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Microbial proteinase inside human cells as anti-mitochondrial activity: A new virulence factor in infectious diseases?

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Summary Both bacteria and fungi produce extracellular proteinases since they need aminoacids for optimal reproduction. This may also occur inside host cells. Viral proteinases are produced during propagation inside host cells to supply amino acids for rapid synthesis of viral proteins, and/or to split poly-protein molecules into single protein molecules, e.g., capsid, and matrix and/or envelope proteins.

In host cells the most profound, microbial proteinase-mediated effect is thought to be damage of the mitochondria, the site of oxidative energy generation. Two major effects can be imagined: (i) damage of proteinase-susceptible extra-mitochondrial membrane-associated proteins (razor blade effect), e.g., of mitochondrial transport proteins and of ATP:ADP translocase, and (ii) damage of intra-mitochondrial proteinase-susceptible proteins that are involved in the energy-generating processes. Although proteinases are not thought to invade and destroy mitochondria and essential intra-mitochondrial structures involved in energy generation, they can destroy non-mitochondrial encoded mitochondrial proteins during transport to the mitochondria, i.e., before incorporation inside the mitochondria in intra-mitochondrial structures.

A secondary effect may be damage of liver cells that effect hepatic gluconeogenesis, the process that is involved in the synthesis of glucose from lactic acid. The proteinase may bring about inactivation of specific gluconeogenesis enzymes. This means that accumulated amounts of lactic acid cannot rapidly be reduced and consequently, such inactivation will increase intracellular and later even systemic acidification that may finally result in death.

We postulate that both direct and indirect proteinase-mediated damage of mitochondrial and gluconeogenesis enzymes, and consequently of human cellular energy generation, is an essential element in acute (e.g., influenza) and chronic (e.g., hepatitis B) intracellular infections.

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Introduction

The beneficial effect of HAART (highly active antiretroviral therapy) that is based on inhibitors of viral transcriptase and HIV-proteinase [1], made us look at the effect of microbial proteinases on energy generation in intracellular infections. Previously Anand et al. reported on progress in designing specific anti-SARS drugs, i.e., inhibitors of Corona virus proteinase [2]. We postulate that proteinase-mediated damage of mitochondria, and consequently of human cellular energy generation, is an essential element in acute (e.g., influenza) and chronic (e.g., hepatitis B) intracellular infections.

Microbial agents and the specific mitochondrial target

Proteinases

Human cells and microbial pathogens produce proteinases that break down proteins. These proteinases make amino acids of proteins available for reuse or even catabolism. Protein breakdown can occur both inside and outside the cell. Intracellular proteinases are located inside proteolytic bodies, the proteasomes [3], and thus, intracellular damage of relevant proteins is prevented. Redundant, often old and degenerated, proteins inside the cell are labelled with ubiquitin molecules for recognition by the proteasomes [3]. This does not apply to pancreatic and microbial proteinases. Pancreatic proteinases are excreted into the gut to depolymerise nutritional proteins. Bacteria and fungi produce extracellular proteinases since they need aminoacids for optimal reproduction. This also occurs inside host cells. Viral proteinases are produced during propagation inside host cells to supply amino acids for rapid synthesis of viral proteins, and/or to split poly-protein molecules into single protein molecules, e.g., capsid, and matrix and/or envelope proteins. For adequate propagation of intracellular pathogens during infection, proteinases have to be freely present inside host cells.

Mitochondria as energy-generating organelles

Mitochondria in eukaryotic cells are bacteria-like organelles that contain their own mtDNA, mtRNA, (mt)ribosomes, structural and functional proteins and energy generation system. Nevertheless, they contain also non-mitochondrial proteins that are coded by genes of nuclear chromosomes. These organelles are the powerhouses of all aerobic eukaryotic organisms. By oxidative dissimilation of 1 mol glucose, palmitic acid or stearic acid, they generate about 38, 132 or 144 ATP, respectively. Also in humans they are indispensable for high-energy output.

Primary pathogenic effect of proteinases on mitochondria

Mitochondrial damage

The most profound, microbial proteinase-mediated effect is thought to be damage of the mitochondria, the site of oxidative energy generation. Two major effects can be imagined: (i) damage of proteinase-susceptible extra-mitochondrial membrane-associated proteins (razor blade effect), e.g., of mitochondrial transport proteins and of ATP: ADP translocase, and (ii) damage of intra-mitochondrial proteinase-susceptible proteins that are involved in the energy-generating processes. Although the proteinases are not thought to invade and destroy mitochondria and essential intra-mitochondrial structures involved in energy generation, they can destroy non-mitochondrial encoded mitochondrial proteins during transport to the mitochondria [4], i.e., before incorporation inside the mitochondria in intra-mitochondrial structures, e.g., in cytochrome oxidase.

Damaged energy generation

The more the microbial proteinase is active, the more mitochondrial structures will be damaged, and the more the mitochondria-mediated, oxidative dissimilation rates will decrease. Infected cells will tend to compensate the increasing loss of this efficient energy generation by means of inefficient fermentation of sugars. In this process the energy generation is limited to glycolysis, which frees only 2 mol ATP from 1 mol of glucose (with simultaneous production of 2 mol lactic acid). Thus, large amounts of sugars are needed for little energy, while simultaneously large amounts of lactic acid are produced that must be excreted into blood and finally spread throughout the whole body.

Secondary pathogenic effects of intracellular proteinases

Conversion of lactic acid

The large amount of lactic acid that arises from fermentative energy production due to damaged mitochondria lowers intracellular pH. In turn, this
intracellular acidification is counteracted by both urinary excretion and by hepatic gluconeogenesis, an important process that produces 1 mol of glucose from 2 mol lactic acid [5]. This makes that only a rather small part of all lactic acid produced inside the cell is measured in the blood, but also that the serum glucose is still present, be it that its concentration is rather low.

**Damaged gluconeogenesis**

Since the normal energy supply from carbohydrates and fatty acids for muscular and neuronal activity (in nerves and brain) will be strongly reduced due to the mitochondrial damage, the patient becomes tired. Once the infection has been overcome, energy production will normalise and patient will recover. In the meantime it may have happened that intracellular proteinases have inactivated specific gluconeogenesis enzymes in the liver. Such inactivation may lead to a rapidly increasing intracellular and later systemic acidification that may result in death.

**Loss of detoxification capacity**

During intracellular multiplication microbial proteinases may also destroy intracellular detoxification enzymes. Loss of detoxification capacity may exacerbate the toxic effects of microbial fermentation products, *e.g.*, ethanol and acetaldehyde, which may arise inside cells or enter the body from the intestine. The more these detoxification systems are damaged, the more the body will suffer from toxic fermentation products.

**Discussion**

Since many viruses produce one or more specific proteinase(s), the greatest effect of many viral diseases seems to be a decrease in energy generation. Some severe intracellular infections are caused by bacteria, *e.g.*, meningococci, *Yersinia pestis*, *Salmonella typhi* and *Legionella pneumophila*; they excrete proteinases into the host cell cytoplasm. The proteolytic effect does not essentially differ from that of viral proteinases. The same applies for parasites, *e.g.*, *Leishmania* spp., *Toxoplasma gondii* and *Plasmodium* spp. that are present inside cells. Thus, proteinase-mediated intracellular effects of plasmodia are limited to the hepatic schizont, *i.e.*, asexual multiplication inside hepatic cells.

In contrast to bacteria and parasites all viruses must multiply inside specific host cells. Therefore, the mentioned hypothesis primarily concerns viral infections. Intracellular inhibition of viral proteinase prevents direct production of viral proteins from polyproteins and/or indirect production from host proteins. Consequently, the production of intact virulent virions and viral spread is limited or even prevented. Intracellular inhibition of the proteinases of bacteria or parasites may slow down multiplication of these organisms in a similar way and thus reduce the spread and infectivity. We suggest that specific proteinase inhibitors, other than proteasome inhibitors [6], may become important antimicrobial agents to be used against intracellular pathogens, especially viruses [1].

The presented way of thinking may be extrapolated to other semi-autonomous, eukaryotic organelles, such as plastids, *e.g.*, chloroplasts in green plants. Also these organelles may be damaged in a similar way as mitochondria. Whereas the biochemical effect is quite different, the final result is still a diseased organism, but now it is a plant.

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