Review

ROS and Innate Immunity

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Abstract. *Oxygen is converted into reactive oxygen (RO) by radiation, light, the electron transport system in mitochondria, or by other enzymes and is regulated by the action of antioxidative enzymes which convert RO into an inactive state. Reactive oxygen species (ROS) have a biocidal effect on invading bacteria and they can also injure the cells of the host. For this reason, RO is considered as a general cause of aging and contributes to lifestyle-related diseases and cancer. However, for any organism that uses oxygen as an energy source, RO is inevitably produced and has important biological significance. Apart from the direct activity of RO, recent studies have shown that it functions as a second messenger of signal transduction. In this review, the recent findings related to ROS/nitric oxide (NO) and especially of its relationship to innate immunity are summarized.*

Innate Immunity and ROS

The immune system can be divided into innate immunity and acquired immunity and the two types are closely related to each other. While innate immunity is possessed by all kinds of multi-cellular organisms, acquired immunity only exists in vertebrates. In acquired immunity, the dominant forces are antigen-antibody reactions and T-cells and B-cells play important roles. Conversely, innate immunity is a primitive prophylactic system for eliminating non-self, where macrophages, neutrophils and dendritic cells are the important cells. The cells involved in innate immunity recognize foreign substances such as bacteria with toll-like receptors (TLR), the receptors for innate immunity, and regulate the activation of other cells by the production of various cytokines. Cells may phagocytose foreign bodies and then activate the acquired immunity system by presenting a part of the phagocytosed and digested foreign substances on their membrane surfaces (1). During this recognition and response process (phagocytosis), reactive oxygen species (ROS) and nitrogen oxide (NO) are produced (2)

The reactive oxygen (RO) that appears during phagocytosis is produced on the membranes of the endosome of the phagocytosing cells (3) with the involvement of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the cellular membranes (4, 5). NADPH oxidase is an enzyme formed by the complex of $gp91^{phox}$ (NOX2), $p22^{phox}$, $p40^{phox}$, $p67^{phox}$, $p47^{phox}$ and Ras-related C3 botulinum toxin substrate 2 (rac2) (6). Phox is defined as an oxidase of phagocytes. Usually, the structural subunits of phox are separated. They become a complete complex by activation and separate NADPH into NADP and H^+ and simultaneously transform oxygen into the radical (Figure 1). Although charged RO has difficulty passing through the membrane, hydrogen peroxide (H_2O_2) passes through it easily and enters the cytoplasm (5). Interestingly, when TLR4 binds with lipopolysaccharide (LPS), TLR4 is reported to bind with a NADPH receptor; thus activation of TLR4 possibly conjugates NADPH oxidase (7) (Figure 1).

In a rare hereditary disease, chronic granulomatous disease (CGD), a defect in one of the subunits of NADPH oxidase leads to a failure in the production of RO by

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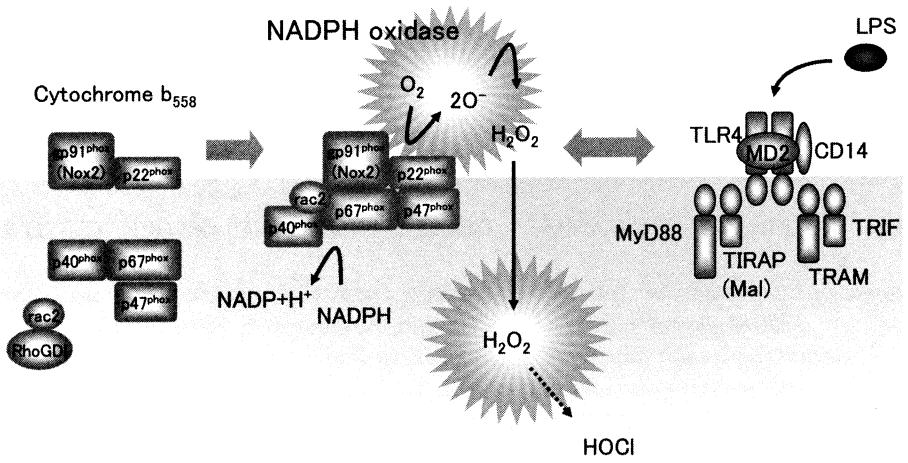

Figure 1. *Generation of ROS during stimulation with foreign substances. LPS, lipopolysaccharide; NADP, nicotinamide adenine dinucleotide phosphate; TLR, Toll-like receptor; MyD88, myeloid differentiation factor 88; TRIF, TIR-domain-containing adapter-inducing interferon-β; TIRAP, TIR domain-containing adaptor protein; TRAM, TRIF-related adaptor molecule; rac2, ras-related C3 botulinum toxin substrate 2; RhoGDI, dissociation inhibitor for Rho proteins.*

phagocytes (8). This results in bacterial or yeast infections such as pneumonia, abscesses, suppurative arthritis and osteomyelitis. Granulomas are formed as a result of the failure to eliminate these bacteria. In other words, the action of NADPH oxidase and its role in the production of RO is indispensable for preventing infection.

LPS Signal and ROS

Apart from the direct role of RO in phylaxis, recent evidence has indicated that it has a role in signal transduction related to innate immunity (9). For example, after TLR4 binds with LPS on the surface of macrophages, adaptor molecules associate with the TLR4 in the cytoplasm. This triggers phosphorylation or activation of various proteins which then transmit signals and finally induces the expression of certain genes (after activated transcription factors move into the nucleus). Based on the expression of these genes, functional activation occurs in the macrophages (1). During this process, the macrophages secrete various cytokines to activate other immune cells so that the whole state of an organism is thus regulated. As described above, in the binding of LPS with TLR4, there is a possibility of conjugation of NADPH oxidase. It has become clear that RO works as a second messenger in the signal transduction pathway of LPS.

Two signal transduction pathways are known for LPS, myeloid differentiation factor 88 (MyD88)-dependent and non-dependent. The MyD88-dependent pathway is further linked to two other pathways, activator protein-1 (AP1) and nuclear factor-κB (NF-κB) (1). ROS are involved in both sets of pathways (Figure 2). First, in the mitogen-activated protein (MAP) kinase pathway, apoptosis signal-regulating kinase (ASK1) (MAPKKK) requires phosphorylation (10). ASK1 is blocked with thioredoxin. As hydrogen peroxide changes the structure of thioredoxin, it is isolated from ASK1 and ASK1 is phosphorylated (11). As a consequence, downstream p38 is also phosphorylated (2) (Figure 2). In the NF-κB pathway, phosphorylation of IκB (an inhibitor of NF-κB) is reported to be augmented by RO (12-14) (Figure 2). This mechanism is not well known but IκB kinase (IKK) might be activated or direct phosphorylation of IκB might be accelerated by IKK in a non-dependent manner.

TNF Signal and ROS

After the transcription factors (AP1, NF-κB and IFN-regulatory factor (IRF3)) are activated, various cytokines are produced (1). Tumor necrosis factor (TNF) is a representative cytokine produced by activated macrophages. RO is reported to be produced in the course of action by TNF with other cells, and is involved in its signal transduction.

Three kinds of signal transduction pathways of TNF bound to the TNF receptor1 (TNFR1) are known, NF-κB, MAP kinase and caspase (15). The dominant pathway depends on the kind or state of the cells. When activation of NF-κB becomes dominant, proliferation of the cells is promoted, while dominance of the caspase pathway leads to cellular death. When MAP kinase is the dominantly activated in TNF signal it is connected to inflammation, apoptosis or necrosis (16).

RO is produced by binding of TNF with its receptor. This RO is not produced by NADPH oxidase on the cellular membranes, but is produced in the cytoplasm, in the

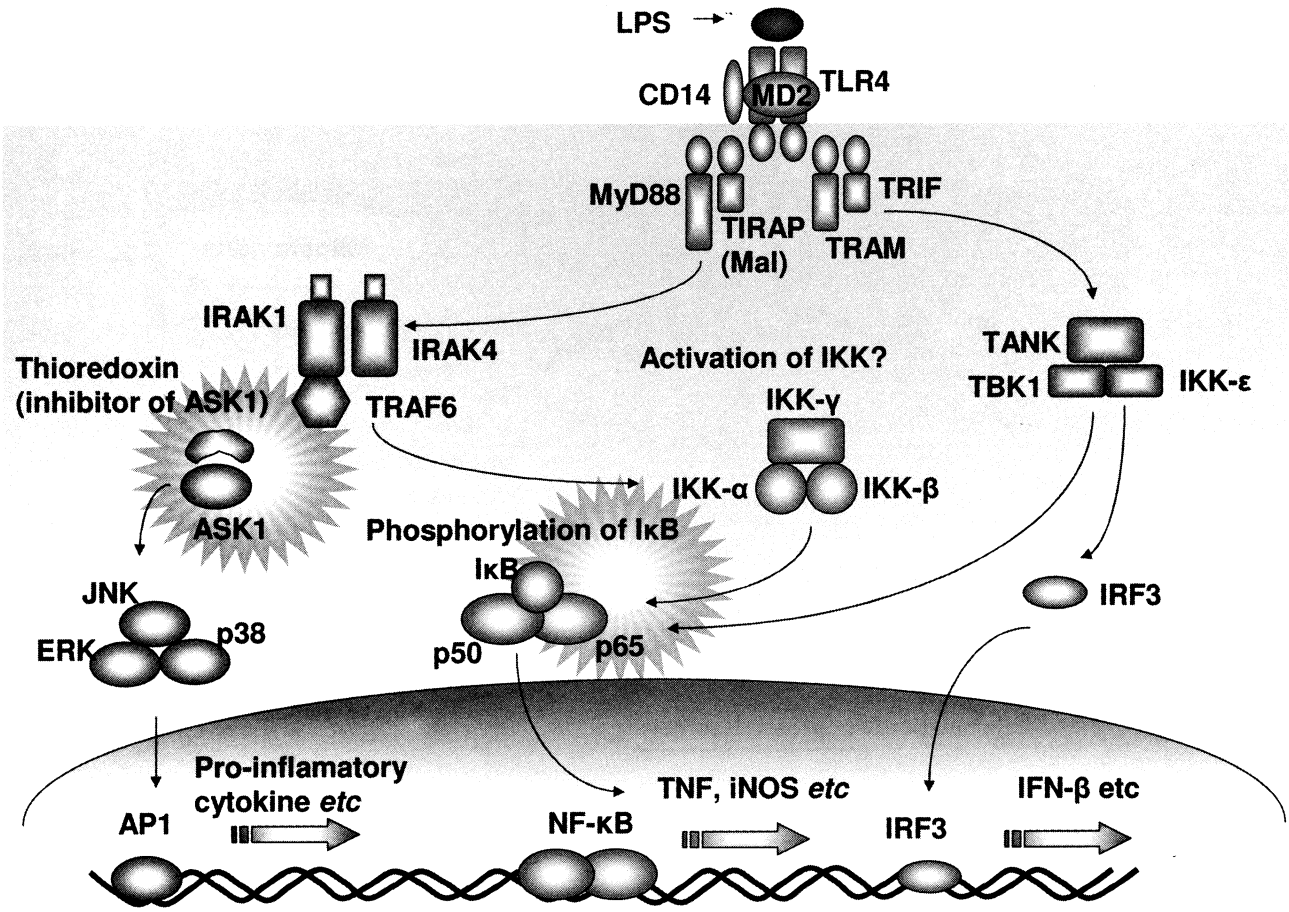

Figure 2. *LPS signal and ROS. IRAK-1, Interleukin-1 receptor-associated kinase 1; TRAF, TNFR-associated factor; ASK, apoptosis signal-regulating kinase; JNK, c-Jun NH2-terminal kinase; ERK, extracellular signal-regulated kinase; AP-1, activator protein-1, NF-κB, nuclear factor κB; IκB, inhibitor of NF-κB; IKK, IκB kinase; TANK, TRAF-family-member-associated NF-κB activator; TBK, TANK binding kinase; IRF, IFN-regulatory factor; IFN, interferon.*

mitochondria (3). As described above, RO is related to the activation of NF-κB. It is reported that under certain conditions in which NF-κB is suppressed, RO inactivates the phosphatase so that the kinase becomes dominant leading to continuous activation of c-Jun NH_2-terminal kinase (JNK) and induction of apoptosis or necrosis (17) (Figure 3). In this way, RO is induced by the stimulation of cytokines and is related to the pariticular pathway activated.

Innate Immunity and NO

Both RO and NO are produced during the activation of innate immunity. NO is produced in the process of conversion of arginine to citrulline by nitric oxide synthase (NOS) (18). This NOS is either structural or induced by the stimulation of foreign substances. NO itself is not a highly toxic substance. The reaction of NO with RO produces ONOO, a highly reactive substance with biocidal effects. The effect of NO is not limited to disinfection; NO also relaxes vascular smooth muscle and therefore decreases blood pressure. Also, in the nervous system it is known to be involved in the regulation of neurotransmission (19).

Innate Immunity, ROS and Cancer Therapy

In studies in our laboratory, when LPS derived from *Pantoea agglomerans* (IP-PA1) was administrated orally or intradermally to human and animals carrying various diseases, IP-PA1 was revealed to have curative properties for many diseases (20). As an example, in an experiment with Meth A fibrosarcoma implanted to BALB/c mice, tumor growth was suppressed by intradermal administration of LPS (21) (Figure 4): 5/8 mice survived, whereas there were no survivors (0/8) in the control group. In this experiment, TNF was shown to be involved in the tumor lesions. RO induced by TNF might have been part of the beneficial process, and

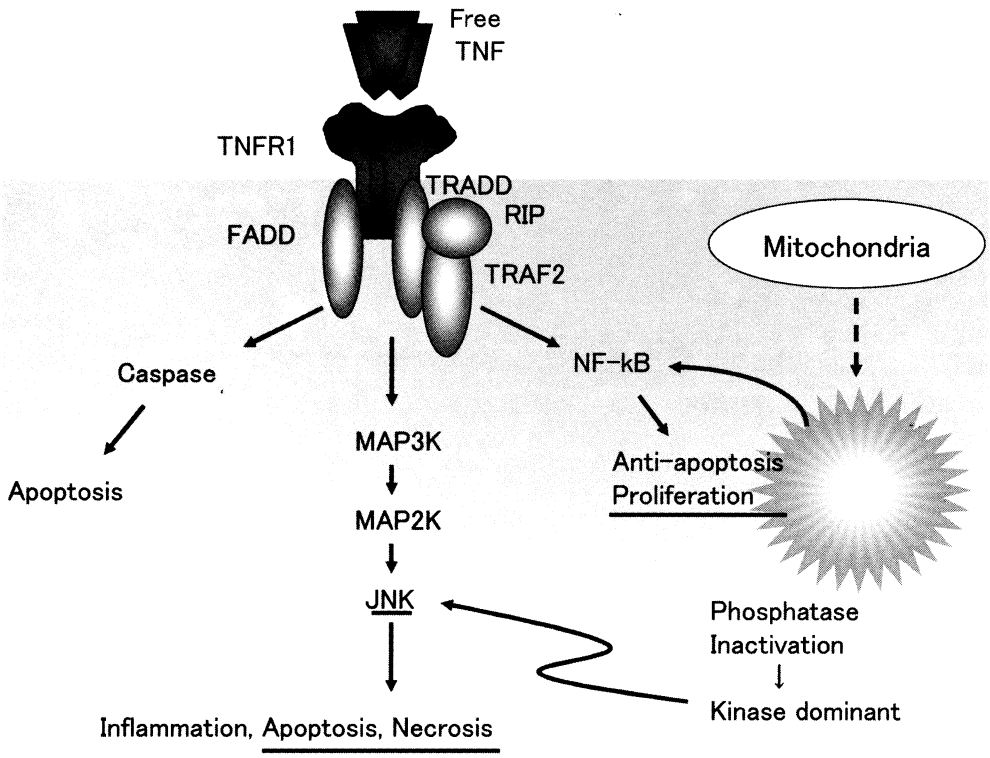

Figure 3. *TNF signal and ROS. TNF, tumor necrosis factor; TNFR, TNF-receptor; FADD, Fas-associated death domain protein; TRADD, TNF receptor-associated death domain protein; RIP, receptor-interacting protein; TRAF, TNFR-associated factor; NF-κB, nuclear factor κB; MAPK, mitogen-activated protein kinase; JNK, c-Jun NH_2-terminal kinase.*

it is believed that it was the total effect of TNF and RO that caused the suppression of tumor growth. In this setting, RO and TNF could be used in biotherapies, including the treatment of cancer, assuming quantity and localization can be controlled.

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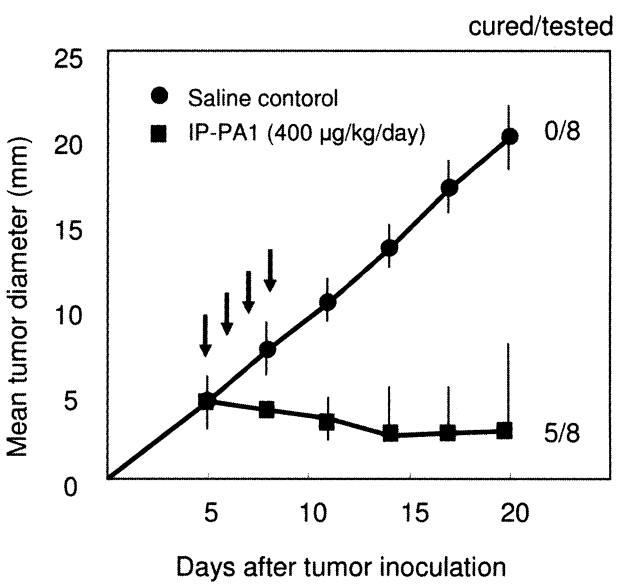

Figure 4. *Therapeutic effect of intradermal administration of LPS on cancer. Meth A cells (2×10^5) were inoculated intradermally into the abdomen of BALB/c mice. Saline (●) or IP-PA1 (Pantoea LPS) (■) was injected i.d. on days 5, 6, 7, and 8. Symbols and bars represent mean values and SD of eight individual mice. Number of complete response (CR) mice/number examined on day 20.*

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