the mucosal or skin epithelial cells themselves, which may synthesize antibiotic agents similar to some of those found in the mobile phagocytes. The focus of this section will be on the antimicrobial molecules of phagocytes.

Targeting Phagocytes to Sites of Microbial Invasion

Phagocytes (Greek: eating cells), the cells especially equipped for seeking out and destroying intruding microorganisms, were discovered and named by Ilya Metchnikoff more than 100 years ago.

The phagocytes are either localized as tissue macrophages and wait for the microorganisms to be brought by them (fixed macrophages in spleen, lungs and liver), or are migrating phagocytes that are able actively and rapidly to localize to microorganisms that are gaining access to tissues. The most numerous of the mobile phagocytes in the human body is the neutrophilic granulocyte. This is also the fastest migrating of the mobile phagocytes and the first to arrive at sites of microbial entrance. Neutrophils are able to sense the presence of microorganisms via bacterial products, *N*-formylated peptides, that elicit a chemotactic (directed movement along a chemical gradient) response in the neutrophils. The highly specific guidance of neutrophils towards invading microorganisms (which may result in an abscess) is the result of a combined action of the vascular lining (endothelial cells) and the active and directed migration of the neutrophils. Intruding microorganisms elicit a localized activation of cells in the vascular bed. (This results macroscopically in local swelling and redness.) The activated endothelial cells of the postcapillary venules put out adhesion proteins (selectins) on their luminal surface. These capture circulating neutrophils and make it possible for the neutrophils to emigrate from the blood into tissues where they subsequently establish physical contact with the microorganisms. This is mediated by receptors that may either recognize specific carbohydrate structures on the microorganisms or recognize opsonins (components of the complement system and specific antibodies that are

proteins which our adaptive humoral immune system has generated to cover microorganisms and make them suitable for binding to receptors on phagocytes). The addition of substances (specific antibodies and/or complement) that enhance the subsequent uptake of microorganisms by phagocytes is called opsonization.

Microbicidal Acts of Phagocytes

Phagocytes engulf microorganisms by a specific process called phagocytosis. Microorganisms are sequestered in an invagination of the plasma membrane called the phagocytic vacuole and become exposed to microbicidal agents that may be generated locally (reactive oxygen derivatives) or transported to the phagocytic vacuole from storage granules in the phagocyte. .

The neutrophil contains two essentially different types of storage granules: peroxidase-positive granules, which contain most of the preformed antibiotic substances of the neutrophil in addition to proteolytic enzymes and the important enzyme, myeloperoxidase, which is a marker protein for these granules (peroxidase positive granules); and peroxidase-negative granules, which contribute especially to the microbicidal activity by the flavocytochrome protein complex $gp91^{phox}/p22^{phox}$, which is part of the superoxide anion generating the reduced form of nicotinamide–adenine dinucleotide phosphate (NADPH) oxidase. In addition, specific granules contain proteins that, either alone or in combination with proteins from azurophil granules, contribute to the killing of ingested microorganisms (**Figures 1** and **2**).

Antibiotic Molecules

Antibiotic molecules range from complex proteins that are stored intracellularly in granules to short-lived reactive molecules such as hydroxyl radicals and nitrous oxide generated in response to specific activation of the phagocytes. In this entry, antibiotic molecules will be classified according to their chemical nature and mode of action (**Table 1**).

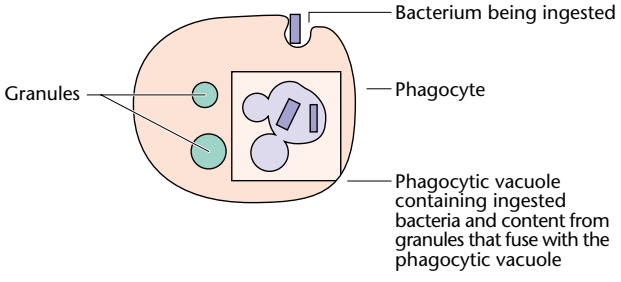

Figure 1 A neutrophil phagocytizes microorganisms and takes them into a phagocytic vacuole (inset, **Figure 2**), into which the two major granule subsets empty their contents to create a microbicidal milieu.

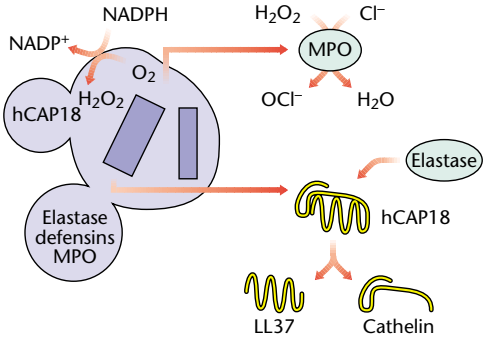

Figure 2 Inset from Figure 1 showing how major bactericidal systems are generated by combining the contents of peroxidase-positive and peroxidase-negative granules. Myeloperoxidase (MPO) works on hydrogen peroxide, the end product of the NADPH oxidase from specific granule membranes. Elastase from peroxidase-positive granules activates the cathelicidin hCAP18 from specific granules.

Pore-forming Antibiotic Molecules

Evolution, through different ways, has reached the same solution to the problem that faces all higher organisms – how to protect themselves against microorganisms. Although the antibiotics that we use in medicine are largely effective and work like penicillin and quinolones as inhibitors of specific and essential enzymes of bacteria, the problem is that they are not active against a wide range of microorganisms and, more importantly, resistance develops quite easily – a major and increasing problem. The agents that our body relies on in defence against microorganisms must work by more general mechanisms in order to be effective throughout evolution. The pore-forming antibiotic peptides seem ideal, as testified by the fact that the same overall structures have evolved in different unrelated species, and from no common ancestral gene. The basic principle is that antibiotic peptides permeabilize the surface membrane of microorganisms. This is achieved by assembling small peptides that associate on the membrane and insert and form pores. This offers several advantages. Being small, the peptides are rapidly made and less likely to be attacked by proteases. Also, being small and not individually able to form a lytic pore, the peptides retain the capacity to be both water-soluble and lipophilic, as opposed to being an integral membrane protein, which would be the alternative if the lytic pore were formed by one protein. Furthermore, a large pore-forming protein would be likely to be highly sensitive to mutations that would affect the three-dimensional structure and thus its function, whereas lytic pores composed of multimers of smaller peptides are less likely to be sensitive to mutations in the 'building blocks'.

Table 1 Antibiotic molecules

Pore-forming antibiotic molecules
Cecropins
Defensins
Magainins
Cathelicidins
Antibiotic molecules that work by stoichiometric mechanisms other than pore formation
Bactericidal permeability-increasing protein (BPI)
Cationic protein of 37 kDa (CAP-37)
Myeloid related proteins 8/14 (MRP-8/14)
Antimetabolic antibiotic molecules
Lactoferrin
Natural resistance-associated macrophage protein (Nramp)
Vitamin B_{12}-binding protein
Antibiotic molecules with enzymatic activity
Lysozyme
Phospholipase A_2
Chitinases
Elastase
Reactive oxygen derivatives
Superoxide anions
Hydrogen peroxide
Hypochlorous acid
Chloramines
Reactive nitrous derivatives
Nitric oxide

The antibiotic peptides are in general amphiphilic peptides that have a positively charged domain, which is necessary for binding to the negatively charged phospholipids of the bacterial surface membranes, and a hydrophobic domain that allows the peptides to insert into the phospholipid bilayer. The antibiotic peptides may then organize as multimeric complexes that constitute a membrane pore with ion channel activity that leads to dissipation of the intracellular ionic milieu and death of the microorganism. The effective concentration of antibacterial peptides is in general, around $1–10\,\mu g\,mL^{-1}$.

Since phospholipids are part of the surrounding membrane of all organisms, the antibiotic peptides that work by binding to the phospholipids and by insertion into the phospholipid bilayer are generally very broad in their antimicrobial spectrum. i.e. active against both Gram-positive and Gram-negative bacteria and fungi. Some are even toxic to mammalian cells, but in general most are more active against microorganisms than against eukaryotic cells. This is a reflection of differences in the composition of the membrane phospholipids of microorganisms and of eukaryotic cells. The antibiotic peptides are in general more lytic the more anionic the phospholipids. Some antibiotic peptides are inhibited by cholesterol, which is found in the phospholipid bilayer of eukaryotic cells but not in microorganisms (Boman, 1995).

Cecropins

Cecropins were the first antibacterial peptides identified (Steiner *et al*., 1981). They were isolated from the immune haemolymph of the *Hyalophora cecropia* moth; hence their name, which does not reflect a specific structure but an origin. Antibiotic peptides from other insects have been named differently. (To add to the confused terminology, which is a characteristic of antibiotic peptides, a mammalian 'cecropin', cecropin P1, has been isolated from the small intestines of pigs; however, its cellular source has not been identified.) Cecropins are 31–39-amino acid residues long, strongly basic peptides. Cecropins form an amphipathic α helix, and may assemble into complexes on the surface of microorganisms to form pores. Cecropins are active against both Gram-positive and Gram-negative bacteria. The mammalian cecropin does not aggregate on the lipid surface. This may relate to its reduced antibacterial activity against Gram-positive bacteria compared with the insect cecropins. Cecropins are not stored intracellularly but their synthesis is induced in response to infection or injury, like most of the epithelial defensins.

Defensins

Defensins are small 3–4-kDa peptides (Ganz and Lehrer, 1995). They are active against both Gram-positive and Gram-negative bacteria, against fungi and against some enveloped viruses. They are present in a wide spectrum of organisms, from plants to insects to mammals. Defensins share a structural motif of three disulfide bonds, which are essential for maintaining the hydrophobic β sheet that endows defensins with the ability to insert into the lipid bilayer. The vertebrate defensins are divided into two main families, the classical defensins (or α-defensins) and the β-defensins. This classification is based on the pairing of six canonical cysteins to form three disulfide bridges. Insect and plant defensins further segregate out as subgroups of defensins with yet different organizations of the disulfide bonds. The different subgroups of defensins have not evolved from the same ancestral gene, but most likely represent the independent but successful solution by different species to the same basic problem: how to get the best defence against microorganisms.

The classical defensins (first identified in rabbits) are stored in peroxidase-positive granules of neutrophils and in Paneth cells of the small intestines. In mice, which lack α-defensins in neutrophil granules, α-defensins known as cryptdins are found in crypt cells of the intestinal mucosa. In contrast, most β-defensins are not stored but induced in epithelial cells in response to infectious or inflammatory events, and have been demonstrated in airway epithelial

cells as tracheal antibacterial peptide (TAP), in tongue epithelium, and in the epithelium of the genitourinary tract. Insect defensins are induced in the haemolymph in response to microbial challenge, and plant defensins, like insect defensins, are largely induced in response to microbial infection.

Amphibian antibacterial peptides (magainins)

The first amphibian antibacterial peptide identified was named magainin (Hebrew: shield) (Zasloff, 1987). Magainins are representative of a vast number of largely similar antibacterial peptides that are stored in neuroepithelial cells of amphibians and secreted in response to injury or infection to form a highly antiseptic (bacteria-resistant) mesh that covers the epithelial surfaces. Like cecropins, the antibacterial activity of amphibian antibacterial peptides lies in an amphipathic α helix. The antimicrobial activity is very broad. Although many different antibiotic peptides are found in amphibians, they do not seem to have a specialized function against certain microorganisms. Instead, the antibacterial activity works by general mechanisms, as discussed above, that prevent resistance developing. It is not clear whether the multitude of antibacterial peptides represents a redundancy or back-up, or whether the general theme, small amphipathic α-helical peptides are of such basic structure that evolution does not put a specific stress on refining the structure.

Cathelicidins

Cathelicidins are a family of antibacterial peptides that are found only in mammals (Zanetti *et al*., 1995) There are now some 40 members described. Cathelicidins seem to represent the accumulated wisdom acquired by less developed organisms during evolution, as they use most of the varying mechanisms of antimicrobial activity that are found in other families of antibiotic peptides. Despite working in a manner similar to peptides, 11–19-kDa cathelicidins are actually polypeptides.

The N-terminal part of cathelicidins, named after cathelin, a protein purified from porcine neutrophils, is highly conserved and thus qualifies the members of the cathelicidin family. The parts of cathelicidins, C-terminal to the cathelin-like segment, are highly divergent; the shortest are 12 amino acids and the longest over 100 amino acids. Cathelicidins are stored in peroxidase-negative granules of neutrophils and most, with the exception of the p15s from rabbits, are without antibacterial activity until the C-terminal part is proteolytically removed from the cathelin part. For most cathelicidins this has been shown to be executed by elastase, a proteolytic enzyme present in the azurophil granules. The meeting point of contents from azurophil and specific granules is the phagocytic vacuole, the convenient place where microorganisms (**Figure 2**) are sequestered inside phagocytes to be exposed to high concentrations of antimicrobial agents.

Some of these antibacterial C-terminal parts of cathelicidins have the same basic structure as magainins and cecropins, i.e. amphipathic α helices, and some have adopted a defensin-like amphipathic β sheet. In the latter case the β sheet is maintained by two disulfide bonds and not three, as in defensins. The cathelicidins which use this structure are known as protegrins. In addition, some C-termini of cathelicidins have long stretches of repeated prolines and arginines. These are in general not bactericidal but bacteriostatic, and do not work by forming pores, but their mechanism of action is largely unknown.

Cathelicidins in general are active against both Gram-positive and Gram-negative bacteria and fungi. The antibacterial activity is only unleashed after cleavage of the C-terminal peptide from the cathelin part. However, both the intact peptide and the antibacterial C-terminus bind the lipopolysaccharide (LPS) coat of Gram-negative bacteria (endotoxin) and may neutralize this important inflammatory mediator. While several cathelicidins are present in neutrophils of ruminants, only one human cathelicidin exists, hCAP-18, also called LL-37 from the size and initial amino acids of its active C-terminal peptide. In addition, hCAP-18 has been shown to be induced in the skin of patients with psoriasis, eczema and atopic dermatitis.

Antibiotic Molecules that Work by Stoichiometric Mechanisms Other than Pore Formation

Bactericidal permeability-increasing protein (BPI)

BPI is a 57-kDa protein with a highly positive charged N-terminal half and a neutral but highly hydrophobic C-terminal half. BPI is a constituent of azurophil granules of neutrophils. It binds to LPS in the outer membranes of Gram-negative organisms and induces a lethal increase in permeability in these microorganisms. Thus, the protein is only active against LPS containing Gram-negative microorganisms. In a cellular extract from neutrophils, nearly all antibacterial activity against *Escherichia coli* can be ascribed to BPI. BPI has significant structural homology to the LPS-binding protein of plasma. A recombinant form of the LPS-binding part of the protein is now in clinical use as an LPS-neutralizing agent for patients with Gram-negative sepsis (Elsbach, 1998).

Cationic protein of 37 kDa (CAP-37)

CAP-37, also known as azurocidin and heparin-binding protein, is a cationic protein of 37 kDa found in azurophil granules of neutrophils (with BPI, myeloperoxidase, elastase, and α-defensins among others). The protein has some antibacterial activity against Gram-negative microorganisms, although the mechanism of action is not known. Currently, the protein attracts attention as a strong chemoattractant for monocytes.

Myeloid-related proteins (MRPs)

MRP-8/14 is a complex of two proteins at 8 and 14 kDa that constitute almost 50% of the total protein of neutrophil cytoplasm. MRPs are also known as calgranulin A and B, as calprotectin, and as cystic fibrosis antigen. MRPs belong to the S-100 family of calcium-binding proteins. The specific function of MRP-8/14 is not known, but a potent fungistatic activity, especially against *Candida albicans*, has been noted (Murthy *et al*., 1993). The complex is antifungal at 1 μmol L^{-1} concentration (approximately 30 μg mL^{-1}). This should compare with an intracellular concentration of approximately 300 μmol L^{-1} in neutrophils. MRP-like molecules (likely MRPs) have been identified on inflamed epithelial cells. Whether this represents *de novo* synthesis in nonmyeloid cells or 'postmortem' activity from disintegrated neutrophils is not known.

Antimetabolic Antibiotic Molecules

Lactoferrin

Lactoferrin (80 kDa) is so named from the source where it is found in highest quantity, namely milk. It is particularly rich in the first milk, the colostrum, which is also particularly rich in antibodies. Lactoferrin is believed to cover the gastrointestinal lining of infants and to protect against microbial infection by depriving microorganisms of iron. It is possible, however, that this is not the most important role of lactoferrin in the innate immunity of the newborn. It has been shown that lactoferrin is degraded by pepsin, one of the digestive enzymes that is necessary for breaking down food proteins to smaller peptides and eventually to amino acids, which may then be absorbed. Pepsin can generate a 25-amino acid residue, highly basic bactericidal peptide segment called lactoferricin (Hoek *et al*., 1997). In addition to milk and other secretions, such as tears and saliva, lactoferrin is also a prominent constituent of peroxidase-negative granules of neutrophils. As other specific granule proteins, lactoferrin is released to the phagocytic vacuole and to the outside of the neutrophils during phagocytosis.

Natural resistance-associated macrophage protein (Nramp)

Nramp is a 65-kDa protein that was initially discovered as missing from strains of mice known to be particularly susceptible to infections by mycobacteria (Cellier *et al*., 1995). A human Nramp also exists. The protein is present as an integral membrane protein in macrophages and neutrophils. Its synthesis is induced by interferon (INF)-γ, an important mediator of inflammation. The mechanism by which Nramp offers protection against mycobacteria is unknown. A plant analogue of Nramp has been identified. Nramp may function as a transport protein that removes iron from the phagocytic vacuole.

Vitamin B_{12}-binding protein

This is a member of the haptocorrin family of vitamin B_{12}-binding proteins. It has no direct antibacterial activity but is able to sequester vitamin B_{12}, which is an essential cofactor for folin synthesis and thus essential for continued growth of microorganisms. There are no direct data that allow estimation of the significance of either B_{12}-binding protein or lactoferrin to the overall antimicrobial activity of neutrophils.

Antibiotic Molecules with Enzymatic Activity

Lysozyme

Lysozyme (muramidase) is a 14-kDa nonglycosylated protein which specifically cleaves the peptidoglycan of bacterial cell walls, thereby converting bacteria to spheroplasts that burst, owing to differences in osmotic pressure across the plasma membrane of the bacteria. In addition, lysozyme degrades the chitin lining of fungi. Lysozyme is a widely expressed protein present in avian as well as mammalian species. In humans, lysozyme is stored in both types of neutrophil granules and in monocyte granules. Lysozyme is found in plasma and most secretions, such as tears and saliva, in a high concentration, which may contribute to the primary defence. In some human leukaemias, the lysozyme concentration in plasma is exceedingly high.

Chitinases

Chitinases are found widespread in plants, insects and nematodes where they are important in protection against fungal infection. Chitinases are capable of cleaving the specific chitin structure of fungal membranes (Renkema *et al*., 1995). A chitinase called chitotriosidase has been identified in macrophages and a structurally related but

enzymatically inactive protein, YKL-40, is present in the peroxidase-negative granules of human neutrophils.

Phospholipase A_2

Phospholipase A_2 is a 14-kDa protein of azurophil neutrophil granules that may be important in dissolution of ingested microorganisms and itself is bactericidal. It seems to potentiate the bactericidal activity of some of the nonenzymatic bactericidal molecules of the azurophil granules, in particular of BPI (Weiss *et al*., 1994).

Elastase

Elastase is a 32-kDa protein that is active in degrading extracellular matrix. This is important for the resolution of the inflammatory process, but may on the other hand contribute significantly to tissue damage, as occurs in chronic obstructive pulmonary disease and rheumatoid arthritis, where neutrophil infiltration is prominent. Recently, however, elastase has been shown to have an antibacterial activity by itself. The direct antibacterial activity of elastase is not dependent upon its catalytic activity, but the mechanism is unknown. A specific activity of elastase against the spirochete *Borrelia burgdoferi*, the agent responsible for Lyme disease, has been noted. Mice genetically deficient in elastase are more susceptible to infection with a variety of mainly Gram-negative bacteria and fungi than normal mice. It is possible that the increase in bacterial and fungal infections is due to the lack of direct antimicrobial activity of elastase and/or the lack of elastase-dependent proteolytic activation of cathelicidins.

Reactive Oxygen Derivatives

A respiratory burst is elicited in neutrophils, eosinophils and monocytes when these are stimulated, as happens during phagocytosis (Chanock *et al*., 1994). This respiratory burst is nonmitochondrial. The significance of the respiratory burst became evident when deficiency in generating a respiratory burst was shown to be fundamental in children suffering from chronic granulomatous disease, which is a disease characterized by inability of phagocytes to kill a wide variety of microorganisms.

The respiratory burst is the result of activity of an NADPH oxidase that is assembled from several components during activation of neutrophils. Three cytosolic components, $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$, and the membrane integrated flavocytochrome complex $p22^{phox}$ and $gp91^{phox}$, are assembled into a functional NADPH oxidase which releases superoxide as the primary product. The assembly of the NADPH oxidase to a functional unit is initiated by activation of a cytosolic G protein, Rac, which further mediates phosphorylation of $p47^{phox}$ on serine and thereonine residues. $P47^{phox}$ and $p67^{phox}$ then associate with the flavocytochrome complex and together form the NADPH oxidase. Superoxide anions are generated on the outer aspect of the cell or in the phagocytic vacuole, and dismutate to form hydrogen peroxide. This is a proton-consuming process which may lead to alkalinization of the phagocytic vacuole, if the consumption of protons is not equalized by a proton-translocation mechanism that shuttles protons from the cytosol to the extracellular medium. The NADPH oxidase has been shown also to be a proton channel to facilitate this equilibration (**Figure 3**).

Hydrogen peroxide is a substrate of myeloperoxidase which may generate hypochlorous acid and chloramines that may have effects remote from their site of production. Short-lived but very reactive oxygen species like hydroxyl radicals and singlet oxygen may also be generated as a secondary product of superoxide anions and contribute to microbicidal activity of the phagocytes. Although mainly a product of phagocytes, the components of the NADPH oxidase are now identified in endothelial and other cells, although only in smaller quantities, and their functional significance remains to be determined. Chronic granulomatous disease has been associated with defects in the structure or expression of $p47^{phox}$, $p67^{phox}$, $p22^{phox}$ and $gp91^{phox}$.

Nitric Oxide

Nitric oxide was first identified as endothelial-derived relaxation factor, whose function as a signalling molecule in vascular tone is well recognized (Bredt and Snyder, 1994). Nitric oxide is the product of the constitutively active nitric oxide synthase (NOS) of endothelial cells. It is generated from L-arginine and molecular oxygen, with electrons contributed by NADPH. The products are nitric oxide, L-citrulline and $NADP^+$. Macrophages and neutrophils can be induced to generate quantities of nitric oxide that far exceed those active in signalling. The molecular basis is the inducible NOS (iNOS) or NOS2, which is a homodimeric protein (flavo-haem) of 220 kDa.

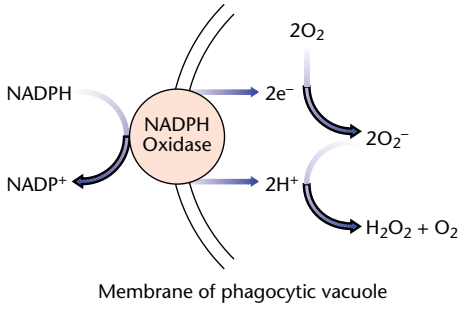

Figure 3 The function of the NADPH oxidase as a combined electron and proton transporter.

The iNOS gene is localized to chromosome 17 and under the control of INF, LPS and tumour necrosis factor (TNF). Thus it is activated by primary or secondary messengers of bacterial infections (LPS is a component of the bacterial cell wall of Gram-negative bacteria). Glucocorticoids and transforming growth factor (TGF)-β suppress the synthesis of iNOS.

Both mice with targeted disruption of the iNOS gene (iNOS knockouts) and inhibitor studies have demonstrated the importance of iNOS for control of microbicidal infection. Mice deficient in iNOS are susceptible to certain bacteria such as *Listeria monocytogenes* and mycobacteria, and to intracellular parasites such as *Toxoplasma gondii*.

The exact mode of action of nitric oxide as an antibacterial agent is not clear. It can bind to iron present in iron-sulfur enzymes. It influences iron metabolism by interfering with iron regulation factor, an iron-sulfur protein that interacts with iron response elements in the messenger RNA (mRNA) of key enzymes in iron metabolism. Nitric oxide may contribute to anaemia of chronic disease.

Evasion of Microbicidal Activity

Cell-associated microbicidal peptides are activated when exposed to microorganisms. Microorganisms that have few binding sites for opsonins (encapsulated bacteria) may evade immune recognition and not be taken up by phagocytes.

Some microorganisms produce toxins that destroy the phagocytes, e.g. streptolysin, which binds specifically to cholesterol and forms pores in mammalian cells, including phagocytes. Some microorganisms produce toxins that are able to block cellular activation, e.g. pertussis toxin, which adenosine biphosphate (ADP)-ribosylates heterotrimeric G proteins associated with chemokine receptors. Others generate substances which interfere with granule–phagosome fusion, thus preventing delivery of bactericidal substances to the phagosome (Spitznagel, 1984).

Summary

Antibiotic molecules are needed by all living organisms to protect their cells against microorganisms. This protection is provided by a multitude of different antibiotic molecules that act individually by very different mechanisms, although some are variations on a common theme. Many different antibiotic agents come into play at the same time and place, and microorganisms are therefore exposed to a cocktail of antibiotic molecules when trying to access the body. To gain knowledge about their mechanism of action, the antibiotic molecules are often studied in isolation, but their full function may well depend on their interaction with each other, and this may be the reason for the plethora of antibiotic molecules discovered so far. Yet, some of these seem powerful enough that we may be able to make good use of them as antibiotic drugs. Even more exciting, we may in due time develop means to instruct the body to induce synthesis of antibiotic molecules in organs of patients who suffer from infections.

References

Boman HG (1995) Peptide antibiotics and their role in innate immunity. *Annual Review of Immunology* **13**: 61–92.

Bredt DS and Snyder SH (1994) Nitric oxide: a physiologic messenger molecule. *Annual Review of Biochemistry* **63**: 175–195.

Cellier M, Privé G, Belouchi A *et al.* (1995) Nramp defines a family of membrane proteins. *Proceedings of the National Academy of Sciences of the USA* **92**: 10089–10093.

Chanock SJ, El Benna J, Smith RM and Babior BM (1994) The respiratory burst oxidase. *Journal of Biological Chemistry* **269**: 24519–24522.

Elsbach P (1998) The bactericidal/permeability-increasing protein (BPI) in antibacterial host defense. *Journal of Leukocyte Biology* **64**: 14–18.

Ganz T and Lehrer RI (1995) Defensins. *Pharmacology and Therapeutics* **66**: 191–205.

Hoek KS, Milne JM, Grieve PA, Dionysius DA and Smith R (1997) Antibacterial activity of bovine lactoferrin-derived peptides. *Antimicrobial Agents and Chemotherapy* **41**: 54–59.

Murthy ARK, Lehrer RI, Harwig SSL and Miyasaki KT (1993) In vitro candidastatic properties of the human neutrophil calprotectin complex. *Journal of Immunology* **151**: 6291–6301.

Renkema GH, Boot RG, Muijsers AO, Donker-Koopman WE and Aerts JMFG (1995) Purification and characterization of human chitriosidase, a novel member of the chitinase family of proteins. *Journal of Biological Chemistry* **270**: 2198–2202.

Spitznagel JK (1984) Nonoxidative antimicrobial reactions of leukocytes. *Contemporary Topics in Immunobiology* **14**: 283–343.

Steiner H, Hultmark D, Engstrom A and Boman HG (1981) Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* **292**: 246–248.

Weiss J, Inada M, Elsbach P and Crowl RM (1994) Structural determinants of the action against *Escherichia coli* of a human inflammatory fluid phospholipase A_2 in concert with polymorphonuclear leukocytes. *Journal of Biological Chemistry* **269**: 26331–26337.

Zanetti M, Gennaro R and Romeo D (1995) Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS Letters* **374**: 1–5.

Zasloff M (1987) Magainins, a class of antimicrobial peptides form Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proceedings of the National Academy of Sciences of the USA* **84**: 5549–5453.

Further Reading

Duclohier H (1994) Anion pores from magainins and related defensive peptides. *Toxicology* **87**: 175–188.

Fukui T, Ishizaka N, Rajagopalan S *et al.* (1997) p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circulation Research* **80**: 45–51.

Levy O (1996) Antibiotic proteins of polymorphonuclear leukocytes. *European Journal of Haematology* **56**: 263–277.

MacMicking J, Xie QW and Nathan C (1997) Nitric oxide and macrophage function. *Annual Review of Immunology* **15**: 323–350.

Martin E, Ganz T and Lehrer RI (1995) Defensins and other endogenous peptide antibiotics of vertebrates. *Journal of Leukocyte Biology* **58**: 128–136.

Roos D, Deboer M, Kuribayashi F *et al*. (1996) Mutations in the x-linked and autosomal recessive forms of chronic granulomatous disease. *Blood* **87**: 1663–1681.

Schonwetter BS, Stolzberg ED and Zasloff MA (1995) Epithelial antibiotics induced at sites of inflammation. *Science* **267**: 1645–1648.

Zhao C, Liu L and Lehrer RI (1994) Identification of a new member of the protegrin family by cDNA cloning. *FEBS Letters* **346**: 285–288.