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Biology of Disease

Cell and Tissue Responses to Oxidative Damage

YVONNE M. W. JANSSEN, BENNETT VAN HOUTEN, PAUL J. A. BORM, AND BROOKE T. MOSSMAN

Department of Pathology, University of Vermont, Medical Alumni Building, Burlington Vermont; and the Department of Health Risk Analysis and Toxicology, University of Limburg, Maastricht, The Netherlands

INTRODUCTION

Although oxygen is a necessary requirement for aerobic organisms, its more reactive metabolites, active oxygen species (AOS), are implicated in a number of diseases. Bacterial and mammalian cells have elaborate defense mechanisms against AOS including enzymatic and nonenzymatic antioxidants (1–3). However, under certain conditions, these defense systems may be inadequate, and cell damage and/or disease ensue.

Currently, much attention is focused on AOS and their deleterious effects on cells (lipid peroxidation, DNA damage etc.). Moreover, biomarkers for prediction of disease are being developed to measure the status of oxidative stress in individuals (4–10). However, little is known about cellular responses to AOS-mediated damage. For example, some cells may respond to AOS-mediated stress by altering expression of certain genes and synthesizing proteins involved in scavenging or repair of AOS-induced lesions. These phenomena may reflect adaptive responses. Other cell types may be deficient in repair of damage induced by oxidant stress. In this review, pathways of generation of AOS and various diseases associated with elaboration of AOS and/or altered DNA repair will be addressed briefly. Lastly, we will focus on specific responses of mammalian cells to oxidative stress and their implication in cellular defense, adaptation and protection from disease.

FORMATION AND REMOVAL OF AOS

During reduction of molecular oxygen to water in cellular respiration, partially reduced oxygen species are produced which are very reactive with protein, lipids and DNA (3). These AOS are formed in the mitochondrial electron transport chain (11), the cyclooxygenase pathway, and by cellular enzymes such as cytochrome P450 oxidase, xanthine oxidase and NADPH oxidase (3, 7, 11, 12). AOS have important physiological roles in the metabolism of various xenobiotic compounds (7) and in bacterial killing by phagocytes. During phagocytosis, a membrane bound enzyme (NADPH oxidase) is activated in neutrophils and macrophages which evokes an oxidative burst and the formation of various AOS (Fig. 1). Neutrophils contain myeloperoxidase which converts hydrogen peroxide (H_2O_2) into hypochlorous acid (HOCl), a relatively long-lived oxidant (7, 10). In cellular and acellular systems, the presence of iron or other divalent cations can convert superoxide (O_2^-) and hydrogen peroxide (H_2O_2) to the hydroxyl radical ($OH^\cdot$), one of the most reactive AOS (13). Another potential source of O_2^- is the peroxisomal enzyme, xanthine oxidase (14), which

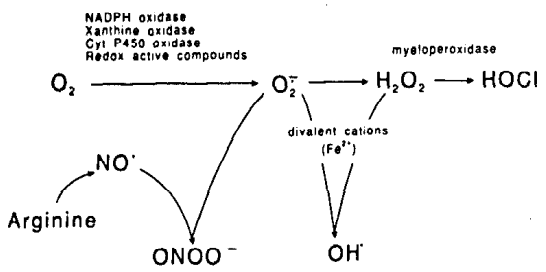

FIG. 1. Pathways of formation of active oxygen species (AOS). O_2^-, superoxide anion; OH·, hydroxyl radical; H_2O_2, hydrogen peroxide; NO·, nitric oxide; HOCl, hypochlorous acid; ONOO-, peroxynitrite anion.

generates O_2^- and H_2O_2 during the metabolism of xanthine or hypoxanthine to uric acid (7, 15). Cellular conversion of xanthine dehydrogenase to xanthine oxidase occurs in a variety of cell types after exposure to specific stimuli, including phorbol esters, tumor necrosis factor-α (TNF) or ischemia-reperfusion injury (15, 16). Nitric oxide (NO·), formed during oxidation of arginine, can react with O_2^- to form peroxynitrite (ONOO-), a stable intermediate which decomposes into a strong oxidant with reactivity similar to OH· (17).

A variety of exogenous chemical and physical agents also cause production of AOS (3, 13, 18–21). These include mineral dusts, ozone, nitrogen oxides and other gases, ultraviolet radiation, ionizing radiation, etc. Other compounds constituting an important source of oxidant stress are phytoalexins or plant stress metabolites such as rose bengal, psoralen and quinones (22). Some of these species can undergo redox cycling within the cell resulting in a continuous generation of AOS (22).

AOS are potentially harmful to cells because they interact with and modify a spectrum of biomolecules (7, 12, 18, 23). Some biochemical effects of AOS resulting in progressive cell damage include lipid peroxidation, oxidative modification of proteins, and DNA alterations. Lipid peroxidation consists of a series of reactions resulting from the interaction of AOS with polyunsaturated fatty acids. In a chain reaction, a range of AOS can be formed including OH·, H_2O_2, singlet oxygen, peroxyl and alkoxyl radicals (10). Numerous studies use products of lipid peroxidation as indicators of oxidative stress, and sensitive techniques are available to measure the extent of lipid peroxidation in cells, lavage fluids and urine (6, 24).

AOS also activate or inactivate proteins. For example, stimulated neutrophils which release AOS can inactivate glutamate synthetase and several other endogenous enzymes (25) including copper zinc-containing superoxide dismutase (CuZnSOD) (26). Alternatively, guanylate cyclase is activated following sulfhydryl oxidation by H_2O_2 resulting in the production of the second messenger, cyclic GMP (27). Various AOS also inactivate certain antioxidant enzymes or generating systems directly (26, 28–31). For example, inactivation of xanthine oxidase by H_2O_2 might provide a negative feedback mechanism for prevention of cellular or tissue injury (32).

Elaborate defense systems against AOS exist in bacterial and mammalian cells (1, 3, 19, 33, 34). The functions of some relevant antioxidant enzymes are outlined in Figure 2. Mammalian tissues contain three forms of superoxide dismutase (SOD), an enzyme converting O_2^- to H_2O_2. These include two CuZnSODs, one localized extracellularly (ECSOD) (35) and the other within peroxisomes (36) and the cytoplasm (37). The third form of SOD contains manganese (MnSOD) and is localized almost exclusively in the mitochondria (38). The SODs and other antioxidant enzymes, including catalase and glutathione peroxidase (GPX), are complemented by a number of non-enzymatic factors located both intra- and extracellularly (34, 39). Sulfhydryl-containing molecules such as glutathione (GSH), vitamins C and E, albumin, ceruloplasmin, bilirubin and uric acid are components of the non-enzymatic antioxidant system (12). Metallothionein and heme oxygenase are proteins induced after exposure to agents that cause oxidative stress (40, 41). Although not considered traditionally as antioxidants, both proteins have antioxidant functions. Metallothionein, a sulfhydryl-rich protein involved in metal homeostasis, is a scavenger of OH· *in vitro* (40). Heme oxygenase, an enzyme catalyzing conversion of heme to biliverdin, leads to a reduction of the cellular pool of heme and heme containing proteins, thus removing potential pro-oxidant catalysts. Furthermore, bilirubin, the end product of the heme degradation pathway, is a molecule with antioxidant properties (41, 42). This combination of enzymatic and nonenzymatic sources provides an important protective system against various oxidant stresses. Normally, a balance exists between formation of AOS and antioxidants. However, oxidative stress, cell injury and disease may ensue after excessive production of oxidants or deficient functioning of antioxidant defenses (3, 10, 39).

DISEASES ASSOCIATED WITH AOS

Various pathological conditions are associated with AOS-mediated events, including cancer, aging, rheumatoid arthritis, various pulmonary disorders including

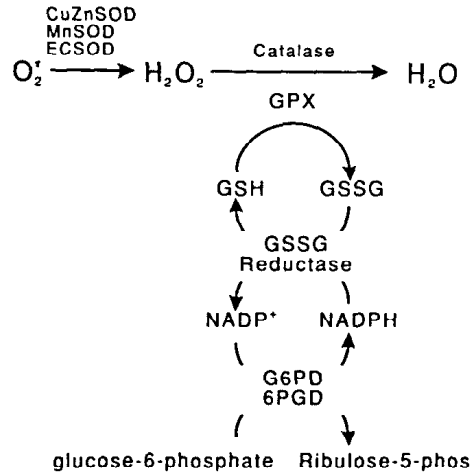

FIG. 2. Detoxification of AOS by antioxidant enzymes. This interrelated system is complemented by a number of non-enzymatic antioxidants. CuZnSOD, copper zinc-containing superoxide dismutase; MnSOD, manganese-containing superoxide dismutase; ECSOD, extracellular superoxide dismutase; GPX, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; G6PD, glucose 6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase.

adult respiratory distress syndrome (ARDS), and pulmonary fibrosis, ischemia-reperfusion injury, and immune complex-mediated disease (1, 3, 7, 18, 43–45). Of these diseases, the involvement of AOS in carcinogenesis has been studied most intensely in a number of experimental models.

Carcinogenesis is a multi-stage process which classically is subdivided into several phases (1, 2). Initiation involves the interaction of a carcinogen with DNA resulting in a persistent lesion which can lead to mutations and heritable changes within the cell. Subsequently, selection and clonal proliferation of initiated cells occur during promotion. In the final stages of carcinogenesis, progression of benign lesions and subsequent genetic and phenotypic changes culminate in the establishment of a malignant neoplasm (1).

Strong evidence exists that AOS play an important role in all stages of tumorigenesis (1, 2, 7, 10, 46–49). For example, direct interaction of AOS with DNA is implicated in the initiation and progressional stages of carcinogenesis (1, 3, 47, 48). AOS cause formation of oxidized bases (7, 8, 10) and a spectrum of DNA lesions including base damage, single strand breaks, double strand breaks, crosslinking of DNA, and damage to the deoxyribose moiety (Fig. 3, Table 1) (10, 50). Since neither O_2^- nor H_2O_2 appears to cause strand breaks or base modifications in DNA under physiological conditions, it has been proposed that conversion of these species to $OH^\cdot$ in the presence of metal ions via a Fenton reaction is responsible for DNA damage (9, 10, 51, 52).

A number of new techniques have become available for sensitive measurement of oxidative lesions in DNA. For example, gas chromatography/mass spectrometry with selected ion monitoring can be used to characterize the spectrum of DNA damage after exposure to xenobiotics or AOS, thus providing a method for fingerprinting

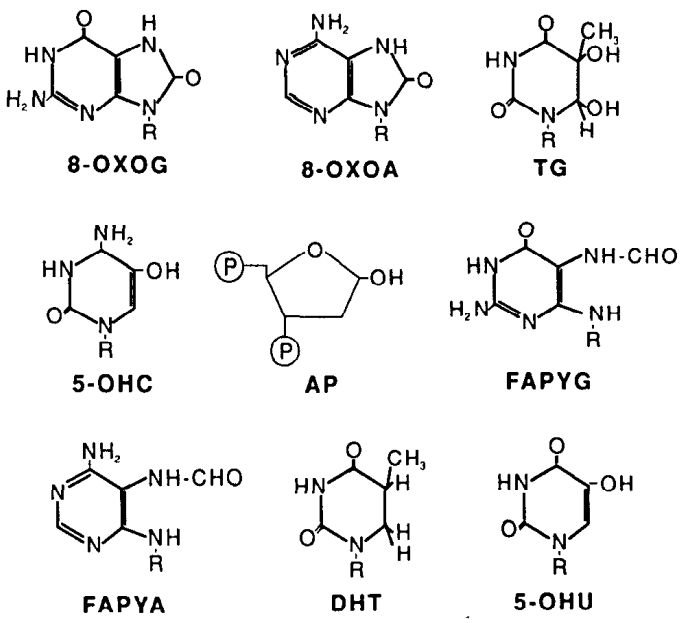

FIG. 3. Structure of common base damages following exposure of DNA to AOS. Some of the known DNA lesions include: 8-oxoguanine (8-oxoG) or 8-oxoadenine (8-oxoA); thymine glycol (TG); 5-hydroxycytosine (5-OHC); apurinic/apyridinic abasic site (AP), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPYG), 4,6-diamino-5-formamidopyrimidine (FAPYA), dihydrothymine. (DHT), 5-hydroxyuracil, (5-OHU).

DNA damage (10). One of the most prevalent types of endogenous DNA damage is the oxidative lesion, 8-oxodeoxyguanine (8-oxoG), also referred to as 8-hydroxyguanine (8, 53). 8-oxoG is a major mutagenic lesion and a suspect lesion in the formation of both spontaneous cancers and those induced by a number of different agents. For example, 8-oxoG formation is observed in DNA after exposure to the known carcinogens, 4-nitro-

TABLE 1. COMMONLY DETECTED DNA BASE DAMAGES ASSOCIATED WITH ACTIVE OXYGEN SPECIES

Damage	Repair pathways[a]	Consequences
8-oxoguanine	FAPY glycosylase(*MutM*) 8-oxoGC 8-oxo GTPase(*MutT*) A glycosylase(*MutY*) BER[b] NER[c] (?)	G → T transversions
8-oxoadenine	Not known NER (?)	Not known
FAPYG[d]	FAPY glycosylase(*MutM*)/BER NER (?)	Cytotoxic lesions
FAPYA[e]	FAPY glycosylase(*MutM*)/BER NER (?)	None known
Thymine glycol	Endonuclease III(*nth*)/BER	Cytotoxic lesions
Dihydrothymine	Endonuclease III(*nth*)/BER	None known
5-hydroxycytosine	Not known	Not known
5-hydroxyuracil	Not known	C → T transitions
Abasic sites	AP endonucleases(*nth*, *xth*, *nfo*)/BER NER	Insertion of A

[a] Most of the repair pathways have been characterized in *E. coli* (64). The *E. coli* genes encoding respective enzymes are *italicized* in *parentheses*. Homologous enzymes in mammalian cells have been identified, but have not been well characterized.
[b] BER, base excision repair.
[c] NER, nucleotide excision repair.
[d] FAPYG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine.
[e] FAPYA, 4,6-diamino-5-formamidopyrimidine.

quinoline oxide, ionizing radiation, $KBrO_3$, and 2-nitropropane (18, 54).

The involvement of AOS in tumor promotion is indicated by studies showing that generating systems of AOS mimic some of the biochemical actions of tumor promoting compounds (47, 48, 55). These effects include increased activity of ornithine decarboxylase (ODC), a rate limiting enzyme in the biosynthesis of polyamines integral to cell proliferation (56, 57), increased incorporation of ^{3}H-thymidine (54), and activation of protein kinase C (PKC) (58). Moreover, phorbol ester tumor promoters can stimulate endogenous formation of AOS in several cell types and cause a decrease in cellular antioxidant defenses (7). Lastly, it has also been shown that antioxidants are anticarcinogens and inhibit tumor promotion and/or progression in experimental models of carcinogenesis (1, 2, 49, 59).

The critical role of DNA repair in the prevention of cancer development is indicated by several syndromes in which specific repair pathways are blocked. The disease xeroderma pigmentosum (XP) is characterized by a defect in a nucleotide excision pathway which renders patients extremely sensitive to UV-induced DNA damage and skin cancer (60). Fibroblasts from patients with ataxia telangiectasia (AT) are hypersensitive to the lethal effects of ionizing radiation, implying that these cells are defective in the ability to process radiation-induced DNA damage (60). Moreover, patients with AT are extremely sensitive to X-rays and have an increased incidence of cancers of the lymphoreticular system (60). Other repair deficiencies and their consequences in man have been reviewed elsewhere (60–62).

Repair of Oxidative DNA Damage in Mammalian Cells

Repair of other biomolecules such as lipids and proteins may occur after oxidative injury, but most research has focused on mechanisms of DNA repair. AOS-induced DNA damage is not a rare event, and it is estimated that a human cell sustains an average of 10^3 'oxidative hits' per day due to cellular oxidative metabolism (63). However, DNA is functionally very stable, and the incidence of cancer is much lower than one would expect considering the high frequency of oxidative attacks. This protection is accomplished in part by a broad array of enzymes involved in the repair of DNA lesions (reviewed in 64–68). Table 1 summarizes DNA base damages and repair pathways associated with AOS and the known biological consequences of these base damages. The importance of these repair systems is indicated by the existence in cells of multiple enzymes involved in the repair of single lesions and the high degree of homology of DNA repair genes in various species.

Base damage induced by AOS can be repaired by two general pathways in both bacterial and mammalian cells. These include base excision repair and nucleotide excision repair (67). Base excision repair represents the removal of oxidative base damages by specific glycosylases followed by the action of apurinic/apyrimidinic (AP) endonucleases which cleave the phosphodiester backbone resulting in the loss of the abasic sugar. The nucleotide gap is then filled in and sealed by the action of DNA polymerase and DNA ligase.

The five general steps in the base excision repair pathway of DNA damage in bacterial and mammalian cells are shown in Figure 4. Base damage is recognized by a glycosylase which excises the base resulting in an abasic site. The cell contains many glycosylases, some of which also contain AP-endonuclease activity recognizing a specific type of base damage. Abasic sites are recognized by endonucleases. Dual action of 5′ and 3′ AP-endonucleases and/or subsequent action of 5′→3′ exonuclease generates gaps in the strand. During the resynthesis step, DNA polymerase fills in these gaps, and DNA ligase seals the strand and completes the DNA repair process.

Base excision repair is also referred to as "short patch repair" as it involves resynthesis of 1–3 nucleotides. Another repair pathway of DNA damage which exists in both bacterial and mammalian cells involves resynthesis of larger patches of DNA, thus is referred to as "long patch" or nucleotide excision repair (69). The molecular events of this repair process have not been well characterized in mammalian cells, but involve the coordinate action of a number of different proteins at sites of numerous bulky DNA lesions.

Several other repair enzymes are induced in mammalian cells in response to DNA damage (70–73). DNA strand breaks induced by a variety of oxidants activate a nuclear enzyme (poly-ADP ribose polymerase) involved in repair of DNA lesions (72, 74, 75). However, activation of this enzyme diminishes cellular NAD and ATP levels and is associated with decreased cell survival (75). Inhibition of poly-ADP ribose polymerase activity in mammalian cells exposed to lethal concentrations of H_2O_2 prevents the sequence of events that eventually lead to cell lysis (72). These observations illustrate a circumstance where induction of a DNA repair enzyme is associated with cell injury and death (72, 75).

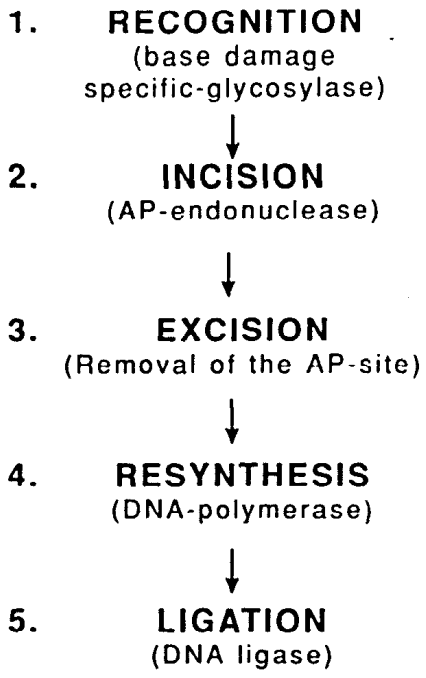

FIG. 4. Base excision repair of DNA damage in mammalian cells.

Alterations in Gene Expression and Proteins after Specific Oxidative Stresses in Mammalian Cells

After exposure to oxidants, mammalian cells may express stress-induced genes or genes encoding antioxidant enzymes and related proteins. One approach used to elucidate alterations in gene expression following oxidative stress is subtractive hybridization (76, 77). In this method, RNA isolated from cells exposed to oxidants is hybridized to RNA from control cells, and unique RNA species which remain present following the subtraction process can be used to elucidate genes expressed selectively after AOS exposure (78).

Other investigators have used two-dimensional gel electrophoresis to determine alterations in protein synthesis after oxidative stress (79, 80). Moreover, several laboratories have studied the appearance of new enzymes or altered enzymatic activities (81). With the use of these techniques, molecular responses have been investigated after exposure to various oxidant stresses including UV radiation, ionizing radiation, phorbol ester tumor promoters and hyperoxia. Some patterns of response after exposure to various agents are similar (82). However, other pathways of activation appear to be unique to the type of oxidative stress. Some recent findings and their implications in adaptation and/or disease are described below.

Ultraviolet (UV) Radiation. Exposure to UV causes intracellular production of AOS (20) and formation of thymidine dimers which are repaired through a "long patch" nucleotide excision repair pathway (83). The 'UV response' has been defined as the spectrum of genetic changes in mammalian cells following irradiation with UV (or treatment with other DNA damaging agents) including increased expression and amplification of UV-responsive genes (82). Cellular responses to UV have been divided into three phases: (a) primary interactions between the DNA damaging agent and the cell; (b) signal transduction and molecular targeting; and (c) long-lasting cellular consequences (82, 84).

Several pieces of evidence support the hypothesis that DNA is the primary target of the UV response. For example, induction of gene expression in XP skin fibroblasts requires a much lower dose of UV than that required in normal skin fibroblasts. Moreover, transfection of oxidant-damaged DNA into a cell elicits the UV response (82, 84).

Exposure to UV also causes activation of gene regulatory factors intrinsic to cellular replication including transcription factors binding to UV-responsive elements (URE) (82, 84–87). Moreover, UV activates several transcription factors (*i.e.*, AP-1 and NFκB) by post-translational modification (82, 84).

UV radiation also induces a number of proto-oncogenes, including c-*fos* and c-*myc* in a variety of cell types (84, 88, 89). Since increases in mRNA expression of c-*fos* have been reported in CHO cells after exposure to a number of DNA-damaging agents, including UV radiation, increased c-*fos* expression may be a general stress response of the cell (88). In addition to induction of certain proto-oncogenes, UV stimulates levels of p53 cellular tumor antigen, a potent transcription factor that also mediates arrest of the cell cycle at G1 (90, 91).

Several DNA damage-inducible transcripts have been identified using subtractive hybridization in mammalian cells following UV exposure (77). In CHO cells, two of 20 sequenced clones matched known gene sequences identified as metallothionein (MT) I and II. The induced transcripts could be separated into two general classes: class 1 transcripts [induced specifically by UV irradiation and *N*-acetoxy-2-acetylaminofluorine (AAAF)] and class 2 transcripts [induced by UV irradiation, AAAF and methyl methanesulfonate (MMS)]. Many class 2 transcripts were also induced by H_2O_2 and various alkylating agents, but not by heat shock, phorbol ester or DNA-damaging agents not producing high levels of base damage. Induction of these DNA damage-inducible (DDI) transcripts is most likely a direct response to DNA damage since induction is rapid and occurs only after exposure to certain types of DNA damaging agents (77). In other studies, MT I and MT II are rapidly and coordinately induced by UV (84, 92, 93) but not by X-rays and H_2O_2 (92). Thus, MT induction appears to be fairly specific to the UV response (92). The signals responsible for induction of class 1 or 2 transcripts are unknown. Class 1 transcripts may be induced by helical distortion or a step in the nucleotide excision repair pathway whereas class 2 transcripts may be induced by agents which induce high levels of base damage in DNA (UV, MMS, AAAF, H_2O_2) and other alkylating agents (77).

In most cells, one effect of DNA damage is a transient inhibition of DNA synthesis and arrest of cell growth (94). Several of the class 2 transcripts expressed after UV exposure (77) encode transcripts increased by other growth cessation signals (94). The genes encoding for these transcripts are designated as gadd (**g**rowth **a**rrest and **D**NA **d**amage inducible) and may be coordinately regulated. Gadd genes may represent part of a novel regulatory pathway involved in the negative control of mammalian cell growth (94, 95). Although UV-mediated damage is associated with growth arrest and leads to induction of growth arrest (gadd) transcripts, UV also paradoxically stimulates increased expression and activity of ODC, an enzyme involved in proliferation (96–98). ODC is a rate-limiting enzyme in the synthesis of polyamines, essential for cellular division and growth (99).

Increased expression of various proteases, including collagenase and plasminogen activator (PA), have been demonstrated in a variety of cell types following UV exposure (84, 100, 101). PA is induced by UV in human fibroblasts deficient in repair of UV-induced DNA damage, but not in repair-proficient cells (100, 101). Inhibition of DNA repair potentiates UV-stimulated PA induction, suggesting the importance of prolonged DNA damage in inducibility of PA (101).

UV A (320–380 nm) radiation, H_2O_2, sodium arsenite, cadmium chloride and menadione induce a 32 kd stress protein in human skin fibroblasts identified as heme oxygenase (41, 42, 102). Heme oxygenase plays an essential role in heme catabolism by cleaving heme to biliv-

erdin which is subsequently converted to bilirubin by biliverdin reductase (42). Bilirubin is an effective scavenger of singlet oxygen and is able to react with O_2^- and peroxyl radicals. Moreover, when bound to albumin, bilirubin can prevent oxidative damage to albumin and prevent lipid peroxidation of albumin-bound fatty acids (42). Thus, induction of heme oxygenase may constitute an important cellular defense mechanism against oxidative damage.

A probable signal for induction of heme oxygenase appears to be modified or reduced levels of GSH (42, 103). Thus, induction of heme oxygenase may contribute to cell defense provided by endogenous GSH against the cytotoxic consequences of UV A and H_2O_2. Since induction of heme oxygenase and cell death in human skin fibroblasts by these agents can be prevented by addition of the iron chelators, o-phenanthroline or desferrioxamine, generation of $OH^\cdot$ by an iron-catalyzed Haber Weiss reaction is probably involved in both induction of the stress response and cell killing (104). Agents which induce heme oxygenase can be categorized as either oxidants and/or substances modifying cellular glutathione levels (105). High resolution two-dimensional gel electrophoresis has also been used to study protein synthesis occurring after exposure of human fibroblasts or keratinocytes to UV C (200–290 nm) or other DNA damaging agents (79). UV, some alkylating agents, and chemical carcinogens induce synthesis of human major histocompatibility class-1 (MHC-1) proteins. Thus, one possible consequence of induction of MHC-1 is targeting of damaged cells for immune recognition and somatic cell selection (79). The proinflammatory cytokine, interleukin-1 (IL-1), also is induced after exposure of keratinocytes to UV (106).

In summary, DNA damage appears to be a critical event in governing responses of cells to UV-induced damage. Certain genes affected by UV contain regulatory elements (UREs) in their promoter region which control transcription. Genes induced after UV exposure are diverse and appear related to both increases in proliferation (protooncogenes, ODC) and arrest of cell growth (gadd genes). Other genes (MT and heme oxygenase) may be associated with antioxidant defense mechanisms or stimulation of immune responses.

Ionizing Radiation. Exposure to ionizing radiation leads to formation of $OH^\cdot$ as a result of homolytic fission of oxygen-hydrogen bonds in water (10, 62). The carcinogenic effects of ionizing radiation may be mediated by base pair changes and frame shift mutations (10). Accordingly, expression of several genes is altered in Syrian hamster embryo cells following low doses of ionizing radiation (neutrons, X-rays or gamma rays). Levels of β-actin-specific mRNA decrease within 15 minutes after exposure, and a decrease in accumulation of ODC mRNA is observed within 1 hour (107). Down-regulation of these genes may be associated with the arrest in DNA synthesis observed in fibroblasts and other cell types following exposure to high doses of radiation (107, 108).

Inhibition of replication after ionizing radiation may be mediated by a trans-acting factor enabling cells to repair radiation damage (109). In this respect, ionizing radiation and AOS-generating compounds cause the appearance of a 43 kd DNA binding protein in human cells which may function as a negative regulator, reducing transcription of several genes (110).

The effect of ionizing radiation on transcription and c-*fos* gene expression has been studied in normal and radiation-sensitive mice bearing an autosomal recessive mutation and developing a disease similar to AT in humans (111). Immediately after irradiation, total transcription and c-*fos* expression is depressed in gut tissue of repair-proficient mice. However, transcription is doubled and depression of c-*fos* mRNA is delayed in radiation-sensitive mice suggesting abnormal regulation following exposure to ionizing radiation. A trans-acting factor defective or absent in cells from radiation-sensitive mice may be responsible for transcriptional inhibition in cells following radiation exposure (108, 110).

The proinflammatory cytokines, TNF and IL-1, are produced by a number of different cell types in response to ionizing and UV radiation and other stimuli (103, 106, 112–114). These cytokines activate inflammatory cells resulting in an oxidative burst. In addition, TNF causes conversion of intracellular xanthine dehydrogenase into xanthine oxidase thereby increasing the AOS-generating ability of the cell (16, 115). Cytotoxic effects of TNF are ameliorated under anaerobic conditions and by antioxidants, and cell lines resistent to the cytotoxic effects of TNF exhibit increased levels of antioxidants (115–117). A number of transcription factors, including NFκB and AP-1 (c-*fos*/c-*jun*), also are activated after exposure to TNF. TNF also up-regulates expression of the *gro*, c-*fos*, c-*jun* and c-*myc* oncogenes (115–117).

Recently, several investigators demonstrated that TNF, IL-1, and bacterial lipopolysaccharide (LPS) selectively induce the mitochondrial enzyme, MnSOD (118–122). MnSOD may be an important determinant of resistance to TNF and mitochondrially generated O_2^-, a key component of TNF-mediated cell killing (118, 119, 123). The importance of MnSOD in defense against oxidative stress has been shown in yeast mutant strains lacking MnSOD (124). In the absence of oxygen, mutants grow as rapidly as wild type cells, but increasing oxygen concentrations lead to growth inhibition. This study provides direct evidence that MnSOD contributes to cellular protection from oxygen toxicity (123).

Rats pretreated with TNF and IL-1 before exposure to hyperoxia show less pulmonary injury and mortality in comparison to hyperoxia-exposed rats. This adaptation to hyperoxic injury is associated with increased activity of antioxidant enzymes in lung (125, 126). In addition to MnSOD, TNF also up-regulates expression of other genes (ferritin, MT) which might have protective functions (115). On the other hand, TNF also can induce a program of self destruction (apoptosis) involving DNA fragmentation (115). Thus, some cytokines may cause either increases in antioxidant defense mechanisms or cell killing.

Phorbol Ester Tumor Promoters. Phorbol ester compounds are powerful activators of O_2^- and H_2O_2 production in phagocytes (7). These tumor promoters exert their biological effects by inducing an altered program of gene expression involving activation of PKC and the transcription factor, AP-1 (127–130).

Modulation of gene expression in response to the potent tumor promoter, 12-O-tetradecanoyl phorbol-13-acetate (TPA), has been examined in normal human fibroblasts after construction of cDNA libraries (93). cDNA clones were found which code for a precursor form of the secreted protein XHF1, a product of the collagenase gene (93, 131). The sequence of another clone corresponded in part with the sequence of the metallothionein IIa (MT II) gene. The use of cycloheximide to examine the involvement of protein synthesis in the induction of gene expression by TPA revealed that some genes (MT II) respond directly, whereas activation of others (XHT1) requires protein synthesis (81, 93).

In mouse epidermis, application of TPA induces expression of several mRNA species (132). Of 56 isolated cDNA clones, 32 were identical to MT I and II or endogenous retroviral-like sequences (VL30). Immunohistochemistry and time kinetic studies on mRNA levels in mouse epidermis showed that increases in MT and VL30 mRNAs coincided in time with a TPA-induced transient block in basal cell proliferation (132). However, increases in mRNA expression of *c-fos* and *ODC* were observed at early time points.

Aside from the role of MTs in heavy metal detoxification, MTs maintain cellular Zn^{2+} homeostasis and are thought to be indirectly involved in the regulation of a variety of Zn^{2+}-dependent processes, *e.g.* transcription, DNA replication, DNA repair and protein synthesis. Thus MT expression in mouse epidermis after TPA treatment may be linked mechanistically with a transient block in DNA synthesis as opposed to enhanced proliferation (132). In these experiments, MT mRNA levels and immunoreactive protein return to control levels after 24 hours, a timepoint when the epidermis undergoes proliferation. mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) increase at times correlating with TPA-induced hyperproliferation. Thus, increased GADPH expression is most likely associated with an enhanced rate of cell proliferation (132).

Increased expression of proteases also occur in cells after TPA exposure (133). Fibroblasts from patients with XP or AT show increased expression of procollagenase and prostromelysin, extracellular matrix-degrading metalloproteinases. Altered expression of these proteases could play an important role in the pathogenesis of tumors in individuals with these genetic diseases (133). In summary, the increased expression of MT and proteases after exposure to TPA mimic, in part, the patterns of cellular response observed after exposure of various cell types to UV and ionizing radiation.

Hyperoxia. Cellular remodeling occurring in the lung in response to hyperoxic injury also may be due to changes in gene expression resulting in the synthesis of new proteins. Subtractive hybridization of RNA from hyperoxic and control rabbit lungs yields three hyperoxia-induced clones (76). One clone was identified as a tissue inhibitor of metalloproteinases (TIMP), synthesized and secreted by a variety of cell types including fibroblasts, macrophages and erythroid colony-forming blood cells. The role of TIMP in the response to lung injury remains to be elucidated, but may be important in remodeling of the extracellular matrix. Other clones induced after hyperoxia were identified as MT II (134) and surfactant protein A (sp-A) (135).

Exposure of rodents to hyperoxia for prolonged periods of time can be fatal, dependent on the age of the animal and the exposure regimen. However, pretreatment of adult rats with TNF and IL-1 prior to hyperoxia (>99%) causes an adaptive response resulting in tolerance to subsequent high oxygen concentrations (125, 126). This adaptive response, attributed to increased lung antioxidant defenses, is also observed after pretreatment of rats with LPS (136), sublethal levels of hyperoxia (137), normobaric or hypobaric hypoxia (138), cadmium (139) or ozone (140). Transgenic mice overexpressing Cu-ZnSOD show decreased mortality after exposure to hyperoxia in comparison to control animals (141). Increased survival was more pronounced in the young (2.5 months of age) animals compared to older (5.5 months of age) mice (141). Moreover, administration of polyethylene glycol (PEG)-conjugated, or liposome-encapsulated antioxidant enzymes to rodents or to lung cells *in vitro* protects against hyperoxia-induced toxicity (142–145). These observations may be important in designing clinical strategies for prevention of pulmonary oxygen toxicity.

Studies examining regulation of antioxidant enzymes in cells of the lung after exposure to hyperoxia demonstrate variable changes in gene expression, immunoreactive proteins and activities of antioxidant enzymes. Regulation is complex and appears to be exerted at different levels (146). After 3 and 5 days of exposure to 85% hyperoxia, adult rats show increased MnSOD mRNA levels in lung which return to baseline levels by 14 days. No changes in CuZnSOD or catalase mRNA levels are observed (147).

Discrepancies in documented responses of cells *in vitro* to hyperoxia may occur because endogenous levels of certain antioxidant enzymes diminish after cell isolation (148). Exposure of rat pulmonary epithelial or fibroblast cell lines to hyperoxia for 24 hours has no effect on CuZnSOD or MnSOD mRNA levels (122). Alternatively, increases in mRNA levels, protein and activity of Cu-ZnSOD, as well as increases in GPX mRNA levels and enzyme activity are observed in human endothelial cells following hyperoxic exposure for 3 or 5 days (146). Although catalase mRNA increases, immunoreactive protein and enzyme activity are decreased. Variable increases in MnSOD mRNA, but no changes in MnSOD activity, are demonstrated revealing the complex nature of regulation of antioxidant enzymes following hyperoxic exposure.

Recent reports also show increases in gene expression and activities of antioxidant enzymes, including MnSOD and CuZnSOD in rat lungs after ozone exposure (21, 149), a reactive environmental pollutant which also generates AOS.

Mineral Dusts. Occupational exposure to mineral dusts such as asbestos or silica can lead to a variety of pulmonary disorders including malignant and nonmalignant disease (150). Experimental studies indicate that AOS may act at various stages in the pathogenesis of these diseases (13, 151, 152). For example, AOS can be generated in the lung after encounter of phagocytic cells

(macrophages and neutrophils) with these minerals and consequent phagocytosis of particles. These cells then undergo a respiratory burst that may be prolonged after exposure to larger fibers or particles which are incompletely phagocytized (13, 153). Moreover, some fibrogenic minerals generate AOS by acellular mechanisms. For example, asbestos fibers, many of which contain iron, generate AOS at the surface in a Fenton-like reaction (154–156). Similarly, silica, especially when freshly ground, can release AOS catalyzed by redox reactions on the particle surface (157, 158).

Evidence for a role for AOS in mineral dust-induced pulmonary toxicity and disease stems from numerous studies (reviewed in 152). Cytotoxicity and lipid peroxidation caused by asbestos or silica *in vitro* can be prevented or diminished by concomittent exposure to antioxidants (151, 159–161). Moreover, administration of PEG-conjugated catalase to rats during inhalation of asbestos ameliorates pulmonary toxicity, inflammation and the extent of pulmonary fibrosis (162).

Since AOS appear to be important mediators of asbestos or silica-induced toxicity, we investigated the gene expression, protein and enzyme activities of antioxidant enzymes in rat lungs after inhalation of the fibrogenic dusts, crocidolite asbestos or cristobalite silica. These animal models of pulmonary fibrosis show specific alterations in gene expression of antioxidant enzymes which may involve post-translational modification (163). For example, inhalation of crocidolite asbestos causes increases in steady-state mRNA levels of MnSOD, catalase and GPX, increases in immunoreactive proteins, and overall increases in enzyme activities. However, striking and more dramatic increases in MnSOD mRNA and immunoreactive protein are observed after inhalation of the silicon dioxide particle, cristobalite, although increases in enzyme activity of total SOD (both MnSOD and CuZnSOD) are not observed (163). Immunocytochemical localization of MnSOD reveals selective localization in the mitochondria of type II epithelial cells, whereas, other cell types exhibit little accumulation of MnSOD immunoreactive protein (164). Increased gene expression and protein levels of MnSOD in whole lung tissue is directly related to the extent of lung inflammation in these models (163, 164), an observation supporting the concept that MnSOD is a sensitive indicator of pulmonary inflammation and lung injury.

Since TNF is causally implicated in the development of silicosis (165), and this cytokine selectively induces mRNA expression of MnSOD in a number of cell types, we also examined gene expression of TNF in the lungs of these animals. Both crocidolite and cristobalite inhalation caused increases in steady state mRNA levels of TNF in rat lung indicating that increases in MnSOD mRNA levels might be mediated by TNF (166).

Examination of human pleural mesothelial cells, the progenitor cells of mesothelioma *in vitro*, shows that both crocidolite and chrysotile asbestos, as well as the AOS-generating system, xanthine plus xanthine oxidase, cause increases in mRNA levels of MnSOD and heme oxygenase whereas CuZnSOD is unaffected (167). Moreover, comparative experiments using human lung fibroblasts show similar but less striking induction of MnSOD and heme oxygenase after exposure to these oxidant stresses (167). Interestingly, human mesothelial cells are more sensitive than human lung fibroblasts to the cytotoxic effects of asbestos or xanthine/xanthine oxidase indicating that increases in antioxidant defenses in mesothelial cells are not sufficient to protect these cells from asbestos-induced damage.

The studies described above demonstrate that exposure to fibrogenic mineral dusts causes increases in specific antioxidant enzymes, most notably MnSOD. However, exposure to asbestos at nontoxic concentrations also results in increased steady state mRNA levels of ODC, a marker of cell proliferation in rat lung (168). Antioxidants can inhibit asbestos-induced ODC mRNA expression and activity, indicating the importance of AOS in asbestos-induced ODC activation and cell replication (55). Asbestos also appears to cause cell proliferation by persistent induction of c-*jun* and/or c-*fos* protooncogenes and AP-1 DNA binding activity in tracheal epithelial cells and pleural mesothelial cells (169). Whether persistent induction of the early response gene pathway by asbestos is mediated by AOS is currently under investigation.

Genetic Regulons Induced after Oxidative Stress

Genetic elements responsible for induction of proteins or enzymes after oxidative stress in mammalian cells have not been well characterized. However, a number of distinct regulons governing responses to oxidative stress have been identified in bacterial cells (170–174). For example, the *SoxR* and *OxyR* regulons are activated in bacteria after exposure to O_2^- and H_2O_2, respectively (170, 171). A number of signaling molecules indicative of oxidative stress also have been identified (175, 176).

The aromatic hydrocarbon [Ah]-responsive gene battery may represent a coordinated response system to oxidative stress and DNA damage which resembles the SOS response in bacteria (22). Several regulatory elements referred to as aromatic hydrocarbon responsive elements (AhRE), xenobiotic responsive elements (XRE), and antioxidant responsive elements (ARE), have been identified in genes of the Ah family (22, 177–179). More detailed analysis of the ARE has shown that they are responsive to H_2O_2 and phenolic antioxidants undergoing redox cycling. Thus, ARE could represent part of a signal transduction pathway allowing eukaryotic cells to respond to oxidative stress (180).

Proteins which bind to AhRE, XRE or ARE elements remain to be identified. Some evidence exists that gene activation occurs after post-translational modification of a protein constitutively bound to the ARE after interaction with electrophilic or redox active compounds. These interactions may then lead to altered conformation of the trans-acting protein and increased transcription of the gene (179).

Interestingly, a similar mechanism of gene activation following oxidative stress has been characterized in bacteria. AOS, by altering the redox state of constitutively bound OxyR protein, change its conformation and activate transcription of H_2O_2-inducible genes (170).

Studies using mice with deletion of a fragment of chromosome 7 indicate that this portion of the chromosome contains regulatory elements modulating basal and inducible expression of a number of genes located on other chromosomes (22). A gene in the deleted portion of this chromosome might encode a trans-acting positive regulatory factor for induction of MT and other genes, as well as a trans-acting negative regulatory factor (repressor) for induction of certain genes of the Ah battery (22). Interestingly, three of the class II transcripts previously identified after UV-induced DNA damage and gadd transcripts in CHO cells (77, 94) are also markedly elevated in these mice indicating some overlap of these regulatory pathways (22). This missing region of mouse chromosome 7 may contain a master gene "switch" that responds to oxidative stress (22).

Another post-translational modification which could activate gene expression is protein phosphorylation. For example, activation of PKC occurs after exposure to various oxidants (TPA, asbestos, UV, ionizing radiation) (181, 182). The ability of PKC to activate the transcription factor, AP-1, demonstrates a second messenger pathway leading directly to altered expression of distinct sets of genes (82, 183, 184).

As discussed previously, several classes of DNA damage inducible (DDI) and growth arrest and DNA damage inducible (gadd) genes have been identified. Furthermore, genes induced by UV or TPA contain elements (UREs and TREs, respectively) which control expression of these genes (127, 129, 131). These observations indicate the existence in mammalian cells of distinct genetic elements involved in responses to oxidative stress. Undoubtedly, a finite number of other regulons involved in responses to oxidant injury remain to be elucidated.

SUMMARY

A broad array of oxidative stresses modulates gene expression in a variety of mammalian cells. One goal of this review was to characterize cellular responses to oxidative injury, how these processes are regulated, and the outcome for a particular cell or tissue. Many genes induced in response to specific oxidant stresses have been identified and include transcription factors, replication proteins, proteases, protease inhibitors, proteins affecting cell proliferation and various antioxidants, *i.e.* heme oxygenase, MT, and MnSOD. The latter enzyme is induced after a number of cytokines and oxidant stresses including hyperoxia and mineral dusts causing inflammation (118–120, 147, 163). Moreover, increases in mRNA levels of TNF and IL-1, cytokines inducing MnSOD, are observed after exposure to UV and ionizing radiation (106, 113, 183). Since increased electron flow could lead to generation of more AOS within mitochondria, increased levels of MnSOD might be necessary to maintain normal functioning of the mitochondria after oxidative stress.

Alterations in cell growth are intrinsically related to the pathogenesis of many diseases. Paradoxically, some of the responses of cells to oxidative stress reflect cytotoxicity and cytostasis, whereas others result in increased cell proliferation. For example, induction of gadd genes observed after oxidative stress is related to growth arrest of cells (94), a response which might enable the cell to repair oxidative damage prior to replication. This phenomenon might prevent fixation of mutations associated with oxidative DNA damage. On the other hand, increased mRNA expression and activity of ODC, observed after exposure of cells to UV (96–98) or asbestos (55) is associated with increased cell proliferation. In addition, increased mRNA expression of cellular proto-oncogenes observed after exposure to oxidants (185, 186) could also be related to increased DNA synthesis or proliferation.

Figure 5 provides a general scheme of cell responses to oxidative stress and possible ramifications. AOS can react with a number of target molecules including proteins, lipids, and DNA. These interactions elicit a number of signals including activation of gene regulatory factors (transcription factors) which in turn activate

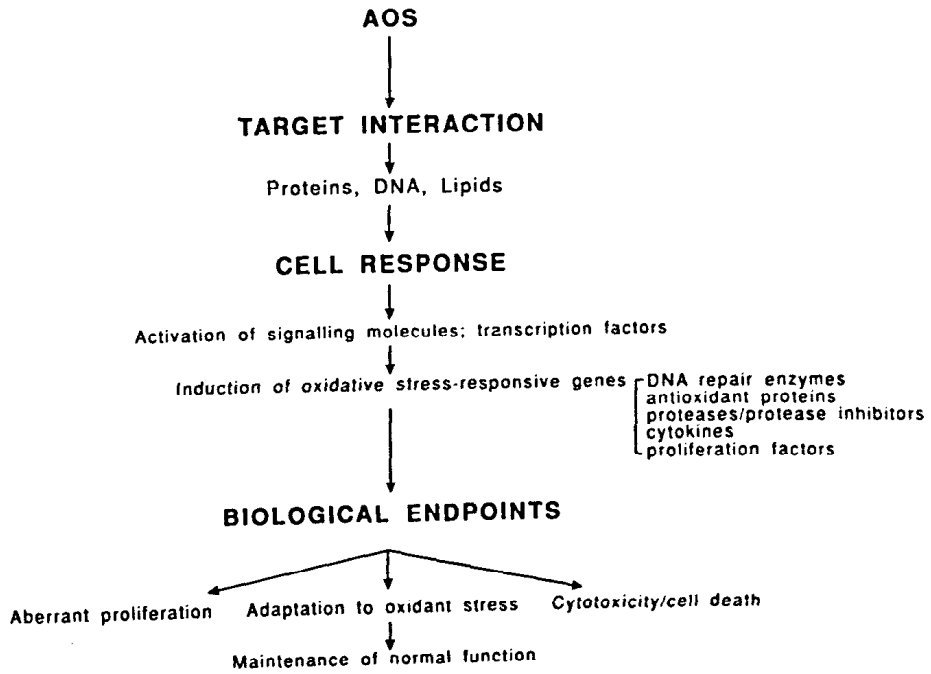

FIG. 5. Summary of cell responses to oxidative damage in relationship to homeostasis and the development of disease.

oxidative stress-responsive genes or regulons. Consequently, a number of proteins are produced with distinctive functions including DNA repair enzymes, antioxidants, proteases or protease inhibitors, cytokines and proteins affecting cell proliferation. These cellular responses to AOS can lead to restoration of normal cellular function and adaptation to oxidative stress, cell death or aberrant proliferation. It is the latter two responses which can lead to a variety of disease states including cancer. The overall outcome of the cellular response to oxidative stress is complex and might depend on the status of cellular differentiation and phase of the cell cycle and/or antioxidant content.

Expression of genes and synthesis of new proteins in response to oxidative stress appear to be, for the most part, cell- and tissue-specific (81). Subtractive hybridization studies have yielded multiple cDNA clones encoding mRNA transcripts which are either increased or induced after oxidative stress. Identification of these clones might improve our understanding of cellular responses to oxidative stresses and their implications in repair of oxidative damage and adaptation to injury.

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Address reprint requests to: Brooke T. Mossman, Professor, Department of Pathology, Medical Alumni Building, University of Vermont, Burlington, VT 05405.

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