ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 3, 2002

Forum Review

Redox-Linked Cell Surface-Oriented Signaling for T-Cell Death

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ABSTRACT

T-cell death, which occurs either for ontogenic T-cell selection or for activated T-cell elimination, is normally induced through binding of a specific ligand to cell-surface T-cell receptor for crosslinkage. Heavy metals and carbonyl compounds that bind to protein-reactive groups such as cysteine sulfhydryl groups and lysine ε-amino groups may also induce crosslinkage of cell-surface proteins, in part replacing or modifying the ligand-mediated action. This chemical event has been found to accompany clustering of membrane rafts, to which signal-transducing elements such as glycosylphosphatidylinositol-anchored proteins and Src family protein tyrosine kinases (PTKs) are attached, and to trigger the signal transduction for apoptotic T-cell death, inducing mitochondrial membrane potential reduction, caspase activation and DNA fragmentation. As signals potentially upstream of this signaling, activations of PTKs and mitogen-activated protein (MAP) family kinases and production of reactive oxygen species (ROS) were induced following the cell-surface event, and crucial roles of activation of c-Jun amino-terminal kinase and apoptosis signal-regulating kinase 1 by a redox-linked mechanism in the cell-death signaling were demonstrated. Intriguingly, ROS production as well as PTK/MAP family kinase activation occurred in a membrane raft integrity-dependent manner. The redox-linked and cell surface-oriented signal delivery pathway demonstrated here may play an important role in induction of immune disorders by protein reactive group-binding chemicals. *Antioxid. Redox Signal.* 4, 445–454.

INTRODUCTION

THE FUNCTIONS OF T-CELLS are normally regulated by a network of cell–cell interactions between different cell types, including antigen-presenting cells, which transduces signals intracellularly for determining cell survival/activation or cell death. This occurs in T-cells during either ontogenic development for positive or negative selection or immune responses to antigens for activation or induction of activation-induced cell death. The cell death-inducing signals are normally transduced following binding of specific ligands such as antigens coupled with MHC molecules on antigen-presenting cells to cell-surface receptors such as T-cell receptor (TCR) for crosslinkage. Recent evidence, however, suggests that cell surface receptors can be crosslinked/aggregated by chemicals that bind to protein-reactive groups such as cysteine sulfhydryl (SH) groups and lysine ε-amino groups. These protein-modifying chemicals (PMCs) may dysregulate normal T-cell functions and cause immune disorders such as immunodeficiency, allergic and autoimmune diseases, and tumor development.

Here we review the results of our recent studies on a cell-surface chemical modification-mediated, ligand-independent signal transduction cascade

PMCS AND INDUCTION OF T-CELL DEATH

Among the various PMCs, heavy metals such as mercury and arsenic have been extensively studied because of their harmful effects on human health (21, 59, 79). It has been shown that exposure of humans and animals to higher concentrations of $HgCl_2$ induces rapid tissue damage, but that exposure to lower concentrations results in immune dysfunctions

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leading to allergy and autoimmune diseases, represented by increased serum immunoglobulin, autoantibody production, immune complex deposit, and lymphocyte proliferation (20, 26, 35, 63, 83). Numerous studies focusing on characterization of the immunotoxic properties of mercuric compounds on T-cells to determine the underlying mechanism have indicated that both organic and inorganic mercury compounds induce death of the cells as a result of apoptosis induction (1, 6, 55, 76–78). Compared with resting T-cells, activated T-cells show a reduced susceptibility to mercury for cell death (18). Lower concentrations of $HgCl_2$ have, however, been shown to promote T-cell growth, partially in association with a TCR-ligand interaction-mediated signal (22, 55), or to protect against CD95/Fas-mediated apoptosis in Jurkat T-cells (92).

Arsenic has also been found to cause immunotoxicity, immunosuppression, lymphoproliferative disorders, and increased risk of cancer (9, 13, 16, 98). Recently, arsenic has attracted much interest because of its effectiveness as an anticancer drug for patients with acute promyelocytic leukemia (81). The usage of arsenic as a drug has been based on its action in inducing apoptotic death of leukemia cells. Thus, although arsenic is a well known carcinogen, there has been increasing evidence recently that arsenic is a potent chemical for inducing growth inhibition and apoptosis in both normal (14, 32) and malignant (25, 100) lymphocytes. This action on normal T-lymphocytes seems to underlie the reported immunomodulatory effects of arsenite.

Another category of PMCs that attack cell-surface receptors is carbonyl compounds. They include glyoxal (GO), methylglyoxal (MGO), 4-hydroxynonenal (HNE), and acrolein, which are produced during the metabolism of carbohydrates and lipids by both oxidative and nonoxidative mechanisms. Carbonyl compounds interact with proteins through formation of Schiff base or Michael addition adducts to ultimately form stable products called advanced glycation end products (AGEs) in a long and complex reaction pathway (12, 50, 51). Progressive accumulation of AGEs in tissue proteins has also been found to be associated with aging, diabetic complications, uremic complications, and atherosclerosis lesions (7, 29–31, 50, 51, 54). Not necessarily related to the late stage of AGE formation, MGO itself is known to be toxic to several cells and to induce cell death in a dose-dependent manner. Treatment of human prostatic cancer PC-3 cells with MGO at ≥3 m*M* caused severe growth inhibition, resulting in nearly 100% cell death (49). Toxic effects of MGO on melanoma cells (5) and cortical neurons (42) have also been reported. The addition of 250 μ*M*, but not <200 μ*M*, MGO to culture media of Jurkat cells, a human leukemia T-cell line, caused induction of typical apoptosis accompanying intranucleosomal DNA fragmentation, whereas MGO at a cencentration of >500 μ*M* induced necrosis (23). Depending on its dose, HNE also shows cytotoxicity to many types of mammalian cells, including human fibroblasts (38), endothelial cells (39), Chinese hamster ovary cells (10), and Ehrlich ascites tumor cells (28). HNE at micromolar concentrations causes cell death, but the effects of HNE at lower concentrations vary greatly depending on the experimental conditions, such as the cell type, cell density, incubation time, and method of determination of cell death. The cytotoxicity of HNE on human leukemia T-cell lines was recently shown to depend strictly on its concentration (37, 46). HNE at concentrations of >100 μ*M* caused swift cell death, and HNE at concentrations of 10–50 μ*M* caused slow cell death; however, HNE at concentrations of <10 μ*M* did not cause any detectable damage to cells.

BIOCHEMISTRY OF PMCS

The structures of the mercuric and aesenic compounds commonly used in our experiments are shown in Fig. 1A.

The induction of immune disorders or T-cell death by $HgCl_2$ and $NaAsO_2$ is possibly mediated by chemical modification of signal-transducing molecules, particularly at SH groups of protein cysteines, as shown in Fig. 1B. Mercury has an exceptionally high affinity for SH groups (89) and is therefore capable of strong binding to free SH groups of pro-

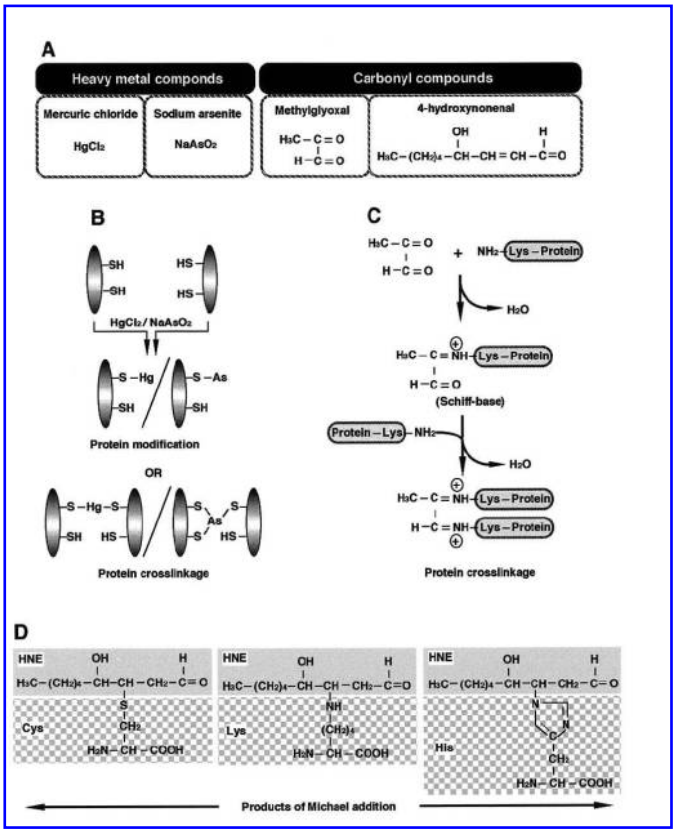

FIG. 1. Structures of chemicals and their reactions with proteins for modification. (A) Structures of mercuric chloride, sodium arsenite, methylglyoxal (MGO) and 4-hydroxynonenal (HNE) are shown. **(B)** Protein modifications by $HgCl_2$ and $NaAsO_2$ are shown. Note that both $HgCl_2$ and $NaAsO_2$ can modify proteins by directly binding to any free protein SH groups. Divalent mercury and trivalent arsenic crosslink proteins by bridging two intermolecular SH groups. **(C)** MGO reacts with the ε-amino group of a protein lysine to form a Schiff base adduct, which may react further with a second protein lysine to crosslink them. **(D)** Products of Michael addition reactions of HNE with cysteine, lysine, and arginine residues in proteins. Note that the free carbonyl groups of these products may form a Schiff base with another protein lysine for inducing crosslinkage.

teins to form mercury–protein complexes. Some of these chemically modified proteins may activate the immune system through T-cell recognition of newly produced modified self-epitope(s) on the mercury–protein complexes, leading to generation of autoantibodies in predisposed individuals (26). However, in addition to this classically suggested mechanism, exposure of murine T-cells or human leukemic Jurkat cells to low concentrations of $HgCl_2$ has been found to result in signal transduction for either T-cell activation or T-cell death, depending on conditions, through a mechanism independent of epitope modification (1, 22, 55). It is therefore likely that activation of effector T-cells or/and abrogation of regulatory T-cells, neither of which depends on epitope modification but can work in parallel with the epitope-dependent mechanism, underlies mercury-mediated induction of autoimmune-related (26, 63) or allergic (83) diseases. Arsenic, another SH group-reactive chemical, also seems to exert its biological activities through modification of SH-rich proteins or enzymes. The protective effects of thiols, such as glutathione (GSH), cysteine, and dithiothreitol, against the toxicity of arsenic suggest that arsenic toxicity results from the formation of reversible bonds with the SH groups of regulatory proteins. A possibly important second mediator for mercury- and arsenic-mediated cellular signal transduction for cell death is reactive oxygen species (ROS), which predispose the cells to death or growth arrest by influencing vital regulatory molecules linked to those processes (17, 32, 76–78, 94).

The cytotoxicities of MGO and HNE are thought to be due to their high reactivity with cellular proteins, and they irreversibly modify proteins under physiological conditions (47, 69). In *in vitro* systems, several studies have shown that MGO interacts with the ε-amino group of lysine in the side chains of proteins to form Schiff bases (47, 48, 52). As MGO possesses two reactive carbonyl groups, it can cause protein crosslinkage as shown in Fig. 1C. In addition, MGO also interacts with SH and guanidine groups of protein cysteine and arginine, respectively (47). On the other hand, HNE as an unsaturated alkenal undergoes Michael addition reactions with cysteine, lysine, and histidine residues in protein (69). The resulting Michael addition products of HNE with these amino acids are shown in Fig. 1D. Like MGO, HNE can also induce crosslinking of proteins through formation of a Schiff base between the free carbonyl group of a Michael addition product (see Fig. 1D) and the ε-amino group of a protein lysine. Although protein modification by MGO and HNE has been implicated in diabetic complications, aging, vascular dysfunction, and atherosclerosis (31, 54, 61, 79, 87), its role in T cell-linked diseases is still unclear. Recently, MGO-modified proteins were found to be increased by severalfold in $CD45RA^+$ memory T-cells in diabetic patients (27). It was also recently shown that HNE binds to surface and cytoplasmic proteins of human T-lymphocytes for regulation of their growth (15).

CELL-DEATH SIGNALING BY PMCS

Recent evidence suggests that a number of PMCs, including heavy metals and carbonyl compounds, have a common activity to trigger signal transduction for cell death.

Apoptotic cell death generally occurs through transduction of death signals that cause morphological changes and affect a number of intracellular key effector molecules step-by-step during the whole process. Electron microscopic analysis of mercury-treated human T-cells has revealed the morphological changes that occur during apoptosis, including chromatin condensation, membrane integrity reduction, loss of organelle structure, and swelling of mitochondria (76, 77). The cell death signal transduction triggered by mercury results in exposure of phosphatidylserine residues at the cell surface detected by binding of annexin V to cell membranes (76), intacellular acidification (77), reduction of Bcl-2 expression (78), reduction of mitochondrial membrane potential (18, 76–78), release of cytochrome *c* (77, 78), activation of capases represented by proteolysis of poly(ADP-ribose) polymerase as a substrate of caspase 3 (78), and fragmentation of nuclear DNA (1, 6).

Basically the same signals have been shown to be transduced for T-cell death induction by arsenite, involving mitochondria as a key effector microorgan (14, 17, 25, 32). Furthermore, both MGO (4, 23, 60) and HNE (37, 45, 46, 99), which affect cellular proteins through a different chemical reaction from that by heavy metals, have also been demonstrated to trigger similar signals for cell-death induction.

All of these studies suggest that PMCs, which have distinct chemical structures, display a common activity in inducing apoptotic T-cell death probably through a mitochondrial pathway. However, the signal transduction cascade upstream of the mitochondrial pathway is still not clear. PMCs may or may not directly attack mitochondria to generate signals for cell-death induction. Recent evidence suggests that at least a part of the signaling starts at the cell surface where PMCs bind proteins for modification (2, 32, 55–57, 70).

CELL-SURFACE MODIFICATION BY PMCS

The surfaces of T-lymphocytes contain a huge number of receptor proteins and glycoproteins, including TCR/CD3, CD4/CD8, CD2, CD28, CD45, CD11/CD18, Thy-1, and cytokine receptors. Physiologically, signals are transduced from the cell surface to the cytoplasm when ligands bind to their specific receptors. These cell-surface proteins are likely to interact also with extracellular PMCs. We found that $HgCl_2$ (55), $NaAsO_2$ (32), and GO/MGO (2) induce aggregation of Thy-1, one of the major cell-surface proteins on murine thymic T lymphocytes. Thy-1 is known to anchor with glycosylphosphatidylinositol (GPI) in the membrane and associate with Src family kinases beneath the plasma membrane for signal delivery (82, 85). $HgCl_2$ also induced aggregation of cell-surface CD3 and CD4. As the reaction modes of $HgCl_2$/$NaAsO_2$ and MGO differ, the mechanisms by which they induce Thy-1 aggregation are also likely to differ. Dithiothreitol abolished $HgCl_2$- or $NaAsO_2$-mediated Thy-1 aggregation, indicating that this aggregation occurs following the bridging of SH groups of respective proteins. On the other hand, MGO induces aggregation of cell-surface proteins by a Schiff-base reaction between the carbonyls of MGO and ε-amino groups of proteins. Unlike MGO, HNE participates in cell-surface protein crosslinkage by first undergoing Michael

addition reactions with cysteine, lysine, or histidine residues in protein and then by formation of a Schiff base between the free carbonyl group of the Michael addition product and the ε-amino group of another protein lysine. Such a mechanism may also underlie the HNE-induced clustering of epidermal growth factor receptor (EGFR) (45).

The detergent-insoluble membrane microdomain termed "raft" has received much attention in the last few years because of its diverse roles in T-cell signaling. A raft is a specialized membrane region that is composed of cholesterol, sphingolipid, ganglioside, glycerophospholipid, and certain transmembrane GPI-anchored or membrane-bound intracellular proteins (Fig. 2A) (64). Rafts act as a platform in the cell membrane for signaling molecules like G proteins, phosphatidylinositol 3-kinase, Src-family kinases, and some adaptor proteins (34). Several intracellular, extracellular, and transmembrane constraints and forces influence the size and distribution of the rafts and their cholesterol component (11). Alteration of the cholesterol content or composition of the rafts changes the association pattern and signaling effects of various molecules. Recent studies have shown that clustering or crosslinking of cell-surface molecules on membrane rafts by their ligands induces T-cell and dendritic cell activation

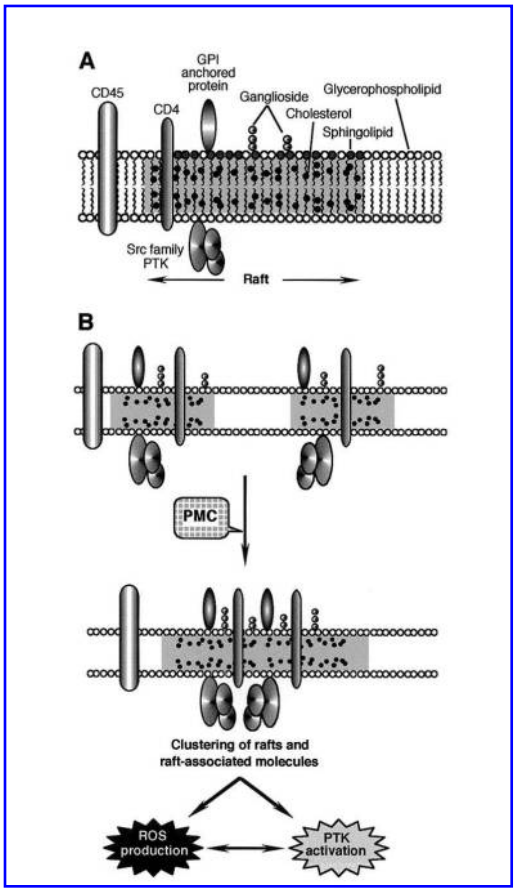

FIG. 2. Cell-surface events triggered by PMC. (A) The architecture of a membrane raft is shown with its constituents as indicated. **(B)** PMCs, such as $HgCl_2$, $NaAsO_2$, MGO, and HNE, induce clustering and aggregation of rafts and raft-associated molecules. This event induces both ROS production and PTK activation for subsequent signal transduction.

(91, 95). We have found that membrane rafts, detected by staining with ganglioside-binding cholera toxin, cluster in association with aggregation of Thy-1 by $HgCl_2$ or $NaAsO_2$ (unpublished observations). We further found that sequestration of cholesterol by β-cyclodextrin from the membrane rafts dispersed arsenic-induced Thy-1 aggregation, suggesting a role of cholesterol as well as integrity of membrane rafts in the mechanism of arsenite-induced Thy-1 aggregation (32).

ACTIVATION OF PROTEIN TYROSINE KINASES

Possibly downstream of the PMC-induced aggregation/clustering of cell-surface proteins/membrane rafts, exposure of lymphocytes to $HgCl_2$ was shown to induce phosphorylation of a number of cellular proteins in a time- and concentration-dependent manner (1, 40, 43, 55, 68, 73). This promotion of protein tyrosine phosphorylation was then found to accompany activation of Src family protein tyrosine kinases (PTKs), such as $p56^{lck}$ (55), $p60^{c\text{-}Src}$ (66, 67) and Hck (71). Exposure of lymphocytes to arsenite was also shown to activate PTKs, in association with its mitogenic and apoptosis-inducing activities (32, 86, 93). Carbonyl compounds, such as MGO (2, 4) and HNE (45, 58, 88), have also recently been shown to induce tyrosine phosphorylation of multiple cellular proteins accompanying activation of $p60^{c\text{-}Src}$ (2) and EGFR (45, 84).

Interestingly, a mutant T-cell line deficient in the GPI anchor was shown to respond poorly to $HgCl_2$ for subsequent intracellular signaling, suggesting that cell-surface proteins are the primary target of $HgCl_2$ to start the signaling for PTK activation (65). Moreover, $NaAsO_2$-mediated signaling for PTK activation was inhibited by treating the cells with β-cyclodextrin for extraction of cholesterol from membrane rafts (32). This suggests that raft integrity is needed for PMC-induced PTK activation (Fig. 2B).

ACTIVATION OF MITOGEN-ACTIVATED PROTEIN (MAP) FAMILY KINASES

Activated tyrosine kinases in most cases convey their signals to the downstream signaling elements in a cascade, including Shc/Ras/MAP kinase family members. Activation of Src family kinases by $HgCl_2$ (55, 66, 71) or by GO/MGO (2) was shown to be accompanied by tyrosine phosphorylation of Shc (1) and phosphorylation/activation of MAP family kinases, such as extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38 kinase (1, 22, 23). Similarly, arsenic has been shown to stimulate the phosphorylation of ERK, JNK, and p38 kinase in murine T-lymphocytes through a PTK-dependent pathway (32). HNE has also been shown to induce activation of JNK (45, 62, 74, 80, 88). Phosphorylation/activation of MAP family kinases by either heavy metals (1, 32) or carbonyl compounds (4, 23, 74, 88) is followed by downstream phosphorylation/activation/expression of the transcription factor c-Jun with elevated AP-1 DNA-binding activity. Hossain *et al.* (32) showed that $NaAsO_2$-induced activations of ERK, JNK, and p38 kinase were all inhib-

ited by treating the cells with β-cyclodextrin, suggesting that PMC-induced activation of MAP family kinases also occurs downstream of raft integrity-dependent cell-surface events.

Activations of JNK and p38 kinase, possibly following activation of apoptosis signal-regulating kinase 1 (ASK1) by a redox-linked mechanism (33, 75), are often linked with the cell-death process (36, 90, 96). It is therefore likely that both heavy metal- and carbonyl compound-mediated activations of JNK and/or p38 kinase are associated with the induction of apoptosis. Partially supporting this view, HNE-induced JNK activation has been shown to accompany intracellular peroxide production (88) and has been suggested to occur through a stress-activated protein kinase/extracellular signal-regulated kinase 1 (SEK1)–ASK1 pathway (80) or through direct HNE–JNK interaction (62). We have shown that transfection of dominant-negative mutant JNK rescues cells from undergoing MGO-mediated apoptosis (23). MGO-induced activation of JNK/p38 kinase has further been shown to be caused by upstream SEK1,which is activated by ASK1 (24). The rate of MGO-mediated apoptosis (DNA fragmentation) was actually enhanced by stable transfection of the cells with wild-type ASK1, whereas apoptosis was noticeably blocked by transfection of the cells with kinase-inactive mutant ASK1 (24), suggesting that ASK1 activity is required for MGO-induced apoptosis.

CHANGES IN CELLULAR REDOX BALANCE

Many heavy metals and carbonyl compounds have the ability to change the cellular redox level, and this change may play a crucial role in the signal transduction for apoptosis induction.

As potent SH group-reactive heavy metals, mercury and arsenite are capable of changing the cellular redox balance, and a change in the cellular redox balance may play a role in signal delivery for cell death. It has been suggested that the underlying basis for the toxic effects of mercury/arsenite involves perturbation of mitochondrial function, leading to generation of ROS (17, 18, 21, 32, 76–78, 94). This generation of ROS is accompanied by a reduction of the intracellular GSH content (19, 72, 76–78). The ROS produced in cells may be involved in activating ASK1, a MAP kinase kinase kinase that lies upstream of JNK and p38 kinase, for promotion of apoptosis.

Glycation reaction of proteins with MGO is thought to be one of the main mechanisms by which MGO induces production of ROS or superoxide (O_2^-) (44, 97). Our work on MGO-treated Jurkat cells showed that O_2^- production is important for MGO-induced apoptosis (24). Using a lucigenin-derived chemiluminescence assay, which is believed to be specific for O_2^-, we found that an appreciable increase in O_2^- occurred shortly after MGO treatment. Furthermore, addition of Mn(III) tetrakis(4-benzoic acid) porphyrin chloride (MnTBAP) alone, a cell-permeable low-molecular-weight superoxide dismutase mimetic, or catalase alone to Jurkat cell culture caused only partial protection of the cells from MGO-induced apoptosis. Intriguingly, MGO-induced apoptosis and activation of ASK1 were greatly decreased by the addition of both MnTBAP and catalase together. The synergism of MnTBAP and catalase for blocking MGO-induced apoptosis suggests that both formation of O_2^- and subsequent increase in H_2O_2 are involved in MGO-induced cytotoxicity (24).

As an end product of membrane lipid peroxidation, HNE itself is a potential source of intracellular prooxidant (88). This explains how HNE mediates oxidative stress-induced cytotoxicity. We have reported that HNE treatment reduces the level of GSH in Jurkat T-cells and that this reduction can be prevented by the addition of *N*-acetylcysteine (46). This reduction of GSH is closely associated with activation of the caspase cascade and DNA fragmentation, demonstrating that alteration of the cellular redox status plays an important role in HNE-induced T-cell apoptosis.

All of the recent studies suggest that ROS produced following exposure of cells to heavy metals or carbonyl compounds play a crucial role as a second messenger in relaying the signal for apoptotic cell death. We lastly investigated whether PMC-induced cell-surface events are somehow involved in the mechanism of ROS generation in cells. We found that sequestration of cholesterol by β-cyclodextrin from the membrane rafts, which dispersed arsenic-induced Thy-1 clustering, inhibited ROS production, PTK/MAP kinase family kinase activation, and caspase activation following $NaAsO_2$ treatment (32). This suggested that cholesterol, as well as integrity of membrane rafts, plays a key role in arsenite-induced signaling for cell-death induction. It is therefore speculated that PMC-induced aggregation or clustering of cell-surface proteins/membrane rafts is the initial event that leads to ROS production and subsequent signal transduction for T-cell death (Fig. 2B). We further observed that the addition of β-cyclodextrin together with excess cholesterol, which prevented chelation of cholesterol from the membrane rafts, allowed arsenite again to produce ROS, suggesting that cholesterol in the membrane rafts is one of the key components for arsenite-induced ROS production (our unpublished observation).

Taken together, the results suggest that some, although not necessarily all, PMCs induce ROS production through a membrane raft integrity-dependent mechanism and that ROS production plays a crucial role in signal transduction for apoptotic T-cell death.

SUMMARY OF SIGNALING FOR PMC-INDUCED T-CELL DEATH

All of the above-described observations suggest that PMCs trigger a probably common signal pathway for T-cell death. PMCs start delivering signals after primarily targeting cell-surface proteins, including GPI-anchoring proteins, for induction of their aggregation and clustering. This event is accompanied by the activation of receptor-type and nonreceptor-type PTKs and the production of ROS. The generated ROS may accelerate activation of PTKs possibly through inactivation of regulatory protein tyrosine phosphatases (53) and/or a redox reaction-mediated structural modification of PTKs (3, 41), or the generated ROS may activate the ASK1–SEK1–JNK pathway, leading to activation of caspases for cell death. ROS production and ASK1/JNK activation

were shown to be crucial for caspase activation. PMC-mediated activation of PTKs causes activation of the Ras–MAP kinase pathway, which is probably not involved in the signal cascade for apoptosis induction, but works for cellular activation or cytokine production. Tentative signaling cascades that are triggered by PMCs are shown in Fig. 3, pending the possibility that other signaling cascades work in parallel, depending on conditions. Importantly, all of the signal transduction events in this scheme, including cell-surface protein aggregation, ROS production, PTK/JNK activation, and caspase activation, were shown to require the integrity of glycosphingolipid/cholesterol-enriched membrane rafts.

The demonstrated PMC-mediated signaling for T-cell death induction may in part be involved in the mechanism of induction of immunodeficiencies, immune disorders, and autoimmune diseases in humans and animals. For example, *in vivo* development of immune disorders could follow selective elimination of regulatory T-cells that are more sensitive to ROS for apoptotic cell death, potentially in association with a lower concentration of PMC-mediated activation of effector T-cells.

PMC-mediated apoptotic cell death can be utilized therapeutically as arsenic has been used in the treatment of acute promyelocytic leukemia. A recent study has also suggested that the synergistic effect of combination therapy of arsenic and interferon induces cell-cycle arrest and apoptosis in human T-cell lymphotropic virus-infected cells (8).

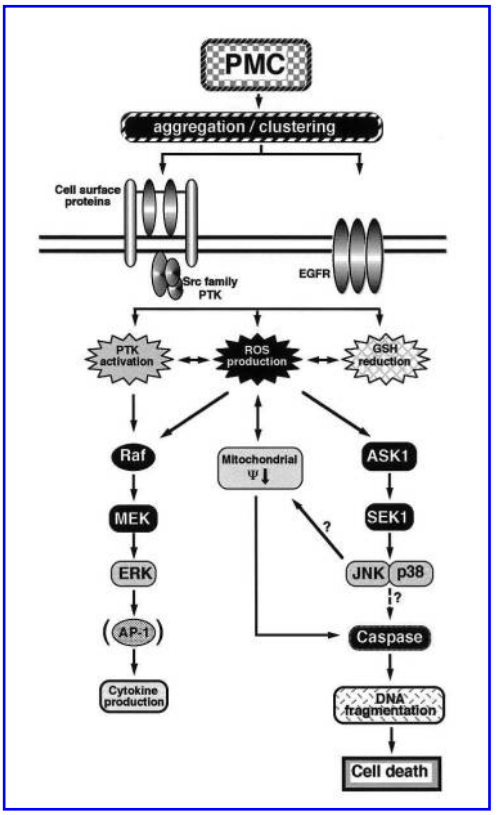

FIG. 3. Schematic representation of signal transduction cascades for PMC-induced T-cell death. Signals are generated soon after PMCs induce aggregation or clustering of cell-surface proteins and rafts. These signals lead to ROS production and PTK activation, both of which then relay the signals to downstream elements for ultimately inducing cell death or promoting cell growth, depending on the conditions.

CONCLUDING REMARKS

PMCs, such as heavy metals and carbonyl compounds, were shown to induce aggregation of cell-surface proteins and clustering of membrane rafts, which delivered signals for inducing apoptosis of T-cells in a membrane raft integrity-dependent manner. The cascade of this signal delivery included ROS production, activation of ASK1 and JNK, reduction in mitochondrial membrane potential followed by caspase activation and DNA fragmentation. This ligand-independent signal transduction pathway may be involved in the pathogenesis of chemically induced T-cell disorders and related diseases.

ACKNOWLEDGMENTS

This work was supported in part by the funds for Comprehensive Research on Aging and Health from the Ministry of Health and Welfare of Japan and by Grants-in-Aid for Scientific Research of Priority Areas from the Ministry of Education, Science, Sports, and Culture of Japan. We thank Y. Umeda and H. Saeki for technical assistance

ABBREVIATIONS

AGE, advance glycation end product; ASK1, apoptosis signal-regulating kinase 1; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GO, glyoxal; GPI, glycosylphosphatidylinositol; GSH, glutathione; HNE, 4-hydroxynonenal; JNK, c-Jun amino-terminal kinase; MAP, mitogen-activated protein; MGO, methylglyoxal; MnTBAP, Mn(III)tetrakis(4-benzoic acid) porphyrin chloride; $O_2^{\cdot-}$, superoxide; PMC, protein-modifying chemical; PTK, protein tyrosine kinase; ROS, reactive oxygen species; SEK1, stress-activated protein kinase/extracellular signal-regulated kinase 1; SH, sulfhydryl; TCR, T-cell receptor.

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Received for publication July 19, 2001; accepted October 26, 2001.

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