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Département de Biologie Animale

spécialité: Immunologie Cellulaire et Moléculaire Master I

Matière: Signalisation Moléculaire et cellulaire

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Chapter I Second part

E. Respiratory burst and intracellular killing

- During phagocytosis there is an increase in glucose and oxygen consumption which is referred to as the respiratory burst.
- The consequence of the respiratory burst is that a number of oxygen-containing compounds are produced which kill the bacteria being phagocytosed. This is referred to as oxygen-dependent intracellular killing.
- In addition, bacteria can be killed by pre-formed substances released from granules or lysosomes when they fuse with the phagosome. This is referred to as oxygen-independent intracellular killing.

1. Oxygen-dependent intracellular killing

1-1 Oxygen-dependent, myeloperoxidase-independent reactions

During phagocytosis glucose is metabolized via the pentose monophosphate shunt and NADPH is formed. Cytochrome B which was part of the specific granule combines with the plasma membrane NADPH oxidase and activates it. The activated NADPH oxidase uses oxygen to oxidize the NADPH.

The result is the production of superoxide anion. Some of the superoxide anion is converted to H2O2 and singlet oxygen by superoxide dismutase.

In addition, superoxide anion can react with H2O2 resulting in the formation of hydroxyl radical and more singlet oxygen.

The result of all of these reactions is the production of the toxic oxygen compounds superoxide anion (O_2-), H_2O_2, singlet oxygen ($^1O2$) and hydroxyl radical (OH•) (Figure 7).

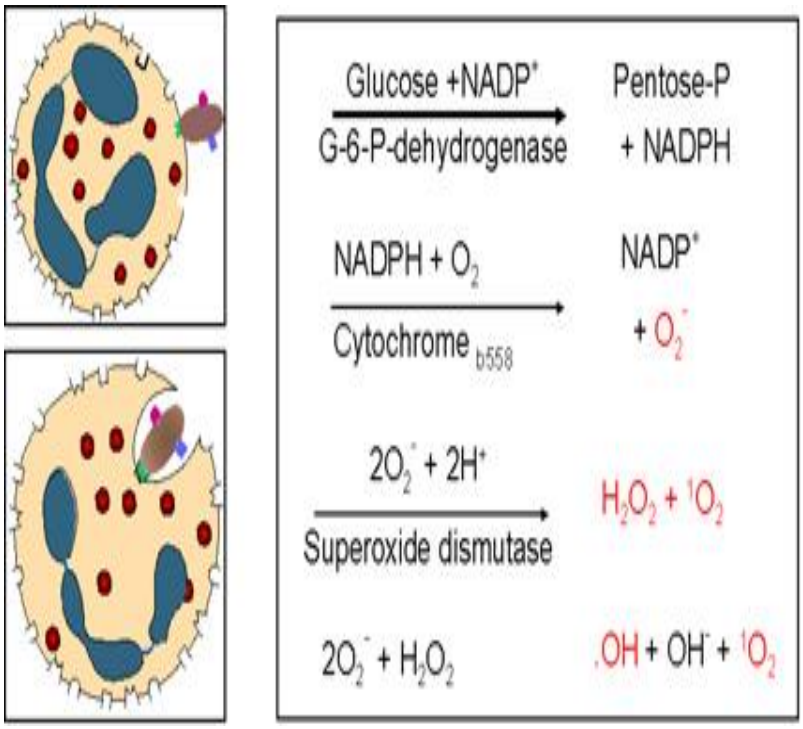

Figure 7: Oxygen-dependent, myeloperoxidase-independent reactions.

1-2. Oxygen-dependent myeloperoxidase-dependent intracellular killing

As the azurophilic granules fuse with the phagosome, myeloperoxidase is released into the phagolysosome. Myeloperoxidase utilizes H_2O_2 and halide ions (usually Cl-) to produce hypochlorite, a highly toxic substance. Some of the hypochlorite can spontaneously break down to yield singlet oxygen. The result of these reactions is the production of toxic hypochlorite (OCl-) and singlet oxygen ($^1O2$) (Figure8).

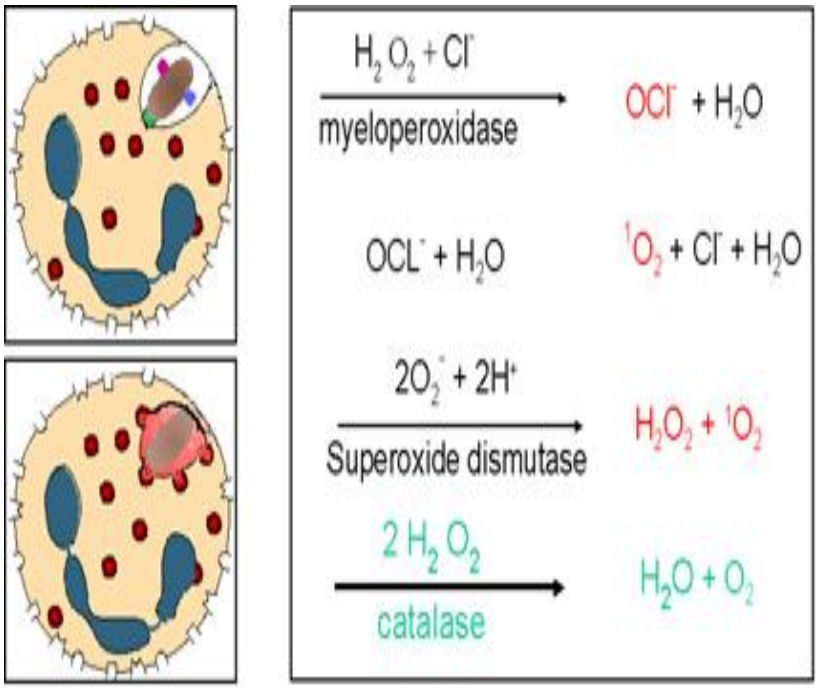

Figure 8: Oxygen-dependent, myeloperoxidase-dependent reactions

*- Free radicals

Free radicals are highly reactive species with one unpaired electron. The most important free radicals in biological systems are reactive oxygen such as O_2- and (OH•)

They are derived from normal cellular respiration and the oxidative burst produced when phagocytic cells destroy bacteria or virus - infected cells.

*-Antioxidants

To cope with the constant generation of potentially damaging oxygen radicals , eukaryotic organisms have evolved many defense mechanisms. These include antioxidants such as glutathione , vitamins C and E which act as free radical scavengers.

The antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) catalyse the reduction of the reactive oxygen intermediates.

Superoxide dismutase is found in two forms , cu^{2+} / Zn^{2+}- SOD which is found in the cytoplasm and nucleus and Mn^{2+} - SOD, which is located in the mitochondrial matrix .

The mitochondrial superoxide dismutase of eukaryotes is similar to the superoxide dismutase of many bacteria with respect to its characteristic content of Mn+2 and many homologie in amino acid sequence.

The cytosol form of superoxide dismutase , on the other hand , has quite a different structure and contains Cu+2 and Zn+2 . These enzymes are present in high concentration and are extraordinarily active.

In addition a ferrienzyme (Fe SOD) has been identified in bacteria.

1-3. Detoxification reactions

Neutrophils and macrophages have means to protect themselves from the toxic oxygen intermediates. These reactions involve the dismutation of superoxide anion to hydrogen peroxide by superoxide dismutase and the conversion of hydrogen peroxide to water by catalase (Table 2) .

When the balance between oxidants and antioxidants is disturbed , a cell or organism is considered to be in a state of oxidative stress. Under these conditions , reactive oxygen intermediates may damage DNA and membrane lipids .

Peroxidation of membrane lipids by free radicals result in the loss of membrane integrity and function . Oxidative damage to DNA can disrupt normal transcription and replication and induce mutations.

Catalase is found in the microbodies of animal cells , also called peroxisomes

Hydrogen peroxide formed by superoxide dismutase is decomposed by the heme enzyme catalase in the reaction (Table2).

Table 2: Detoxification reactions.

Reactions	Enzymes
$H_2O_2 + Cl^- \rightarrow OCl^- + H_2O$	Myeloperoxidase
$OCl^- + H_2O \rightarrow {}^1O_2 + Cl^- + H_2O$	
$2O_2 + 2H^+ \rightarrow O_2^- + H_2O_2$	Superoxide dismutatse
$H_2O_2 \rightarrow H_2O + ½O_2$	Catalase

2. Oxygen-independent intracellular killing

- In addition to the oxygen-dependent mechanisms of killing there are also oxygen–independent killing mechanisms in phagocytes:
- cationic proteins (cathepsin) released into the phagolysosome can damage bacterial membranes;
- lysozyme breaks down bacterial cell walls.
- lactoferrin chelates iron, which deprives bacteria of this required nutrient.
- hydrolytic enzymes break down bacterial proteins.
- Thus, even patients who have defects in the oxygen-dependent killing pathways are able to kill bacteria. However, since the oxygen-dependent mechanisms are much more efficient in killing, patients with defects in these pathways are more susceptible and get more serious infections (Table 3).

Table 3: Oxygen-independent mechanisms of intracellular killing.

Effector molecule	Function
Cationic proteins (including cathepsin)	Damage to microbial membranes
Lysozyme	Splits mucopeptide in bacterial cell wall
Lactoferrin	Deprives bacteria from free iron
Proteolytic and hydrolyic	Digestion of killed organisms

F. NITRIC OXIDE-DEPENDENT KILLING

Binding of bacteria to macrophages, particularly binding via Toll-like receptors, results in the production of TNF-alpha, which acts in an autocrine manner to induce the expression of the inducible nitric oxide synthetase gene (i-nos) resulting in the production of nitric oxide (NO). If the cell is also exposed to interferon gamma (IFN-gamma) additional nitric oxide will be produced (figure 9). Nitric oxide released by the cell is toxic and can kill microorganism.

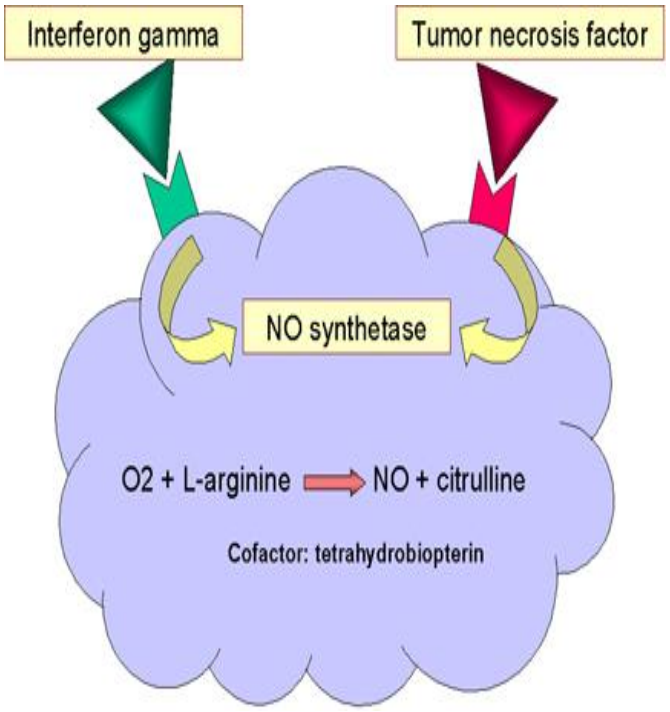

Figure 9 a: Nitric oxide-dependent killing

Nitric oxide

*-Definition

Nitric oxide is a special molecule because , as well as being toxic to pathogens , it also acts as an important messenger .

Nitric oxide is constituvely produced at low levels by neuronal and endothelial cells , it is formed from L- arginine in a reaction catalysed by nitric oxide synthase.

*-The role of Nitric oxide

Nitric oxide has a role as a neurotransmitter and in maintaining vascular tone.

And has various antithrombotic properties ; it inhibits platelet adhesion , Activation and aggregation .

Nitric oxide interact with biologic thiol such as homocysteine , forming S-nitrosothiol or S- nitrosohomocysteine which has vasodilatory and antiplatelet actions.

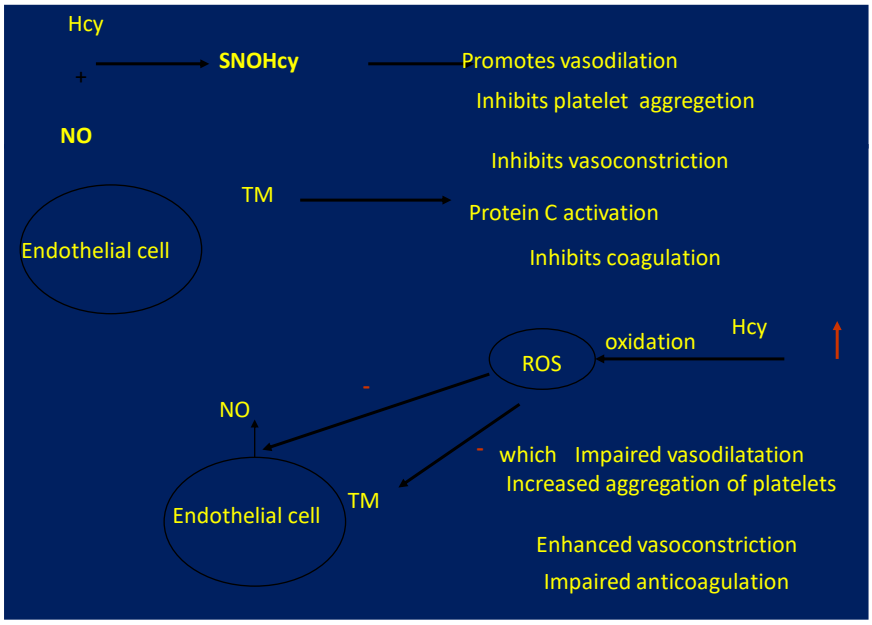

Figure 10.Mechanisms of homocysteine (Hcy) induced vascular damage

Phagocytes can produce high levels of nitric oxide when inducible nitric oxide synthetase is activated . High levels of Nitric oxide reduce vascular tone and cardiac output and contribute to the low blood pressure of septic shock.

There is also evidence that nitric oxide acts as a messenger molecule and can promote the effects of T cells , contributing to chronic inflammation.

Vascular tone at any given time is determined by the balance of competing vasoconstrictor and vasodilator influences (Figure 11).

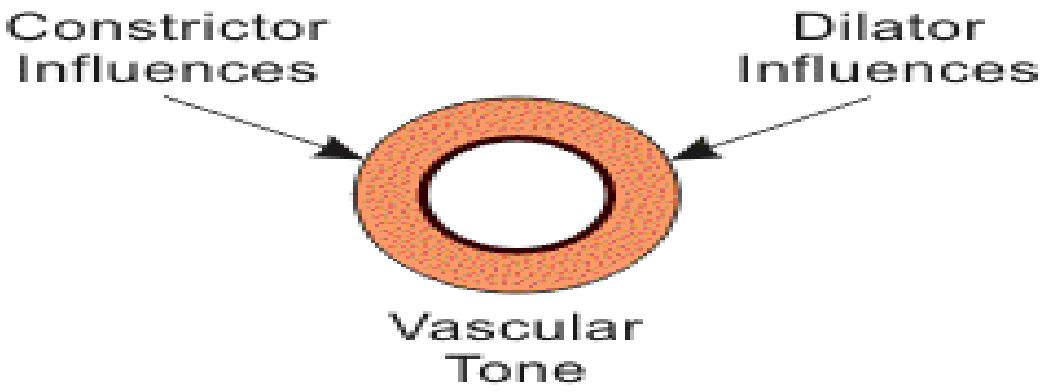

Figure 11 : Shows the vasoconstrictor and vasodilator influences acting on arteries and veins determine their state of vascular tone, which is the balance between constrictor and dilator influences.

V. NON-SPECIFIC KILLER CELLS

- Several different cells including NK and LAK cells are capable of killing foreign and altered self target cells in a non-specific manner. These cells play an important role in the innate immune system.
- Natural killer (NK) cells and LAK cells
- are also known as large granular lymphocytes (LGL) because they resemble lymphocytes in their morphology,
- except that they are slightly larger and have numerous granules.
- NK cells can be identified by the presence of CD56 and CD16 and a lack of CD3 cell surface markers.

- NK cells are capable of killing virus-infected and malignant target cells but they are relatively inefficient in doing so.

However, upon exposure to IL-2 and IFN-gamma, NK cells become lymphokine-activated killer (LAK) cells, which are capable of killing malignant cells. Continued exposure to IL-2 and IFN-gamma enables the LAK cells to kill transformed as well as malignant cells.

LAK cell therapy is one approach for the treatment of malignancies (Figure 11).

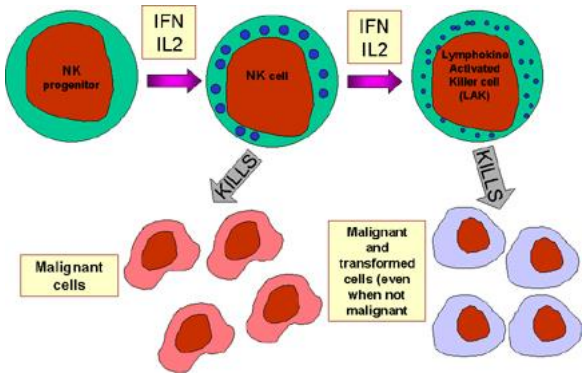

Figure 11: NK cells and their activation