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Forum Editorial

Redox Control of T-Cell Death

IZUMI NAKASHIMA, HARUHIKO SUZUKI, MASASHI KATO, and ANWARUL A. AKHAND

THE CELLULAR REDOX STATUS, which is determined by an equilibrium of levels of reactive oxygen species (ROS) and antioxidants, might physiologically regulate both cell activation and cell death induction in normal T cells during their development in the thymus and immune response in the peripheral lymphoid organs (10, 11).

A low level of ROS is normally produced in cells during respiratory chain chemical reactions in mitochondria, and the cellular ROS level may be increased during cell–cell interaction through binding of inflammatory cytokines to their receptors or exposure to extracellular ROS that are produced by inflammatory cells, including antigen-presenting cells (APC). Exposure to oxidative chemicals including heavy metals from polluted environments, ultraviolet ray irradiation, microbial infections, or genetic abnormality in the antioxidant system may induce an abnormal increase in ROS level/oxidative stress in T cells.

Oxidative stress-linked unusual intracellular signaling can induce T-cell-based immunodeficiency and allergic and autoimmune diseases potentially due to loss of regulatory T cells or T-cell-oriented neoplastic diseases. Use of antioxidants such as *N*-acetylcysteine (NAC) and thioredoxin (Trx) may be effective for treating some of these oxidative stress-induced T-cell disorders. This forum aimed to cover (a) basic understanding of the signal-transduction cascade for T-cell death during normal T-cell development and of the potential involvement of redox-linked mechanisms in the cascade, (b) molecular pathogenesis of abnormal T-cell death induced by environmental oxidative elements, microbial infections, or genetic abnormalities, and (c) therapeutic aspects of antioxidants for revising abnormal T-cell death.

REDOX CONTROL OF CELL DEATH

T-cell death develops and is controlled basically through mechanisms common to death of other cell types (5, 8). Two review articles in this forum are on the general topics of redox control of cell death from different aspects.

Yodoi and his colleagues overviewed results of recent studies on redox control of death signaling by glutathione (GSH) and Trx systems. Cellular redox status is controlled by both GSH and Trx systems, which scavenge intracellular excess ROS. They discussed the possible sites of redox control by GSH/Trx in cell-death signaling. First, they speculated that the cellular redox status determines either of the two types of cell death, necrosis and apoptosis, and that Trx is required for activation of redox-sensitive caspases for apoptosis induction. Second, they suggested that both ROS production in mitochondria and release of cytochrome *c* from mitochondria are regulated by mitochondria-specific Trx (Trx-2). Third, they insisted that Trx plays a crucial role as a negative regulator of apoptosis signal-regulating kinase-1 (ASK1).

Ichijo and his group reviewed updated results on the roles of ASK1-mediated signal pathways in oxidative stress- and endoplasmic reticulum stress-induced apoptosis, focusing on their own findings obtained from ASK1 knockout mice. In their article, they concluded that the decision as to whether cells are committed to death or life depends on the extent and duration of intracellular and/or extracellular stresses and that ASK1 can serve as an initial sensor of excess or prolonged oxidative stress. They demonstrated that ASK1 is crucially involved in the signaling either by ROS-induced oxidative stress or by abnormal protein aggregation-induced endoplasmic reticulum stress and that it controls the mitochondria-dependent pathway for caspase activation through c-Jun N-terminal kinase (JNK)/p38 pathway-mediated regulation of Bcl-2 family molecules.

PHYSIOLOGICAL T-CELL DEATH

An appropriately made decision of cell survival or cell death is crucial for normal development and control of T-cell activity at various stages (12–14), of which abnormal promotion or interruption may lead to development of immunological disorders, such as immunodeficiency, allergic and autoimmune diseases, and neoplastic disorders. The cell survival/cell death decision is particularly important at two steps of early T-cell development in the thymus after the stage when T cells initially express both CD4 and CD8 and low levels of T-cell re-

Department of Immunology, Nagoya University Graduate School of Medicine, Nagoya, Japan.

ceptor (TCR)/CD3. The first step of this decision is for the positive selection of immature T cells with TCR that efficiently transduce the signal for survival through reaction with self MHC on APC. This step is followed by the second step for negative selection of the late stage of CD4/CD8-double positive T cells with TCR that react with self peptides plus self MHC and transduce the signals for cell death. CD4-single positive and CD8-single positive mature T cells then develop, and these cells bear TCR that are ready to react with not-self peptides plus self MHC on APC.

The mature T cells move from the thymus to peripheral lymphoid tissues and continuously receive a signal for cell survival through a yet undetermined pathway until they encounter APC that present antigen plus self MHC to TCR. A combination of the signal transduced through TCR and the signals delivered by interaction between costimulatory molecules such as CD28 on T cells and CD80/CD86 on APC or between cytokines and cytokine receptors decides activation or failure of activation (anergy) of T cells.

The next critical step of decision between survival or death of T cells is at the time when already activated T cells are subjected to further extensive antigenic stimulation. This induces so-called activation-induced T-cell death, which down-regulates excessively high T-cell activity. Many of the activated T cells die through this mechanism, but some of them survive as memory T cells.

SIGNALING FOR T-CELL DEATH

The cascades of signal transduction for positive or negative selection of CD4/CD8-double positive T cells have not yet been clarified. These T cells are known to be highly sensitive to the corticosteroid hormone-mediated signal for cell death, which can be stress-dependent. A recent study has shown that corticosteroid hormone is produced by thymic epithelial cells, suggesting its role in T-cell development. It is, however, still controversial whether the steroid receptor-mediated signaling is crucially involved in the signaling for positive or negative selection of thymic T cells (2).

Cell death receptors such as Fas may (6) or may not (1) be crucially involved in the signal pathway for negative selection of thymic T cells, and it has been suggested that TCR-mediated and mitochondria-dependent pathways play a central role in this step of cell-death induction. Results of a recent study using *in vitro* models suggest that the signal transduction cascade for negative selection includes the MKK6/p38 mitogen-activated protein (MAP) kinase pathway (15), which can be triggered by oxidative stress. More recently, Suzuki *et al.* (unpublished observations) have established an MKK6-deficient mouse line and demonstrated that the MKK6/p38 MAP kinase pathway plays an important role in signaling for negative selection of T cells *in vivo*. This observation suggests that the physiological signal for negative selection of T cells may be subject to a redox-linked control, potentially following ROS generation during T-cell and APC interaction.

In contrast to a relatively low contribution of cell-death receptors in thymic T-cell death, activation-induced T-cell death is mediated mainly by signals through cell-death receptors such as Fas, which became active on activated T cells to receive the cell-death signal from cytotoxic T lymphocytes bearing FasL. As Ichijo and his colleagues describe in their review article, the cell-death receptor-mediated signaling can also be subject to a redox-linked control.

REDOX-LINKED METABOLIC PATHWAYS FOR T-CELL DEATH

Recent evidence suggests that mitochondria play crucial roles (7) in deciding the fate of T cells during the development in the thymus and the immune response in peripheral lymphoid tissues, as suggested by Perl and his colleague in their review article. First, both processes for activation and cell-death induction of T cells require energy provided by ATP, which is synthesized in mitochondria. Second, ROS produced in mitochondria through the reaction of electrons directly with oxygen play important roles in the signal-transduction pathways for either activation or death induction of T cells. ATP synthesis and ROS production in mitochondria and release of cell death-inducing elements from mitochondria are all controlled by mitochondrial membrane potential. Disruption of this membrane potential is thus a critical step for cell-death induction, and this process is regulated by the redox equilibrium of levels of ROS and cellular antioxidants such as GSH and Trx.

Perl and his colleagues further claimed that the pentose phosphate pathway (PPP) plays a crucial role in maintenance of GSH and Trx in a reduced state through production of NADPH. According to them, the levels of glucose 6-dehydrogenase and transaldolase regulate PPP and NADPH levels, and transaldolase activity determines the output of NADPH and GSH and thereby the sensitivity of cells to mitochondria-dependent apoptotic death.

Vitamin C, which is widely used as an antioxidant, also has prooxidant and proapototic properties. Puskus *et al.* demonstrated that dehydroascorbate (DHA), which is an oxidized form of ascorbate, regenerates GSH from its oxidized form, GSSG, through stimulating the activity of PPP enzymes. This action of DHA increases resistance of Jurkat human T cells to H_2O_2-induced cell death. However, DHA was also shown to promote Fas-mediated cell death by increasing cell-surface expression of Fas.

Ma *et al.* showed that menadione, which is also known as vitamin K_3 and has the property of an electron acceptor, biphasically controls cell-death signaling of T cells. They demonstrated that low concentrations of menadione induced cell death through promotion of phosphorylation/activation of JNK. Interestingly, a high concentration of menadione suppressed the high concentration H_2O_2-mediated cell death by inactivating JNK. These results suggest that redox-linked metabolic pathways of signaling for deciding death or survival of T cells are more complex than previously thought.

CHEMICAL DYSREGULATION OF T-CELL DEATH SIGNALING

The signals for deciding cell survival or cell death, which are delivered through TCR and costimulatory molecules, can be modified by oxidative elements from environments, lead-

ing to T-cell dysfunctions (9–11). A number of oxidative elements from polluted environments, such as oxidative chemicals, heavy metals, and ultraviolet rays, could affect T cells for modulating the signals to induce abnormal death.

Heavy metals such as mercuric and arsenic compounds are known to be toxic to cells in the immune system. Exposure of T cells to these compounds may result in either allergic or autoimmune disorders or immunodeficiency depending on conditions (3, 4). Shenker *et al.* studied the action of methyl mercuric chloride (MeHgCl) on mitochondrial function in T cells with respect to ROS production, thiol status, and caspase activation. They noticed that an increase in the ROS level, reduction of the thiol level, and caspase activation in T cells all developed at the late stage of MeHgCl treatment, whereas reduction of the mitochondrial membrane potential, release of cytochrome *c,* and decrease in the GSH transferase/oxidase level occurred at the early stage. Based on these findings, they proposed that there is a tight linkage between change in cellular redox status and caspase activation in MeHgCl-treated cells.

We ourselves (Akhand *et al.*) overviewed our recent results on the initial step of heavy metal ($HgCl_2$/$NaAsO_2$)-induced signaling at the cell surface, which may replace or modify the TCR-mediated signal to induce T-cell death. In this article, we suggested that a membrane microdomain termed a "raft" plays a critical role in the heavy metal-induced triggering of the mitochondria-linked cell-death induction pathway, which includes intracellular ROS production.

GENETIC DYSREGULATION OF T-CELL DEATH SIGNALING

Hereditary abnormalities of molecules that transduce or control cell death-inducing signals may either enhance or prevent T-cell death. It is known that hereditary dysfunctions of cell death receptors (Fas) or their ligands (FasL) in mice cause proliferation of abnormal T cells and production of autoantibodies (16). Ataxia telangiectasia (AT) has cellular and humoral immunodeficiency in addition to neurodegeneration with cerebellar ataxia due to apoptotic loss of naive T cells, as well as cerebellar Purkinje cells. The gene responsible for AT is *ATM,* the product of which carries a phosphatidylinositol 3-kinase catalytic domain in the C-terminus, and AT cells carry functionally inactivated ATM and are thereby sensitive to oxidative stress that leads to apoptotic cell death. Reichenbach *et al.* demonstrated that the levels of oxidative stress-mediated damage of lipids and DNA are elevated in patients with AT. Based on this finding, they proposed that ATM plays an important role in maintenance of cell homeostasis in response to oxidative damage and that control of ROS levels can be therapeutically beneficial for patients with AT.

VIRAL DYSREGULATION OF T-CELL DEATH SIGNALING AND ITS RESTORATION BY ANTIOXIDANTS

Viral infections often cause immunodeficiency and sometimes induce neoplastic change in cells through promotion or prevention of cell death-inducing signaling in association with change in the cellular redox status.

Nakamura and his colleagues reviewed the results of recent studies showing that human immunodeficiency virus (HIV) infection induces systemic oxidative stress, and they proposed that appropriate redox control can provide a rationale for treatment of AIDS. During progression of the disease, levels of intracellular GSH decrease along with an increase in ROS production possibly triggered by inflammatory cytokines such as tumor necrosis factor-α or by HIV tat protein, which down-regulates manganese superoxide dismutase. The oxidative stress thus provoked can augment the replication of HIV through activation of nuclear factor-κB and may promote cell-death signaling. Nakamura *et al.* presented in their review article recent results from the laboratories of Droge, Herzenberg, and others demonstrating beneficial effects of NAC on AIDS.

Morretti *et al.* examined the effects of a combination therapy of antiretroviral drugs [zidovudine (AZT) plus didanosine (DDI)] with an antiapoptotic or antioxidative stress reagent, L-carnitine, on 20 asymptomatic HIV-infected subjects. They showed that the addition of L-carnitine causes reduction of apoptosis and oxidative stress in CD4 and CD8 lymphocytes during treatment with AZT and DDI.

Tax protein of human T-cell leukemia virus type 1 (HTLV-1) is known to play a pivotal role in promotion of immortalization/transformation of infected cells at the onset of HTLV-1-associated adult T-cell leukemia (ATL). Chlichlia *et al.* suggested that Tax displays pleiotropic effects on HTLV-infected cells by inducing oxidative stress and apoptotic T-cell death, which is enhanced by activation of the TCR pathway. Because Tax is expressed only at a low level in already established ATL cells, they speculated that selective pressure for the maintenance of reduced Tax expression contributes to the virus-mediated pathogenesis.

CONCLUSION

Results of recent studies have revealed important roles of redox-linked chemical events in the signaling for normal development and immune response of T cells, as well as for induction of T-cell-oriented diseases, such as immunodeficiency, allergic and autoimmune diseases, and leukemia. Further studies are needed to develop appropriate therapeutic methods for normalizing the cellular redox status in order to prevent the occurrence of oxidative stress-induced abnormal T-cell-oriented disorders.

ABBREVIATIONS

APC, antigen-presenting cells; ASK1, apoptosis signal-regulating kinase-1; AT, ataxia telangiectasia; ATL, adult T-cell leukemia; AZT, zidovudine; DDI, didanosine; DHA, dehydroascorbate; GSH, glutathione; HIV, human immunodeficiency virus; HTLV-1, human T-cell leukemia virus type 1; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; MeHgCl, methyl mercuric chloride; NAC, N-acetylcysteine; PPP, pentose phosphate pathway; ROS, reactive oxygen species; TCR, T-cell receptors; Trx, thioredoxin.

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Address reprint requests to:
Dr. Izumi Nakashima
Department of Immunology
Nagoya University Graduate School of Medicine
Nagoya, Japan

E-mail: inakashi@med.nagoya-u.ac.jp

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