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INVITED REVIEW

CELL DAMAGE IN INFLAMMATORY AND INFECTIOUS SITES MIGHT INVOLVE A COORDINATED "CROSS-TALK" AMONG OXIDANTS, MICROBIAL HAEMOLYSINS AND AMPIPHILES, CATIONIC PROTEINS, PHOSPHOLIPASES, FATTY ACIDS, PROTEINASES AND CYTOKINES (AN OVERVIEW)

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PROLOGUE

Voluminous literature exists on the mechanisms by which cells and tissues are destroyed in infectious and inflammatory sites. Microbial toxins,[1–5] their enzymes[6–9] and cell-wall components,[10–12] leukocyte and platelets-derived hydrolases and oxygen radicals,[13–18] cationic polypeptides,[19–24] arachidonic acid and metabolites, cytokines,[25,26] coagulation factors and fibrinolysin,[27] cytotoxic antibodies and complement components,[28] nitric oxide,[29] platelet activating factors,[30] killer lymphocytes,[31] as well as additional, still undefined, agonists have all been incriminated as putative agents capable of injuring cells. Special attention has, however, been devoted in the last decade to the role of reactive oxygen species (ROS)[13] as the main agonists responsible for causing tissue destruction in inflammatory conditions. Todate, over 100 human disorders have been connected with an excessive generation of ROS[13,14,15,32–47]

The pivotal role played by leukocyte-derived ROS, in cellular injury, was often supported by showing that their removal ameliorated and even totally prevented the initiation of cellular damage (see below). The exact nature of the ROS involved in the initiation of cellular damage is, however, still controversial. While superoxide has been advocated as the main toxic oxygen radical,[32] H_2O_2, OH· ROO·, NO and additional more exotic radicals were considered to be the main culprits.[14,19,33–47] Screening the

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voluminous literature on the role of ROS in cellular injury suggested, however, that these agents, alone, might not be able to reproduce the complex events which contribute to tissue destruction and that a "cross-talk" (see [47]) among ROS and additional proinflammatory agonists (multiple synergism) might possibly be the main cause of cellular damage in inflammatory and in infectious sites.

A support for this hypothesis stems from studies showing the ability of certain agonists (LPS, cytokines) to "prime" leukocytes to secrete excessive amounts of ROS upon stimulation by additional agonists[48,49] and from studies on the mechanisms of cellular damage induced by catalase-negative haemolytic streptococci[50] and by other toxigenic bacteria.[1,6,8,9]

There are striking similarities between streptococci and activated phagocytes. Both cell types secrete numerous extracellular hydrolases and membrane-damaging agents which allow them to depolymerize the extracellular matrix and the inflammatory exudates and to freely migrate in tissues. Both phagocytes and streptococci possess adhesion molecules, Fc receptors for immunoglobulins and receptors for complement. These facilitate adherence to surfaces of targets and the delivery of toxic agents without the interference of inhibitors present in body fluids.[39,51] Most importantly, however, both cell types also generate large amounts of H_2O_2 (see [14,45,50]).

In 1959 it was demonstrated[51] that H_2O_2-producing group A streptococci which also possessed a potent cell-bound hemolysin (streptolysin S), induced membrane-permeability changes in Ehrlich ascites tumor cells. Such injured cells were disintegrated by the addition of sub-toxic amounts of a streptococcus thiol-dependent proteinase (a synergistic phenomenon). Similarly, streptokinase-activated human plasmin also disintegrated tumor cells which had been injured by complement-dependent cytotoxic antibodies.[52] These studies suggested that proteinases could amplify cell damage initiated by membrane-damaging agents (a true synergism).

Therefore, the possibility that by analogy to streptococci, activated phagocytes might also injure target cells by employing a well-orchestrated "cross-talk" among their secreted agonists, is plausible.

The purpose of the present review is, to discuss those publications which have described the role played by combinations among ROS and additional putative proinflammatory agonists (microbial hemolysins and ampiphiles, cationic proteins, proteinases, phospholipases, fatty acids, cytokines, xenobiotics) in cellular injury and in the release of membrane-associated lipids.

In Vitro Models of Cellular Injury

Most in vitro models designed to study the mechanisms of cellular damage in infections and inflammation have been conducted with activated neutrophils (PMNs), eosinophiles and with macrophages. A variety of mammalian cells (targets) grown in monolayers were labeled either by ^{51}chromium (cytotoxicity assay) or with ^{3}H-arachidonic acid (to measure lipid release). Leukocytes were then layered upon the targets to allow adherence and then activated by various agonists (phorbole esters, ionophores, arachidonic acid metabolites, opsonized bacteria, chemotactic peptides, cationic polyelectrolytes, immune complexes). The release of radioactivity from the cells was measured in the supernatant fluids after different periods of incubation. The possible role played by specific leukocyte-derived agonists in cellular injury was evaluated by the inclusion of putative inhibitors (scavengers of ROS, chelators of transition metals, proteinase inhibitors, PLA_2 inhibitors etc). Also, the employment of leukocytes either from patients suffering of chronic granulomatous disease (CGD) or of

myeloperoxidase deficiency (see [14]) yielded important information on the possible role played by selective ROS in cellular injury. It might, therefore be helpful to employ in vitro models, which test the role played by combinations among well-defined agonists representing those released by activated leukocytes and in the presence of appropriate antagonists.

Definitions

Throughout the review, the terms "synergism or synergistic" will be applied to those conditions under which a mixture of two or more of the agonists, tested together, either enhanced cell death or the solubilization of membrane lipids the sum of the values of which was greater than that induced by each of the individual agonists alone. Such coincidental synergy should be differentiated from a true synergism where one toxic agent enhanced the toxic effect of another agent (see [39]).

The term "priming"[48,49] will be used to describe the ability of certain agonists to promote the enhancement of the generation of a certain agonist (e.g. ROS) by leukocytes activated by another agonist (see section K).

Role of Oxidants in Cellular Injury

Voluminous literature has been published on the mechanisms of oxidative cell damage as related to the pathogenesis of inflammatory conditions.[13,19,32,47] Most publications deal with the role played by ROS generated by activated PMNs, by macrophages and by oxidants generated by the xanthine-xanthine oxidase system.[15] A special attention has also been devoted to the biochemical changes induced in mammalian cells by HOCl[14,38,39,53,54] and by H_2O_2[55,68] and to the toxic role played by metal-catalyzed generation of OH· and ROO·.[15,35,36,67] Oxidants might act directly on membrane lipids to cause oxidation and peroxidation injury,[15] and to enhance membrane fluidity and permeability. Indirectly, H_2O_2 caused a steep drop in adenine nucleotides,[62,63,65] it inhibited glycolysis[53,57] it induced mitochondrial swelling[66] and membrane damage,[68] and also caused DNA strand breaks (Reviewed in[55,57]) H_2O_2, also altered signal transduction in mammalian cells.[68] It activated endothelial cells, selectively by up-regulating intracellular adhesion molecules and major histocompatibility complex class-1,[69] it modulated the respiratory burst in human PMNs,[70] it activated complement in normal human serum[71,72] and phospholipase D in endothelial cells.[73] The anti-metabolic effects of H_2O_2 are probably instrumental in lowering the capacity of cells to repair damages. This might expose them to the toxic effects of other leukocyte-derived agonists.

Collaboration Between Microbial Hemolysins and Oxidants

Group A hemolytic streptococci elaborate two potent hemolysins, streptolysin S(SLS)[1,50,74] and a thiol-dependent and porin-forming streptolysin O (SLO).[1,50] In addition to their hemolytic activities, both agents also possessed distinct cytopathic properties and also caused disruption of lysosomes (reviewed in[50]). The mechanisms by which SLS and SLO hemolyzed red blood cells and also killed nucleated cells might be due to an alteration of membrane phospholipids,[1,50] to the elevation of intracellular calcium[87] and possibly to the activation of PLA_2.[81,87] The ability of streptococci to produce large amounts of H_2O_2, suggested, therefore, that synergism between hemolysins and peroxide might explain, in part, their distinct virulence.

Cell killing

Ehrlich ascites tumor cells[51] and rat heart cells in culture[75] which had been exposed either to group A streptococci possessing cell-bound SLS or to cell-free SLS(SLS), developed distinct cytopathic changes. These were characterized by swelling and by the development of large pseudopod-like structures (blebs). On the other hand neither bovine aortic endothelial cells(EC)[76,77] nor monkey kidney epithelial cells (BGM)[78,79] were damaged even by large amounts of SLS (50-100 hemolytic units-H.U/ml). However, a distinct synergistic cell killing occurred if either glucose-oxidase,[76,79] xanthine-xanthine oxidase[77] or paraquat,[78] (agents capable of generating H_2O_2), were employed together with SLS. (distinct synergistic reactions). Cytotoxicity was strongly inhibited either by catalase, by trypan blue (an SLS inhibitor) or by phosphatidyl choline.[50,80] Since EC which had been pretreated with SLS and then washed could still be killed by the addition of H_2O_2,[77] it was assumed that SLS was irreversibly bound to membrane phospholipids,[80] where it caused subtoxic damage which could be amplified by an oxidant (a synergistic reaction). Unlike SLS, streptolysin O (a thiol-dependent pore-forming toxin) was directly cytotoxic even at a very low concentration (1–2 H.U/ml).[81] However, oxidants further increased cell killing which was blocked by cholesterol. Killing of EC and of BGM cells by mixtures either of SLO or of SLS with H_2O_2 was further increased (also in a synergistic manner) by the addition of trypsin,[79,81] (a triple synergy, see below), suggesting that oxidants and membrane perforating agents might have affected both proteins and lipids and prepared the grounds for a protease attack.[82–84] The reasons for the relative resistance of both EC and BGM cells to SLS as compared with SLO, is not known.

BGM cells which had been exposed to non-toxic amounts of AAPH (azo bis amidinopropane dihydrochloride) a generator of peroxyl radical ($ROO\cdot$)[67] were also killed in a synergistic manner by the addition of SLS.[81] Cytotoxicity was further enhanced by the addition of trypsin. BGM cells treated simultaneously with GO, AAPH and trypsin were also killed in a synergistic manner.[81] Cell killing by mixtures of SLS, H_2O_2, and trypsin was inhibited by Mn^{2+},[85] by carrot juice and by a novel PLA_2 inhibitor[86] suggesting that activation of PLA_2 might be involved in cell killing.[87]

E.Coli hemolysin, also a pore-forming cytotoxin, stimulated human PMNs to generate enhanced amounts of superoxide and also induced the release of elastase and PAF.[88] The amounts of toxin employed did not hemolyze red blood cells. This hemolysin also proved to be a potent inducer of phosphoinositide hydrolysis. Inositol phosphate and diacylglycerol formation was found to parallel degranulation, the respiratory burst and lipid mediator generation in human PMNs treated by the hemolysin. Its activity was found to be greater than that induced either by f-MLP, PAF, LTB_4 or by the ionophore A23187. The ability of this hemolysin to generate the release of proinflammatory agonists suggested that these agents might also act in concert to enhance cell damage.

Taken together it might be postulated that other toxin-producing microorganisms (staphylococci, clostridiae) might also destroy host cells by synergism among their secreted toxins.[8,9] These findings might perhaps also shed light on the mechanisms by which activated phagocytes induce cellular damage in infectious and in inflammatory sites (see below).

Release of arachidonic acid and metabolites

Mammalian cells treated, even by large amounts of hydrogen peroxide, did not lose

significant amounts of membrane-associated lipids.[89 92] The possibility that synergism among oxidants and SLS, which killed cells[76 79 81] might also result in the solubilization of membrane associated lipids was investigated. Mixtures of SLS and GO-generated H_2O_2 killed both EC and BGM cells but failed to solubilize significant amounts of arachidonic acid unless a proteinases was also present[79] (a triple synergy). Such mixtures also solubilized large amounts of PGE_2 and 6-keto PGF. The release of arachidonate was strongly inhibited by soybean trypsin inhibitor (SBTI). Although the mechanisms by which trypsin facilitated the release of membrane lipids are not known, it might be speculated that oxidized membrane proteins and lipids might be more readily detached and cleaved by proteinases.[82 84] These results might also suggest that both microbial and leukocyte-derived proteinases might also facilitate the release of large amounts of proinflammatory, arachidonic acid metabolites, even from dead cells which are always present in large numbers in inflammatory sites. This however depends on the additional presence of membrane-damaging agents (hemolysins, phospholipases, free fatty acids, lysophosphatides, cationic proteins).

Collaboration between microbial ampiphiles and oxidants in cell killing

Gram positive and Gram negative bacteria elaborate important surface-associated ampiphiles, lipoteichoic acid (LTA)[12,93] and lipopolysaccharide (LPS).[3 5,94,95] Both ampiphiles can bind spontaneously, via their lipid moieties, to membrane phsopholipids[5,96,97] and to sensitized them to agglutination by antibodies and to a further lysis in the presence of antibodies and complement[96,98 100] (passive immune kill). Both LTA and LPS served as adhesion agents which facilitated the binding of bacteria to cell surfaces and the delivery of toxic agents. LPS also bound via its lipid moiety to LPS-binding proteins (LBP) present in serum.[101] This also enhanced LPS-bearing particles, to associate with the surfaces of monocyte-derived macrophages. Both LTA[102] and LPS[3 5,94,95] activated complement and acted as potent stimulators of the generation of tumor necrosis factor.[4,5,102]

1 Studies with LTA: LTA, which had been released from Gram positive bacteria by phenol, was not cytotoxic to a variety of cells in culture.[50,96 98] However, endothelial cells (EC) which had been sensitized by LTA and then treated with anti-LTA, IgG, in the absence of complement, could be killed by the addition of subtoxic amounts of GO-generated H_2O_2.[79] Paradoxically, however, cell killing also occurred when mixtures of IgG and peroxide were added to EC. Since the toxic activity of the IgG could be removed by adsorption either upon streptococci or upon EC, it was postulated that the IgG preparation might have contained antibodies cross-reactive with EC (Reviewed in[50]).

Human PMNs which had been "primed" by streptococcal LTA, generated synergistic amounts of superoxide, H_2O_2, and luminol-dependent chemiluminescence and also released large amounts of lysosomal enzymes when treated by anti-LTA globulin.[103] However, unlike LPS, LTA failed to prime PMNs for enhanced generation of superoxide when challenged either with the chemotactic peptide, fMLP, or with phytohemagglutinin[103] (see below). Generation of superoxide by LTA-coated PMNs in the presence of anti-LTA IgG was very markedly enhanced by the addition of by-stander, untreated, PMNs. This suggested that migrating PMNs might amplify the respiratory burst by PMNs already coated by LTA.[103] An amplification of superoxide generation by PMN-coated LTA was also observed when small amounts of nuclear histone (a cationic protein) were added together with the anti-LTA, IgG, suggesting

that cationic agents might have "opsonized" the immunoglobulin and facilitated its interaction with the LTA-coated PMN surfaces. Enhanced amounts of superoxide were also generated by PMNs which had been mixed either with LTA-coated fibroblasts or with LTA-coated epithelial cells. This suggested that LTA could be presented to PMNs by non-neutrophilic cells provided that anti-LTA globulin was also present.[103]

LTA derived from a variety of streptococcal and staphylococcal species stimulated peripheral blood monocytes to generate superoxide in the absence of added antibodies.[104-106] This suggested that this ampiphile might act as a cross-linker of receptors for LTA present upon the cells. No experiments, however, were performed to test the possible effect of anti-LTA antibodies on superoxide generation by LTA-treated monocytes. The stimulation of monocytes to generate superoxide depended on extracellular calcium and was accompanied by the release of arachidonic acid.[107]

Since LTA induced the release of ROS, lysosomal enzymes and a variety of additional agonists, including nitrate[103,107] and TNF (102) and since proteinases markedly enhanced the binding of LTA to mammalian cells,[100] it might be speculated that LTA could play an important role in cellular damage caused by Gram positive microorganisms in vivo (see [50]).

2 Studies with LPS: The mechanisms by which LPS primed PMNs for enhanced respiratory burst was studied in detail.[108-113] The effect of LPS was due neither to an increase in its avidity for NADPH oxidase nor to its affinity for NADPH. The LPS effect was associated with early events of signal transduction, and intracellular ca^{2+} was a crucial intracellular messenger in LPS priming.[112] Since LPS priming was accompanied by a rise in PLA_2 activity which could be inhibited by mepacrine, it was postulated that PLA_2 activation was a fundamental component of priming with LPS. The enhanced generation of superoxide and its dismutation to H_2O_2 following LPS priming might contribute to cellular injury. This might be further enhanced, in a synergistic manner, if additional proinflammatory agonists were also present.

Human platelets incubated with small amounts of LPS released a protein which primed human PMNs for enhanced generation of superoxide following stimulation with f-MLP.[110] A short-term exposure of guinea-pig macrophages either to muramyl dipeptide or to LPS caused enhanced release of superoxide in response to immune complexes, but not to PMA.[108] On the other hand, a longer exposure (24 hrs) also resulted in an enhanced superoxide generation by PMA suggesting that the mechanisms of superoxide generation by macrophages stimulated by immune complexes differed from those induced by PMA. Human blood PMNs treated with LPS also showed enhanced expression of C3bi receptor, phagocytosis of opsonized bacteria, and peroxide generation, which were blocked by the inhibition of intracellular calcium-dependent processes.[113]

Taken together, it might be postulated that ampiphiles (LTA, LPS) release either following exposure of bacteria to leukocyte-derived proteinases[114,115] or following bacteriolysis induced either by cationic proteins, phospholipase[115] or by penicillin,[116,117] might occur in vivo. These might contribute to cellular injury via activation of the respiratory burst, the release of lysosomal hydrolases and the activation of the complement cascade. It would also be important to establish whether LTA and LPS which had been released from bacteria, either under physiological or pathological conditions, possessed the same properties as ampiphiles obtained by harsh chemical treatment (e.g. phenol, butanol). Such preparations are traditionally used in experimental models.[3,5,94,95,108-113]

COLLABORATION BETWEEN OXIDANTS AND POLYCATIONS

1 Role of Polycations

Cationic polyelectrolytes are abundantly present in mammalian cells (reviewed and discussed in [20,24,118,131]). Although most of the polycations possess distinct anti-microbial activities[22,24,118,121,124,125] they were also highly cytotoxic to a variety of both normal and malignant cells.[127,131] It is important to note that cationic agents of mammalian cells are always sequestered within membrane-bound structures (azurophilic granules of PMNs, granules of eosinophils, cell nuclei). This prevents them from interacting, by electrostatic forces, with negatively-charged domains present upon all mammalian cells which could cause cell agglutination and damage to their membranes.[20,118] In addition to their microbicidal and cytocidal properties, polycations also function as potent opsonins,[20,132,134] as stimulators of the respiratory burst in leukocytes,[20,133,137] as enhancers of the binding of PMNs to endothelial cells[138] as potentiating agents in immune-complex cytotoxicity,[139,140] as modulators of complement activation[141], as enhancers of tissue permeability,[142] as releasers of histamine from basophils,[143] as stimulators of cytokine generation by a T-cell hybridoma,[144] and as potent stimulators of autolytic wall enzymes in bacteria.[145] In general, polycations were able to agglutinate cells[94] to induce cross-linking of surface components and to alter membrane permeability by forming ion-channels in lipid bilayers.[125,146,148] The activities of polycations could be inhibited by a variety of polanions.[132,149] The most extensive research on the role of polycations as cytolytic agents against mammalian cells had been performed with defensins,[24,121,125,146,148] with eosinophil cationic proteins,[120,122,123,141] and to a lesser extent, with synthetic polycations.[20,118,128,129]

Cytotoxicity induced either by histones[77,79] or by defensins[146,148] depended on their initial binding, by electrostatic forces, to negatively-charged domains present on the cell surfaces (see [118]). This could be inhibited either by heparine,[149] by polyglutamate or by polyaspartate. The toxicity of defensins depended on several metabolic events which could be inhibited either by azide, 2-deoxy glucos, cytochalasin B or by lysosomal and calmodulin-mediated inhibitors.[146] This suggested that internalization of the polycation was essential for its cytotoxicity.[146] This, however was unaffected by inhibitors of protein synthesis.[146] On the other hand, inhibitors of endocytosis failed to inhibit the cytotoxicity to endothelial cells induced by synthetic polycations.[128] Since the cytotoxicity of defensins to targets was strongly inhibited by serum (presumably by albumin),[146] it was postulated that activated PMNs which secreted defensins might injure cells only by an intimate contact, forming intercellular clefts which could exclude inhibitory serum components. (see[37]).

Paradoxically and unexpectedly, internalization of cationized *Candida albicans* by fibrosarcoma cells which did not elicit a cytotoxic effect, very markedly enhanced tumor growth and spread when injected into mice.[133] This enhancement might have been linked with the massive infiltration of neutrophils around the tumors.

The role played by the highly-cationic cathepsin G, elastase, CAP57, Cap 37, lysozyme (cationic antimicrobial proteins) and the slightly cationic, lactoferrin, as microbicidal agents had been recently reviewed[124,126] and will not be discussed in the present review.

2 Collaboration Between Polycations and Oxidants

Since activated PMNs and EO generated ROS and also released granule components,

(defensins, leukocyte cationic proteins, MPO, EPO) the possibility that polycations, might collaborate with ROS to enhance cellular damage, is plausible.

Bovine pulmonary endothelial cells (EC), monkey kidney epithelial cells (BGM), pneumocytes, and tumor cells, which had been labeled with ^{51}Cr and then exposed either to a mixture of subtoxic amounts of H_2O_2, and defensins[150] or to a mixture of peroxide and a variety of cationic agents (histone, polyarginine, lysozyme, ribonuclease, spermine, chlorhexidine, polymyxyn B, CETAB), were killed in a synergistic manner.[77,78] Cell killing could be totally inhibited either by catalase or by polyanions.[77,78] Similarly, schistosomula of *Schistosoma mansoni* which had been exposed either to oxidant-producing eosinophil cytoplasts or to PMNs were killed in a synergistic manner by the addition of eosinophilic cationic proteins.[151] Both the polycations and the oxidant had to be present, simultaneously, to induce synergistic cell killing.[77,78,150] On the other hand BGM cells which had been exposed to large amounts of histone, to allow binding to membranes and then washed, could still be killed by the addition of H_2O_2.[78] However, cells first exposed to glucose oxidase-generated H_2O_2 and then washed, were not killed following the addition of histone.[77] However, treatment of EC by GO which had been cationized by complexing with poly-L-histidine, and then washed could be killed by the addition of histone[78] suggesting that the binding of cationized GO to the cell membrane facilitated the direct delivery of peroxide, upon the cell membrane. Cationized GO was also found to be several fold more toxic than unmodified GO, when instilled into the rat trachea.[85] While serum albumin had a strong inhibitory effect on the killing of targets by mixtures of defensin and peroxide[146] it had only a partial inhibitory effect on the killing of EC induced either by mixtures of histone and peroxide or by mixtures of CETAB and peroxide.[79] Cytotoxicity induced by mixtures of histone and peroxide was totally inhibited by polyanethole sulfonate.[77] The toxicity of mixtures of CETAB and peroxide to EC was further enhanced by subtoxic amounts of crystalline trypsin[79], suggesting that membranes which had been modulated by polycations and by oxidants became more susceptible to proteolysis.[82-84] It was more difficult to demonstrate[57] synergism among H_2O_2, proteinases and histone as the latter was rapidly degraded by the proteinase. This obstacle was overcome by replacing histone by poly-D-lysine which is known to be resistant to trypsin-like enzymes. Under such conditions[81] a distinct synergistic cell killing was also obtained, which was inhibited by soybean trypsin inhibitor (SBTI).

A variety of synthetic polycations (polyarginine, polyhistidine) enhanced the toxicity of immune complexes in a rat model of acute immune complex-mediated tissue damage[139] (reversed Arthus phenomenon). Similarly to cationized bacteria,[134-136] the cationized immune complexes also very significantly enhanced the generation of superoxide by PMN, in vitro. Since the infusion of PEG-SOD prior to the induction of the Arthus reaction markedly depressed skin permeability, it was postulated that ROS generated in vivo were involved in the enhanced permeability observed. Also, the cationized immune complexes bound more avidly to the tissues and persisted there for longer periods (see [136]).

The mechanisms by which polycations synergized with oxidants to enhance cellular damage is not fully known. It might, however be postulated that alterations either of the plasma membrane,[148] the induction of cross-linking of surface structures, or the formation of voltage-dependent ion channels (functional "holes"[147]) might have allowed the free diffusion of ROS into the cell interior to affect intracellular structures and functions (see [55-57]).

3 *Collaboration between myeloperoxidase and oxidants*

Extensive research had been conducted on the toxicity of the highly-cationic neutrophil myeloperoxidase (MPO)[14,38,39,152-156] and the eosinophil peroxidase (EPO)[157-162] both in vitro and in vivo.

The unique finding that MPO, EPO and lactoperoxidase,[14] when combined with H_2O_2 and with a halide (mostly with Cl and Br) could generate hypohalus acids,[152-153] was a major contribution to the understanding of how activated PMNs and eosinophils killed bacteria, parasites but also injured mammalian cells. Both MPO and EPO are highly cationic proteins (PI>9.0). These can avidly bind, by electrostatic forces, to negatively-charged domains present on surfaces of both microorganisms and mammalian cells. While their activities could be inhibited by heme-inhibitors (azide, aminotriazole), the toxicity of HOCl could be inhibited either by taurine or by methionine.[39]

a) in vitro models: The question of whether HOCl generated by activated PMNs could destroy target cells, via the MPO-H_2O_2-halide system, was investigated.[39,156,157] While MPO-deficient PMNs were cytotoxic for target cells, PMNs from CGD patients failed to kill cells. However, the cell-free MPO-glucose oxidase system did destroy EC. This was inhibited by scavengers of HOCl suggesting that a prior binding of MPO released from granules might have secured the direct delivery of HOCl upon the targets.[154,155] It also pointed to the importance of H_2O_2 as a major cytotoxic agent generated by viable PMNs.[53,63]

Either activated leukocytes, cell-free MPO or EPO were cytotoxic to pneumocytes,[158] to tracheal epithelium,[159] and to human nasal epithelium.[160] Cytotoxicity occurred only if the cationic agents had been allowed to bind to the mammalian cells prior to the addition of peroxidase and a halide. Under these conditions toxic oxygen metabolites could be delivered directly upon the targets without the interference of inhibitors.[39] Unlike MPO or EPO, other cationic agents (histone, defensins, poly cations, cationic detergents) (see above) could kill mammalian targets when simply mixed with peroxide.[77,79] The damage induced in cells injured by MPO, EPO-H_2O_2 system was characterized by swelling, by the formation of blebs and by exfoliation. Another important observation was that whereas chloride was the most effective halide for MPO killing,[39] bromine was more effective than chloride for killing by EPO.[162]

The ability of both native and denatured MPO to function as a cationic agent and as an activator of the autolytic wall enzymes in staphylococci, was demonstrated.[163] It suggested that high concentrations either of MPO or of EPO might, under certain conditions (e.g. low pH) also function as polycations rather than as an enzymes. It was demonstrated that egg white lysozyme (also a cationic protein) which lost its catalytic property (lysis of *M. lysodeikticus*) still continued to function as a polycation and to activate the autolysis of staphylococci (a charge-dependent reaction).[145]

Certain cationic aminoglycoside antibiotics (tobramycin, gentamycin) strongly inhibited the killing of epithelial cells by the MPO, H_2O_2, halide system.[164] Protection against MPO and H_2O_2 was also observed with thioether-containing antibiotics (ticarcillin and teftazimide). The aminoglycosides, which converted HOCl to hydrophilic non-toxic chloramines were unable to prevent the oxidation of sulfhydryls and methionine induced by HOCl. The aminoglycosides protected lung epithelial cells against the cationic MPO by binding to negatively-charged surfaces (a competitive phenomenon).

Treatment of articular cartilage either by reagent H_2O_2 or by peroxide generated by GO, inhibited proteoglycan synthesis but had no effect on its degradation.[165] On the

other hand, HOCl, a product of MPO+H_2O_2+Cl system degraded proteoglycan[165,166] and also inhibited its synthesis.[165] Cleavage of the proteoglycan core protein was near the hyaluronic acid binding region but there was no evidence that also cleaved glycosaminoglycan chains.[165] Since this effect was partially inhibited by an elastase inhibitor, it was suggested that HClO might act together with proteinases to cause a net increase in degradation (a synergistic reaction).

In vivo models: The role played by the EPO-H_2O_2-halide system in glomerular injury in the rat,[154,155] in the toxicity to the isolated working rat heart[162] and in bronchoconstriction and vasoconstriction[167] was studied. Tissue alterations depended on the ability of EPO to bind to and to catalyze halogenation of the targets. Since heme inhibitors (azide, aminotriazole) abrogated the pathological changes induced either by MPO or by EPO, it appeared that these highly cationic agents acted not simply as polycations but as catalytic agents which also had the capacity to bind to membranes by electrostatic forces.

4) *Mechanisms of Action of HOCl on Mammalian Cells*

Since HOCl, the compound generated by MPO, was shown to be a potent toxic agent, the elucidation of its mechanism of action on cells and on the matrix of connective tissue might contribute to the understanding of the pathophysiology of inflammation.[38,39,60,153,157,165,166,168]

Murine macrophage-like tumor cells in culture which had been treated by 10–20μM of HOCl showed, 1) oxidation of plasma membrane sulfhydryls, 2) inactivation of glucose and aminoisobutyric acid uptake, 3) loss of cellular K+ and 4) and increased cell volume.[168] At higher concentrations (>50 μM), a general oxidation of SH-methionine and of tryptophane residues and the formation of carbonyls was detected. A loss in ATP was also seen. By comparison, NAD degradation and ATP depletion caused by H_2O_2 preceded cell death by several hours. Formation of DNA breaks, a major feature of H_2O_2-induced injury,[53,55] was not observed with HOCl. Thus, targets of HOCl were distinct from those of H_2O_2 with the exception of glyceraldehyde-3-phosphate dehydrogenase which was inactivated by both oxidants.[53,55]

The question of whether HOCl generation in physiological environment is detrimental to mammalian cells is controversial. It was suggested[39] that despite the absence of tissue toxicity associated with the use of HOCl in the wounded soldiers, this oxidant possessed distinct cytotoxic activity in vitro. At 0.5%, a concentration of HOCl safely used in vivo, a complete in vitro solubilization of skin and liver tissue occurred. However, if this amount of HOCl was added to slices of tissues that had been suspended in wound secretions, the solubilizing action of HOCl was totally lost. In a physiological environment, even millimolar quantities of HOCl preferentially reacted with endogenous amine-containing moieties to yield the derivative chloramine. These newly-generated chloramines exerted strong microbicidal activities but had little, if, any ability to damage normal tissues. The limitation that applied to HOCl-mediated damage in vivo could be circumvented if the highly cationic MPO or EPO could first be bound to their targets where they delivered toxic oxidants, directly upon targets.[39,153,155] Weiss[39] concluded that "given the information, my own enthusiasm for implicating HOCl *alone*, as the primary key to the neutrophil's tissue destructive potential has been blunted by the surfeit of 70-year-old clinical data". If HOCl can be considered a major toxic oxidant only under strict physiological conditions (a prior binding of MPO), then this oxidant might still function in synergy with other proinflammatory agonists

generated by activated leukocytes. One possibility is that HOCl might inactivate α-1-trypsin inhibitors present in plasma and in the interstitial tissues to facilitate the action of leukocyte-derived elastase, collagenase, gelatinase and cathepsin G on cells and tissues. This might be a true synergism where one agent potentiated the action of another agent. (see [39]).

COLLABORATION BETWEEN PROTEINASES AND OXIDANTS

PMNs, eosinophils and macrophages secrete a variety of both acid (cathepsin G) and neutral proteinases (elastase, collagenase, gelatinase) which might depolymerize a wide variety of substrates, including extracellular matrix proteins. Proteinases also activated xanthine dehydrogenase to xanthine oxidase,[169] modulated neutrophil-induced superoxide responses,[170] caused cell detachment from matrices[171 176] and enhanced tumor metasthesis.[177] Proteinases might also facilitate hydrolysis of oxidized proteins.[82 84] The possibility that the simultaneous attack by oxidants, proteinases and by membrane-damaging agents (including xenobiotics) might enhance cellular damage and the release of membrane-associated lipids, is plausible.

a) Role of trypsin: Subtoxic amounts of GO-generated H_2O_2 acted synergistically either with crystalline trypsin, chymotrypsin, cathepsin G or with pancreatic elastase to kill EC in culture.[178,179] Cell killing was markedly inhibited by ROS scavengers (catalase, dimethylthiourea, Mn^{2+}).[79] Crystalline trypsin also enhanced, in a synergistic manner, the killing of EC and of BGM cells when combined with oxidants and with a variety of membrane-damaging agents (PLC, PLA_2 lysolecithin),[79] including ethanol.

b) Role of elastase: Neutrophil-derived elastase might play an important role in the destruction of the matrix of the connective tissue[39,165,180 187]. This highly cationic enzyme can interact, by electrostatic forces, with negatively-charged domains present in connective tissue matrix. Its activity could be greatly facilitated if both serum α-1-proteinase inhibitor and the secretory leukoproteinase inhibitor,[39] were destroyed. Since HOCl generated by the PMN myeloperoxidase-H_2O_2-halide system could destroy these inhibitors it might be postulated that HOCl generated during PMN activation might be delivered directly upon targets.[39] Elastase could also interact with chondrocytes via a specific receptor[182] and endocytosis of elastase (presumably due to its cationic nature) was secondary to cell binding. Studies on the degradation of heparan sulfate proteoglycan of subendothelial matrix, by neutrophil elastase and the MPO-H_2O_2-chloride system[186] showed that neither elastase alone nor the MPO system alone could induce degradation of proteoglycan. However, when a 4-hour exposure to elastase was followed by a 15 minute exposure to the MPO system, the degradative effect was greater than the additive (a synergistic effect). On the other hand, greater than an additive effect was not observed either when elastase followed the addition of the MPO system or when the two agents were added together. The binding of the cationic elastase to sulfated glycosaminoglycans also resulted in a partial inactivation of the enzyme. It was concluded that PMNs which adhered to glomerular endothelial cells and basement membrane released both ROS and granule enzymes, at the point of attachment. This might have caused damage by a collaboration between oxidants and proteinases.

The question whether leukocyte-derived proteinases also acted in concert with oxidants generated by the NADPH oxidase of phagocytes to destroy matrix, is controversial. The chemotactic peptide fMLP induced the release of MPO from PMNs

which generated HOCl.[183] The addition of elastase resulted in the release of more matrix components than induced by the individual agents. On the other hand either intact PMNs or cytoplasts which lacked granules but still generated ROS, failed to significantly enhance either elastin or collagen degradation by purified neutrophil elastase. These findings suggested that matrix proteolysis correlated more closely with the release of azurophilic granules than with the generation of ROS by NADPH oxidase.

ROS generated by the xanthine-xanthine oxidase system mediated elastase-induced injury to isolated lung and endothelium.[184] Lungs isolated from rats that had been fed a tungsten-rich diet had negligible levels of xanthine oxidase (XO) activity and after exposure to hyperoxia, developed less acute edematous injury during perfusion with purified elastase. Also, tungsten-treated bovine arterial endothelial cells in culture produced less superoxide and after exposure to neutrophil elastase leaked less albumin than XO-repleted monolayers. It was postulated that tungsten acted by lowering the amount of XO thereby decreasing the amounts of ROS which might have inactivated proteinase inhibitors. This could facilitate the action of elastase (see [39]).

The mechanisms of PMN damage to human alveolar extracellular matrix (ECM) was studied.[187] Since scavengers or ROS (catalase, SOD) failed to inhibit the solubilization of the ECM, but inhibitors of neutrophil serine protease, elastase and cathepsin G markedly inhibited ECM degradation, it was suggested that ROS might not be involved in matrix degradation.

Lysis of ox RBC by PMA-activated human PMN was reported.[188] RBC lysis correlated with the quantity of a membrane-bound neutral proteinase. Since the solubilized proteinase acted synergistically with added H_2O_2 to lyse the RBC, it was assumed that activated PMNs lysed cells by synergy between oxidants and a cell bound neutral proteinase. This was probably due to a close contact between the RBC and the activated PMN. This is essentially similar to the conditions which allowed the cell bound hemolysins of streptococci to lyse RBC and to kill targets (see above). Also, both MPO and EPO were toxic to targets only if first allowed to bind to the targets.[154,155]

Sarcoma cells (MCA-1) were killed, in a synergistic manner, when first exposed to non-lytic amounts of H_2O_2 and then treated with a macrophage-derived cytotoxic factor possessing neutral serine proteinase activity.[189] On the other hand, exposure of the cells to the cytotoxic factor prior to the addition of peroxide did not cause cell death. It was suggested that the sequence of the addition of the two factors was important to secure cell death. The necessity to follow a strict order of addition of the reagents might be due to the possibility that a prior exposure to the peroxide diminished the capacity of the targets to cope with a protease attack.

Oxygen metabolites and neutrophil elastase were found to synergistically cause edematous injury in isolated rat lungs.[185] Lung injury could also be shown when PMA-activated PMN cytoplasts (which generated ROS but lacked granules) were infused into the lungs in combination with added PMN elastase.[191] It was suggested that elastase was a necessary component for the maximal development of acute edematous injury in isolated lungs perfused with activated PMNs. A similar mechanism might also occur in acute adult respiratory distress syndrome (ARDS). More recently, it was demonstrated that neutrophil elastase acted synergistically with H_2O_2 to inactivate thrombomodulin of endothelial cells. This could contribute to microthrombi formation at the site of inflammation.[191A] It further demonstrates the important role which might be played by a "cross-talk" among leukocyte-derived agonists in cellular damage in infectious and in inflammatory sites.

The possible role played by proteinases as priming agents for the enhanced generation of superoxide was investigated.[170] Cathepsin G and elastase decreased the order

and increased the lateral mobility of the neutrophil membrane lipids and primed a superoxide response to f-MLP and to PMA. The enhanced amounts of superoxide generated and its dismutation to H_2O_2 might contribrute to enhanced cellular damage especially when combined with membrane-damaging agents (see below).

Role of Metalloproteinases

The role played by oxidants in the activation of latent metalloproteinases was investigated.[39,190,195] This issue was studied in detail by Weiss.[39] Metalloproteinases capable of degrading collagen were synthesized in latent, inactive, forms.[39,195] Unlike PMNs from CGD patients, normal PMNs could activate latent collagenase suggesting that ROS might be responsible for this effect. The active ROS responsible for collagenase activation was identified as HOCl which also activated latent gelatinase. The mechanisms of enzyme activation might involve a thiol-driven intramolecular perturbation that unmasked the active site of the metalloproteinase.[195] Thus the activities of both PMN collagenase and gelatinase were highly linked to the MPO system and HOCl generation (a true synergistic effect[39]). Although the in vitro activation of collagenase by HOCl[192,193] was corroborated other authors have reported contrary results.[193a,193b] Furthermore, it was suggested that activation of collagenase was due to cathepsin G and that the role of HOCl was indirect.[193b] While Weiss et al suggested that gelatinase could also be activated in vitro by HOCl,[192] it was recently shown[193a] that the ratio of HOCl to enzyme employed by Weiss *et al.*[193] caused distinct inactivation of gelatinase.[193a] It was recently stressed that in order to activate coliagenase under in vivo conditions a strict ratio of enzyme/HOCl had to be used[193a] and that conditions of enzyme inactivation in vitro might not be relevant under in vivo conditions where HOCl scavengers are present. An endogenous activation of latent collagenase, by plasminogen activator, in rheumatoid synovial cells, was also reported.[196] It suggested that bacteria possessing plasminogen activators (streptococci, staphylococci, clostridia) might also activate metalloproteinases in inflamed sites which might also synergize with oxidants to amplify cell damage. As suggested by Weiss[39] "the latent collagenases are specifically structured so that they can transform the oxidized potential of HOCl into the enzyme-catalyzedy degradation of the interstitial pericellular, and basement membrane associated collagens.

Collaboration Among Phospolipases, Fatty Acids and Oxidants

PLA_2, and PLC are both implicated in many metabolic and in pathological processes ([1,2,6,197–201]) The presence of a membrane-associated PLA_2 in PMNs[202–204] and the involvement of this enzyme in the respiratory burst which lead to enhanced generation of ROS[205–207] suggested that it might participate in cellular injury in infectious and in inflammatory conditions. The highly hemolytic phospholipases A_2 and C (PLC) present in venoms of snakes and bees and in certain microbial species, respectively, probably function as distinct cytotoxins and as virulence factors.[1,2]

Both the free fatty acids and the lysophosphatides which might be released from injured cells following the activation of PLA_2 might injure by-stander cells due to their membrane-damaging properties.

EC and BGM cells which had been pretreated by subtoxic amounts of PLA_2 from pancreas, from snake and bee venoms and by PLC from *Clostridium Welchii*[76–79] were killed in a synergistic manner by GO-generated H_2O_2. Cell killing induced by PLC was further enhanced, also in a synergistic manner, by the addition of crystalline trypsin (a

triple-synergy). Trypsin was also absolutely essential for the release of substantial amounts of tritiated arachidonic acid, PGE_2 and 6-keto PGF from EC treated by mixtures of peroxide and PLC.[79] Both cell killing and the release of lipids were strongly inhibited by SBTI as well as by catalase, Mn^{2+} and by DMTU.[79] A similar synergistic cell killing and the release of membrane lipids also occurred when BGM cells were exposed to mixtures of lysophosphatidyl choline(lysolecithin), with peroxide and trypsin.[79] The possibility that the synergistic killing of BGM cells by mixtures of PLC or SLS with oxidants (H_2O_2, ROO·) was associated with the activation of the membrane-associated PLA_2 was also investigated. Cells which had been preincubated with a novel PLA_2 inhibitor (carboxymethyl cellulose –CMC, coupled to phospahtidylethanolamine)[86] became highly refractory to killing by these agents. The PLA_2 inhibitor also depressed the lysis of RBC by SLS. On the other hand, CMC alone, was inactive.

PMNs which had been primed by subtoxic concentrations either of lysophosphatidyl choline (LL), lysophosphatidyl inositol, or of lysophosphatidyl glycerol, generated enhanced amounts of superoxide and H_2O_2 when further stimulated by a variety of agonists (immune complexes, poly-L-histidine fMLP.).[208,209] These lysophosphatides were also highly hemolytic for human RBC. On the other hand, equi-molar concentrations either of lysophosphatidyl ethanolamine or of lysophosphatidyl serine (phospholipids with polar headgroups) possessed neither hemolytic nor priming activities for enhanced generation of superoxide. However, both these agents when combined with cytochalasin B, became highly stimulatory for superoxide generation.[209] Under similar conditions both arachidonic acid and eicosapentanoic acid also possessed stimulatory activities for the enhanced generation of superoxide when tested with PMA.[209]

The activation of PLA_2 in phagocytes suspended in albumin-containing media might result in the release of free fatty acids, which might synergize with ROS generated by activated phagocytes to kill by-stander cells. Subtoxic amounts either of arachidonic acid, eicosapentanoic acid or of arachidic acid became highly cytotoxic to BGM cells when combined with H_2O_2 and also released substantial amounts of tritiated arachidonated upon the addition of trypsin.[81] Both cell killing and the release of lipids were inhibited by antioxidants as well as by proteinase inhibitors.

Taken together it appears that synergism among phospholipases fatty acids, oxidants and proteinases might lead to cellular damage in inflammatory sites and that lysophosphatides in addition to their direct toxic effect on cells might also serve as priming agents for the enhanced generation of ROS by phagocytes. It might also be speculated that lysophosphatides and free fatty acids released in ischemic and reperfused organs (see [210,211]) might also act in concert with ROS generated either by the xanthine-xanthine oxidases system or by activated phagocytes, to cause cellular damage.

Collaboration Among Ethanol, Oxidants, Taurocholate and Proteases

The consumption of ethanol was found to be accompanied by the generation of ROS[44] but the role of the latter in cellular injury induced, by ethanol, in vivo, is still not clear. The possibility that ethanol might synergize with oxidants and with proteinases to kill targets was investigated with BGM and with fibroblasts monolayers.[212] Cells which had been exposed to subtoxic amounts of ethanol (6-10%) V/V became rounded but were not killed, even after 4 hours of incubation. On the other hand ethanol at these concentrations synergized with subtoxic amounts either of H_2O_2 or of ROO to rapidly

kill the cells. Cell killing was further enhanced, also in a synergistic manner, by the addition of trypsin (also a triple-synergistic effect). Cell killing was markedly inhibited either by catalase, Mn^{2+} or by fresh carrot juice.

BGM cells which had been treated by mixtures of ethanol, peroxide and trypsin also lost substantial amounts of membrane lipids.[81] These results are essentially similar to those obtained with a series of other membrane-damaging agents (SLS, PLC.LL, PLA_2).[77 79]

A quadruple-synergism among ethanol, peroxide, taurocholic acid and trypsin also killed BGM cells.[81] A similar mixture of agonists might be found in stomachs of people who consume ethanol and who also have regurgitation of pancreatic juices.[44,81]

ARE CYTOKINE-PRIMED, PHAGOCYTE-DERIVED OXIDANTS PROTECTIVE OR DESTRUCTIVE?

Since a variety of proinflammatory agonists might act in concert with oxidants to markedly enhance cellular damage, it seems that agents and conditions which enhanced either the synthesis or the secretion of toxic agonists from activated phagocytes or from tissue sources (e.g. xanthine oxidase) might not only better kill microorganisms but might, paradoxically, also destroy the hosts own cells and tissues (*Horor autotoxicus*). Therefore, the flood of investigations on the role played by cytokines as priming agents for the enhanced generation of leukocyte-derived oxygen species,[25,26,213 250] merit a special consideration regarding the adverse effects that cytokine treatment might cause in patients. Cytokine-primed phagocytes also acquired enhanced phagocytic and degranulation properties,[248] higher killing capacities towards a variety of targets[251 253] and enhanced antibody dependent cellular cytotoxicity properties.[254]

The biological significance of the cytokine-enhanced generation of oxygen radicals is, however, controversial. While cytokine treatment improved the capacity of hosts to deal with microbial infections, it was also reported that such an elevation was accompanied by complications attributable to enhanced oxidative stress.[25,26] While some publications showed that chelators of iron were found to inhibit cytokine-enhanced cytotoxicity (involvement of OH·) others, have failed to show such an effect. One publication[255] showed that the toxicity of TNF was markedly reduced in the presence of H_2O_2[255] while another showed that superoxide generation by macrophages was depressed by IL-4[256] suggesting that this cytokine might regulate some of the effects of the inflammatory response.

Despite the presence of a large body of evidence that cytokine-treated phagocytes generated elevated amounts of ROS and also possessed enhanced cytotoxicity properties, little is known whether cytokine-primed phagocytes might perhaps also generate elevated amounts of other proinflammatory agonists (e.g. cationic proteins, phospholipases, proteinases etc) (see [235]). If indeed such an elevation could occur, it might lend support to the assumption that excessive amounts of leukocyte-derived agonists might be deleterious to tissues due to multiple synergistic interactions, as suggested by the models described above.

How Can ROS Generated by Activated Leukocytes Overcome the Antioxidant Systems Present in Targets?

Screening the literature on the mechanisms of cellular damage by activated leukocytes revealed that the amounts of H_2O_2 (micromolar concentrations) generated by activated

PMNs were not sufficient to kill the targets within the regular assay time (2–8 hours). This was especially true if serum proteins were also added to the media. A more significant cell killing occurred after longer periods of incubation (12–18 hrs.) and also necessitated the employment of a high ratio of phagocytes to targets. The high resistance to killing was probably due to the presence of potent antioxidant systems in both targets and in the effector cells.[257 260] What was commonly observed in in vitro assays, even after overnight incubation, was a detachment of the cell monolayers and the formation of clusters of, mostly viable, targets.[171 175] The detachment phenomenon was most probably caused by the exocytosis of leukocyte proteinases. This could be totally inhibited by proteinase inhibitors.[174] In order to facilitate cell killing, inhibitors of catalase (sodium azide, amino-triazole[174]) and of glutathione reductase[175] had to be added. The removal of proteinase inhibitors by HOCl[39] also enhanced cell killing. However, in the absence of such inhibitors, cell killing took a longer time and was probably dependent on synergistic interactions among oxidants, proteinase and additional proinflammatory agonists and perhaps also on a site-specific, radical-induced membrane damage.[261] The assumption that synergism among oxidants and membrane-damaging agents might be the basis for the understanding how phagocytes kill targets by overcoming their antioxidant capacities, was based on experiments with SLS.[81] While relatively large amounts of H_2O_2 (2–5mM) failed to kill targets within 2–3 hours, as little as 50μM of peroxide became cytotoxic within 30 min. if combined with subtoxic amounts of SLS. The mechanisms by which synergism between SLS and peroxide kill cells are still not known. Since SLS could be effectively replaced by other membrane-damaging agents (SLO, fatty acids, lysophosphatides, PLA_2, PLC, cationic proteins, proteinases) (see above) it stands to reason that the synergism phenomenon described is universal and that additional proinflammatory agonists might also be added to the list of agents capable of killing cells.

Morphological Changes Induced by Synergy Among Oxidants Membrane-Damaging Agents and Proteinases

Cellular injury induced by mixtures of oxidants and membrane-damaging agents (lysophosphatides, PLC, SLS, ethanol – see below)[77 79,212] was characterized by the development of blebs (pseudopod-like structures) usually at one cell pole, which in many cases, were totally detached from the cells. These changes were compatible with altered plasma cell permeability (see 75, 77, 212).

EPILOGUE: WHERE DO WE GO FROM HERE?

Taken together, it might be speculated that the destruction of cells and tissues in infectious and in inflammatory sites might be the result of a well-orchestrated and collaborative events which involve at least three major groups of agonists. 1) oxidants, 2) membrane-damaging agents (hemolysins, phospholipases, lysophosphatides, fatty acids, cationic proteins) and 3) proteinases. All these agents are secreted by bacteria and by activated phagocytes, and their presence in inflammatory exudates, had been demonstrated. The coordinated "cross-talk" among the virulence factors of streptococci, in tissue-destruction, lead to the speculation that invasive and toxigenic bacteria might perhaps be looked upon as "forefathers of modern phagocytes".[78,289] H_2O_2[55 60,262 265] and HOCl,[15,39] both long-lasting oxidants, might be the most important toxic agents generated by activated phagocytes. Both oxidants can attack cells from the outside and

also freely diffuse intracellularly to deplete energy sources and to cause DNA breaks.[53-57] Although both OH· and ROO· might be more toxic than either peroxide or HOCl,[266-268] they cannot readily cross the cell membrane unless it had been injured by membrane-damaging agents. Also the effect of OH· might be dissipated by excess water molecules. Furthermore, its action might also be restricted in a metal-catalyzed site-specific manner.[261] However, it seems that one of the most important property of H_2O_2 is its ability, at micromolar, sub-toxic concentrations, to synergize with membrane-damaging agents and with proteinases to overcome the antioxidant capacities of targets, to kill cells and to solubilize their membrane lipids.[77,79,81] Proteinases might act by degrading oxidized proteins more efficiently.[82-84] H_2O_2 is also capable, at subtoxic amounts, to affect signal transduction in cells[68] and at concentrations likely to be generated by leukocytes, in vivo. Thus the synergism mechanisms proposed might involve amplification of minor damages caused by residual amounts of oxidants which survived the scavenging effects of the cell-associated antioxidant.[258,460]

Oxidants might perhaps also be involved in programmed cell death (apoptosis),[269,270] in the oxidation of LDL which become toxic to mammalian cells[271] and in the killing of targets by NK cells and by other killer lymphocytes. Complement-mediated cell killing might also be amplified by peroxide.[81]

To combat the toxic effects of activated leukocytes on tissues, it seems that scavengers of oxidants,[272-279] might not be adequate as sole therapeutic agents (see [276]) and that cocktails containing putative inhibitors of membrane-damaging agents and of other "primers" of leukocytes, should be also included in therapeutic regimens. Such agents might either be delivered via regular or cationized liposomes (Ginsburg and Bernholtz unpublished observations) or coupled to cationic polypeptides carriers.[281-284] Cationization might serve, to increase their adhesiveness and their uptake by targets.[20] Finally, it might also be proposed that the ability to effectively combat the deleterious effects of inflammatory agonists in vivo, might depend on the ability to disrupt, by mixtures of antagonists, the synergistic "cross-talks" among oxidants, proteinases, phospholipases, fatty acids, cytokines, cationic proteins, microbial toxins and additional membrane-damaging agents, as implied by the experimental models reviewed in the present communication.

Because nitric oxide (NO) has been gaining recognition as an important biological mediator[29,284,285] and because NO is implicated in the killing of parasites, by activated macrophages (reviewed in[285]), its role in cellular damage in inflammation should also be considered. NO might interact with superoxide to generate peroxynitrite, a potent cytotoxin, which could degrade to OH·.[288] OH· might then injure targets. Recently, it was demonstrated that BGM cells which had been exposed to subtoxic concentrations of sodium nitroprusside (a generator of NO), were killed, in a synergistic manner, by the addition of mixtures of H_2O_2 and SLS.[81] Hemoglobin, a scavenger of NO, markedly inhibited cell killing. Trypsin further enhanced cell killing, in a synergistic manner, and also markedly enhanced the release of large amounts of membrane lipids. Paradoxically, however, NO was also shown to inactivate superoxide[288] suggesting that under certain conditions NO might also function a scavenger of ROS. This paradox should be resolved.

Finally, the findings that mixtures of oxidants and membrane-damaging agents could overcome the antioxidant capacities of mammalian cells, and could also enhance the release of significant amounts of membrane-associated lipids, if proteinases were also included,[81,289] seem to be important contributions to the understanding the pathophysiology of inflammation. The universal synergy phenomenon described might also be used to devise tissue culture assays for the evaluation of the safety of commonly-used

drugs, pesticides and additional chemicals in common usage. The elucidation of the molecular mechanisms of synergy might also greatly contribute to the formulation of protective therapeutic measures for the purpose of controlling tissue-damaging inflammatory processes.

References

1. A.W. Bernheimer and B. Rudy (1986) Interaction between membranes and cytolytic peptides. *Biochemica Biophysica Acta*, **864,** 123–141.
2. S.M. Scotland (1988) Toxins. *Journal of Applied Bacteriology*, 109S–1295, Suppl.
3. K.F. Bayson and J. Cohen (1990) Bacterial endotoxins and current concept in diagnosis and treatment of endotoxemia. *Journal of Medical Microbiology*, **31,** 73–78.
4. J.E. Diran (1992) Biological effects of endotoxins. In: Gut-derived infectious toxic shock (GITS). In *Current Studies in Hematology and Blood Transfusion*, (eds. H. Cottier and R. Kraft), Karger, Basel, **59,** pp. 66 99.
5. E.T. Rietchel, E.T.S. Kirika, U. Schahde, U. Mamat, G. Schmidt, H. Loppnow, A.J. Ulmer, U. Zahringer, U. Seydel, Di. F. Padova, M. Schreier and H. Brade (1994) Bacterial endotoxin: molecular relationship of structure to activity and function. *FASEB Journal*, **8,** 217–225.
6. I. Holder, ed. (1985) *Bacterial enzymes and virulence*. CRC Press, Boca Raton, Florida.
7. I. Ginsburg (1985) Streptococcal enzymes and virulence. In *Bacterial enzymes and virulence* (ed. I. Holder), CRC Press, Boca Raton, Florida, pp. 121 144.
8. P. Giborowski and J. Jeljazewicz (1985) Staphylococcal enzymes and virulence. In *Bacterial enzymes and virulence* (ed. I. Holder), CRC Press, Boca Raton, Florida,. pp. 146 183.
9. D.J. Hentges and L.D.S. Smith (1985) Hydrolytic enzymes as virulence factors of anaerobic bacteria. In *Bacterial enzymes and virulence* (ed. I. Holder), CRC Press, Boca Raton, Florida, pp. 105 119.
10. I. Ginsburg and M.N. Sela (1976) The role of leukocytes and their hydrolases in the persistence, degradation and transport of bacterial constituents in tissues, relation to chronic inflammatory processes in staphylococcal, streptococcal and mycobacterial infections and in chronic periodontal disease. *Critical Reviews in Microbiology*, **4,** 249–332.
11. I. Ginsburg (1972) The role of lysosomal factors of leukocytes in the biodegradation and storage of microbial constituents in infectious granulomas. In *Lysosomes in applied biology and therapeutics* (eds. J.T. Dingle, P.J. Jacques and I.H. Shaw), Vol. 6, North Holland Publication, Amsterdam, pp. 327–406.
12. E. Schrinner, M.H. Richmond, G. Seibert and U. Schwarz (1988) *Surface structures of microorganisms and their interactions with the mammalian host*, VCH Publishers, Weinheim, Federal Republic of Germany.
13. B. Halliwell and J.M.C. Gutteridge (1989) *Free radicals in biology and medicine*. 2nd edition, Clarenton Press, Oxford.
14. J.I. Gallin, I.M. Goldstein and R. Snyderman (1992) *Inflammation, basic principles and clinical correlates*. Raven Press, New York.
15. S.F. Klebanoff (1992) Oxygen metabolites from phagocytes. In *Inflammation, basic principles and clinical correlates* (eds. J.I. Gallin, I.M. Goldstein and R. Snyderman), 2nd edition, Raven Press, New York, pp. 541 588.
16. J.L. Farber, M.E. Kyle and J.B. Coleman (1990) Mechanisms of cell injury by active oxygen species. *Laboratory Investigation*, **62,** 670 679.
17. P.A. Ward and J. Varani (1990) Mechanisms of neutrophil-mediated killing of endothelial cells. *Journal of Leukocyte Biology*, **48,** 97 102.
18. R.T. Dean, S. Gieseg and M.J. Davies (18 November 1993) Reactive species and their accumulation on radical-damaged proteins. *Trends in Biological sciences*, 437–441.
19. P.M. Henson and R.B. Johnston (1987) Tissue injury in inflammation, oxidants, proteinases and cationic proteins. *Journal of Clinical Investigation*, **79,** 669 674.
20. I. Ginsburg (1987) Cationic polyelectrolytes, a new look at their possible roles as opsonins, as stimulators of the respiratory burst in leukocytes, in bacteriolysis and as modulators of immune complex disease. *Inflammation*, **11,** 489–515.
21. K.J. Hammann, R.L. Barker, R.M. Ten and G.J. Gleich (1991) The molecular biology of eosinophil granule proteins. *International Archives of Allergy and Applied Immunology*, **94,** 202–209.
22. J.K. Spitznagel (1990) Antibiotic proteins of human neutrophils. *Journal of Clinical Investigation*, **86,** 1386–1391.
23. P. Elsbach and J. Weiss (1992) Phagocytic cells; oxygen independent antimicrobial system. In *Inflammation basic principles and clinical correlates* (eds. J.I. Gallin, I.M. Goldstein and R. Snyderman), 2nd ed., Raven Press, New York, pp. 445 470.

24. R.I. Lehrer (1993) Defensins, antimicrobial and cytotoxic peptides of mammalian cells. *Annual Review of Immunology*, **11**, 105–128.
25. S.B. Mizel (1989) The interleukins. *FASEB Journal*, **3**, 2379–2388.
26. A. Cerami (1992) Inflammatory cytokines. *Clinical Immunology and Immunopathology*, **62**, 53–510.
27. D. Collen and H.R. Lijnen (1991) Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood*, **78**, 3114–3124.
28. B.B. Morgan (1989) Complement membrane attack on nucleated cells, resistance, recovery and non-lethal effects. *Biochemical Journal*, **264**, 1–15.
29. C. Nathan (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB Journal*, **6**, 3051–3064.
30. M. Koltai, D. Hosford and P. Braquet (1992) Role of PAF and cytokines in microvascular tissue injury. *Journal of Laboratory and Clinical Medicine*, **119**, 461–466.
31. (1988) Cytotoxic T-cells, biology and relevance to disease. *Annals of the New York Academy of Sciences*, p. 532.
32. J.M. McCord (1988) Free radicals in myocardial ischemia, overview and outlook. *Free Radicals in Biology and Medicine*, **4**, 9–14.26.
33. A.J. Sbarra and R.R. Strauss, eds. (1988) *The respiratory burst and its physiological significance*. Plenum Press, New York.
34. G.D. Groot and A. Littauer (1989) Hypoxia, reactive oxygen and cell injury. *Free Radicals in Biology and Medicine*, **6**, 541–551.
35. B. Halliwell (1989) Free radicals, reactive oxygen species and human disease, a critical evaluation with special reference to atherosclerosis. *British Journal of Experimental Pathology*, **70**, 737–757.
36. B. Halliwell, S. Chirico, H. Kaur, O.I. Aruoma, M. Grootveld and D.R. Blake (1993) In *Oxidative Damage and Repair: Chemical and Medical Aspects* (ed. K.J.A. Davies), pp. 846–853, Pergamon Press, New York.
37. A. Kontos (1989) Oxygen radicals in CNS damage. *Chemico-Biological Interactions*, **72**, 229–255.
38. A. Slivka, A.F. LoBuglio and S.J. Weiss (1989) A potential role for hypochlorous acid in granulocyte mediated tumor-cell toxicity. *Blood*, **55**, 3477–3500.
39. S.J. Weiss (1989) Tissue destruction by neutrophils. *New England Journal of Medicine*, **320**, 365–376.
40. A.J. Jestis, M.T. Ginn, G. Mukhergee, P.A. Ward and E.A. Dratz (1991) Death by oxygen, radical views. *The New Biologist*, **3**, 651–655.
41. R. Seifert and G. Schultz (1991) The superoxide-forming NADP, H oxidase of phagocytes. An enzyme system regulated by multiple mechanisms. *Review of Physiology Biochemistry and Pharmacology*, **117**, 1–510.
42. F. Morel, J. Doussers and P.V. Vignais (1991) The superoxide-generating oxidase of phagocytic cells. Physiology, molecular and pathological aspects. *European Journal of Biochemistry*, **201**, 523–546.
43. A. Van Der Vilt and A. Bast (1991) Role of reactive oxygen species in intestinal diseases. *Free Radicals in Biology and Medicine*, **12**, 499–513.
44. R. Nordmann, C. Ribier and H. Rouach (1992) Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Radicals in Biology and Medicine*, **12**, 219–240.
45. M. Baggiolini, F. Boulay, J.A. Badwey and J.T. Curnutte (1993) Activation of neutrophil leukocytes, chemoattractant receptor and respiratory burst. *FASEB Journal*, **7**, 1004–1010.
46. Y.M.W. Jansen, B. Van Houten, P.A.J. Brom and B.T. Mossman (1993) Cell and tissue responses to oxidative damage. *Laboratory Investigation*, **69**, 261–274.
47. H.A. Lehr and K.E. Arfors (1994) Mechanisms of tissue damage by leukocytes. *Current Opinion in Hematology*, **1**, 92–99.
48. L.C. McPhail, P. Henson and R.B. Johnston (1981) Respiratory burst enzyme in human neutrophils, evidence for multiple mechanisms of activation. *Journal of Clinical Investigation*, **67**, 710–716.
49. I. Ginsburg, R. Borinski, M. Lahav, Y. Matzner, I. Elliasson, P. Christensen and D. Malamud (1985) Poly-L-arginine and N-formulated chemotactic peptide act synergistically with lectins and calcium ionophore to induce intense chemiluminescence and superoxide production by human blood leukocytes. *Inflammation*, **8**, 1–26.
50. I. Ginsburg, I. (1972) Mechanisms of cell and tissue injury induced by group A streptococci: relation to poststreptococcal sequelae. *Journal of Infectious Diseases*, **126**, 294–340, 419–456.
51. I. Ginsburg (1959) Action of streptococcal hemolysins and proteolytic enzymes on Enrlich ascites tumor cells. *British Journal of Experimental Pathology*, **40**, 417–423.
52. I. Ginsburg and N. Ram (1963) Effect of antibodies and plasmin on Enrlich ascites tumor cells. *Nature*, **185**, 328–330.
53. I.V. Schraufstatter, K. Browner, A. Harris, P.A. Hyslop, J.H. Jackson, O. Quehenberger, C.G. Cochrane (1990) Mechanisms of hypochlorite injury to targets. *Journal of Clinical Investigation*, **85**, 554–562.
54. C. Brenofsky (1991) Nucleotide chloramines and neutrophil-mediated cytotoxicity. *FASEB Journal*, **5**, 295–300.

55. I.V. Schraufstatter, P.A. Hyslop, J. Jackson and C.C. Cochrane (1986) Mechanisms of oxidant injury of cells. In *Leukocyte migration and its sequellae* (ed. M. Movat), Satellite symposium of the 6th international congress on immunology, pp. 1150–1160.
56. A. Lichtenstein (1989) Mechanisms of neutrophil-mediated oxidative and non-oxidative tumor lysis. In *New Horizons of Tumor Immunotherapy* (eds. M. Torisu and Y. Yoshida), Elsvier Science Publisher B. V. (Biomedical Division), pp. 79–100.
57. S. Orrenius (1990) Mechanisms of oxidative cell damage. In *Free radicals from basic science to medicine* (eds. G. Poli, E. Albano and M.U. Dianzani), Birkhauser Verlag, Basel, Switzerland, pp. 47–64.
58. P.A. Ward (1991) Mechanisms of endothelial cell injury. *Journal of Laboratory and Clinical Medicine*, **118,** 421–426.
59. A.A. Vlessis, O. Muller, D. Bartos and D. Trunkey (1991) Mechanisms of peroxide-induced cellular injury in cultures and adult myocytes. *FASEB Journal*, **5,** 2600–2605.
60. M.M. Hallecki, J.H. Richburg and F.C. Kaufman (1992) Reversible and irreversible oxidant injury to PC12 cells by hydrogen peroxide. *Free Radicals in Biology and Medicine*, **12,** 137–144.
61. Y.M.W. Janssen, B. Van Houten, P.J.A. Borm and B.T. Mossman (1993) Cell and tissue responses to oxidative damage. *Laboratory Investigation*, **69,** 261–274.
62. M.S. Baker, J. Feigan and D.A. Lowther (1989) The mechanism of chondrocyte hydrogen peroxide damage. Depletion of intracellular ATP due to suppression of glucolysis caused by oxidation of glyceraldehyde-3-phosphate dehydrogenase. *Journal of Rheumatology*, **16,** 7–14.
63. J. Varani, S.E. Phan, D.F. Gibbs, U.S. Ryan and P.A. Ward, P.A. (1990) H_2O_2-mediated cytotoxicity of rat pulmonary endothelial cells, changes in adenosine triphosphate and purine products and effects of protective interventions. *Laboratory Investigation*, **63,** 683–689.
64. R.G. Sprag (1991) DNA strand break formation following exposure of bovine pulmonary artery and aortic endothelial cells to reactive oxygen products. *American Journal of Respiratory and Cell Biology*, **4,** 4–10.
65. T.K. Alto and K.O. Raivio (1993) Mechanisms of adenine nucleotide depletion from endothelial cells exposed to reactive oxygen metabolites. *Free Radicals in Biology and Medicine*, **14,** 177–183.
66. S. Mehrotra, P. Makkar and P.M. Wiswanathan (1991) Mitochondrial damage by oxygen species *in vitro*. *Free Radicals in Biology and Medicine*, **10,** 277–283.
67. Y. Yamamoto, E. Niki, J. Eguchi and H. Shimasaki (1985) Oxidation of biological membranes and its inhibition. Free radical chain oxidation of erythrocyte ghost membrane by oxygen. *Biochemica et Biophysica Acta*, **819,** 29–36.
68. G.M. Vercelotti, S.P. Severson, P. Duane and C.F. Moldow (1991) Hydrogen peroxide alters signal transduction in human endothelial cells. *Journal of Laboratory and Clinical Medicine*, **117,** 15–24.
69. J.R. Bradley, D.R. Johnson and J.S. Pober (1993) Endothelial cells activation by hydrogen peroxide. Selective increases of intracellular adhesion molecule-1 and major histocompatibility complex class 1. *American Journal of Pathology*, **142,** 1598–1609.
70. J.S. Winn, J. Guille, J.M. Gebicki and R.O. Day (1990) Hydrogen peroxide modulate respiratory burst in human neutrophils. *Biochemical Pharmacology*, **41,** 31–36.
71. M. Shingu, S. Nonaka, H. Nisimukai, M. Nobunaga, H. Kitamura and K. Tomooka (1992) Activation of complement in normal serum by hydrogen peroxide and hydrogen peroxide-related oxygen radicals produced by activated neutrophils. *Immunology*, **90,** 72–78.
72. V. Vogt, B. Damerau, I. Von Zabern, R. Nolte and D. Brunahl (1989) Non-enzymatic activation of the fifth component of human complement, by oxygen radicals. Some properties of the activation product C5b-like C5. *Molecular Immunology*, **26,** 1133–1142.
73. W. Natarajan, M.M. Taher, B. Roehm, N.L. Parinadi, H.H.O. Schmidt, Z. Kiss and J.G.N. Garcia (1993) Activation of endothelial cells phospholipase D by hydrogen peroxide and fatty acid hydroperoxide. *Journal of Biological Chemistry*, **268,** 930–937.
74. I. Ginsburg and T.N. Harris (1965) Oxygen-stable hemolysins of group A streptococci. IV. The mechanisms of lysis of red blood cells by the cell-bound hemolysin. *Journal of Experimental Medicine*, **121,** 647–653.
75. Z. Marcus, M. Davies and I. Ginsburg (1966) Oxygen-stable hemolysins of group A streptococci. V. Effect on rat heart and kidney cells grown in tissue culture. *Experimental and Molecular Pathology*, **5,** 93–107.
76. I. Ginsburg and J. Varani (1993) Interaction of viable group A streptococci and hydrogen peroxide in killing of vascular endothelial cells. *Free Radicals in Biology and Medicine*, **14,** 495–500.
77. I. Ginsburg, D.F. Gibbs, L. Schuger, K.L. Johnson, U.S. Ryan, P.A. Ward and J. Varani (1989) Vascular endothelial cells killing by combinations of membrane-active agents and hydrogen peroxide. *Free Radicals in Biology and Medicine*, **7,** 369–375.
78. I. Ginsburg, R. Misgav, A. Pinson, J. Varani and R. Kohen (1992) Synergism among oxidants, proteinases, phospholipases, microbial hemolysins, cationic proteins, proteinases and cytokines. *Inflammation*, **16,** 519–538.

79. I. Ginsburg, R.S. Mitra Jr., D.F. Gibbs, J. Varani and R. Kohen, (1993) Killing of endothelial cells and release of arachidonic acid, synergistic effects among hydrogen peroxide, membrane-damaging agents cationic substances and proteinases and their modulation by inhibitors. *Inflammation*, **17**, 295–319.
80. N. Elias, M. Heller and I. Ginsburg (1966) Binding of streptolysin S to red blood cell ghosts and ghost lipids. *Israel Journal of Medical Sciences*, **2**, 302–309.
81. I. Ginsburg and R. Kohen (in press, 1994) Synergistic effects among oxidants, membrane-damaging agents, fatty acids, proteinases and xenobiotics: Killing of epithelial cells and the release of arachidonic acid. *Inflammation*, **19**, 101–118.
82. K.J.A. Davies, S.W. Lin and R.E. Pacific (1987) Protein damage and degradation by oxygen radicals. IV. Degradation of denatured proteins. *Journal of Biological Chemistry*, **262**, 9914–9920.
83. J. Neuzil, J.M. Gebicki and R. Stocker (1993) Radical-induced chain oxidation of proteins and its inhibition by chain-breaking antioxidants. *Biochemical Journal*, **293**, 601–606.
84. E.R. Stadtman (1992) Protein oxidation and aging. *Science*, **257**, 1220–1224.
85. J. Varani, I. Ginsburg, D.F. Gibbs, P.S. Mukhopadhyay, C. Sulavik, K.J. Johnson, Y.M. Weinberg, U.S. Ryan and P.A. Ward (1991) Hydrogen peroxide induced cell and tissue injury, protective effect of Mn^{2}. *Inflammation*, **15**, 291–301.
86. S. Yedgar, A. Dagan, P. Dan and I. Ginsburg (October 31, 1993) Regulation of cell membrane phospholipase A_2 activity by cell-inpermeable inhibitors. Abstract In *3rd International Conference on Lipid Mediators in Health and Disease*. Jerusalem, p. 25.
87. S.A. Raya, V. Trembovler, E. Shohami and P. Lazarovici (1993) Cytolsins increase intracellular calcium and induce eicodanoids released by pheochromocytoma PC 12 cell culture. *Natural Toxins*, **1**, 263–270.
88. G.F. Grimminger, U. Sibelius, S. Bhakdi, N. Suttrop and W. Seeger (1991) *Escherichia coli* hemolysin is a potent inductor of phosphoinositide hydrogen and related metabolic responses in human neutrophils. *Journal of Clinical Investigation*, **88**, 1531–1539.
89. P.S. Sporn, M. Peter-Golden and R.H. Simon (1988) Hydrogen peroxide-induced arachidonic acid metabolism in the rat alveolar macrophages. *American Journal of Respiratory Diseases*, **137**, 490–496.
90. M.R. Prasad and D.K. Das (1989) Effect of oxygen-derived free radicals and oxidants on the degradation of *in vitro* of membrane phospholipids. *Free Radicals Research Communications*, **7**, 381–388.
91. S.N.S. Murty, C.G. Cooney and H.R. Clearfield (1990) Hydrogen peroxide-induced alteration in prostaglandin secretion in rat colon *in vitro*. *Inflammation*, **14**, 645–661.
92. P.G. Duane, K.L. Rice, D.E. Charboneau, M.B. King, D.P. Gillboe and D.E. Niewoehner (1991) Relationship of oxidant-mediated cytotoxicity to phospholipid metabolism in endothelial cells. *American Journal of Respiratory and Molecular Biology*, **4**, 408–416.
93. A.J. Wicken and K.W. Knox (1975) Lipoteicholic acids, a new class of bacterial antigens. *Science*, **187**, 1161–1167.
94. J.E. Doran (1992) Biological effects of endotoxins in gut-derived infectious-toxic shock (GITS). In *Current Studies in Blood Transfusions* (eds. H. Cottier and R. Kraft), No. 59, Karger, Basel, pp., 66–99.
95. C.R.H. Raetz, R.J. Ulevitch, S.D. Wright, C.H. Sibley, A. Ding and C.F. Nathan (1991) Gram-negative endotoxin: an extraordinary lipid with profound effect on eukaryotic signal transduction. *FASEB Journal*, **5**, 2652–2660.
96. N. Neeman and I. Ginsburg (1971) Red cell sensitizing agent of group A streptococci. II. Immunological and immunopathological properties. *Israel Journal of Medical Sciences*, **8**, 1807–1816.
97. I. Ofek, E.H. Beachey, W. Jefferson and G.L. Campbell (1975) Gel 1 Membrane binding properties of group A streptococcal lipoteichoic acid. *Journal of Experimental Medicine*, **141**, 990–1003.
98. D. Lopathin and R.E. Kessler (1985) Pretreatment with lipoteichoic acid sensitizes target cells to antibody-dependent cellular cytotoxicity in the presence of antilipoteichoic acid antibodies. *Infection and Immunity*, **48**, 638–643.
99. J.H. Winkelstein and A. Thomasz (1978) Activation of the alternative complement pathway for pneumococcal cell-wall teichoic acid. *Journal of Immunology*, **120**, 174–178.
100. T. Dishon, R. Finkel, Z. Marcus and I. Ginsburg (1967) Cell sensitizing products of streptococci. *Immunology*, **13**, 555–564.
101. P.S. Tobias, J.C. Mathison and R.J. Ulevitch (1988) A family of lipolysaccharide binding proteins involved in responses to Gram negative sepsis. *Journal of Biological Chemistry*, **263**, 13479–13481.
102. A. Yamamoto, H. Usami, M. Nagamuta, Y. Sugawara, S. Hamada, T. Yamamoto, S. Kokeguchi and S. Kotani (1985) The use of lipoteichoic acid (LTA) from streptococcus pyogenes to induce serum factor causing tumor necrosis. *British Journal of Cancer*, **51**, 737–742.
103. I. Ginsburg, S.E.G. Fligeil, P.A. Ward and J. Varani (1989) Lipoteichoic acid-antilipoteichoic acid complexes induce superoxide generation by human neutrophils. *Inflammation*, **12**, 525–545.

104. R. Levi, M. Kotob, O. Nagauker, G. Majmudar, M. Alkan, I. Ofek and E.H. Beachey (1990) Stimulation of oxidative burst in human monocytes by lipoteichoic acid. *Infection Immunity*, **58,** 566–568.
105. D. Tarsky-Tsuk and R. Levy (1990) Stimulation of the respiratory burst in peripheral blood monocytes by lipoteichoic acid. The involvement of calcium ions and phospholipase A_2. *Journal of Immunology*, **144,** 2665–2670.
106. Y. Oshima, J. Beuth, H. Yassin, H.L. Ko, and G. Pulverer (1988) Stimulation of human monocyte chemiluminescence by staphylococcal lipoteichoic acid. *Medical Microbiology and Immunology*, **177,** 115–121.
107. R. Keller, W. Fischer and S. Bassett (1992) Macrophage response to bacteria, induction of marked secretory and cellular activities by lipoteichoic acid. *Infection and Immunity*, **60,** 3364–3672.
108. K. Yagawa, M. Kahku, Y. Ichinose, S. Nagao, A. Tanaka and A. Tomoda (1984) Bophasic effects of muramyl dipeptide and lipopolysaccharide on superoxide anion-generating activities of macrophages. *Infection and Immunity*, **45,** 82–86.
109. G.S. Worthen, J.F. Seccombe, K.L. Clay, L.A. Guthrie and R.B. Johnston (1988) The priming of neutrophils by lipopolysaccharide for production of intracellular platelet-activating factor. Potential role in mediation of enhanced superoxide secretion. *Journal of Immunology*, **140,** 3553–3559.
110. G.G. Wright, P.W. Read and G.L. Mandell (1988) Lipopolysaccharide release a priming substance for platelets that augment the oxidative response of polymorphonuclear neutrophils to chemotactic peptide. *Journal Infectious Diseases*, **157,** 690–696.
111. J.R. Forehand, M.J. Papst, W.A. Phillips and R.B. Johnston (1989) Lipopolysaccharide priming of human neutrophils for an enhanced respiratory burst, role of intracellular calcium. *Journal of Clinical Investigation*, **83,** 74–83.
112. J.R. Forehand, J.S. Bomalski and R.B. Johnston (1991) Mechanisms of lipopolysaccharide priming for enhanced respiratory burst in human neutrophils. In *New aspects of human polymorphonuclear leukocytes*, (eds. W.H. Hirll and P.J. Schollmeyer) Plenum Press, pp. 65–73.
113. J.B. Klein, V. Payene, T.M. Schepers and K.R. McLeish (1990) Bacterial lipopolysaccharide enhance polymorphonuclear leukocyte function independent of changes in intracellular calcium. *Inflammation*, **14,** 599–611.
114. M. Ferne, Z. Duchan, S. Rabinovitz, M.N. Seka and I. Ginsburg, (1978) The effect of leukocyte hydrolases on bacteria XII. The release of LPS from *Salmonella typhi* by leukocyte extracts, lysozyme, inflammatory exudates and by serum and synovial fluids and the modulation by anionic and cationic polyelectrolytes of LPS release and the sensitization of erythrocytes. *Inflammation*, **3,** 59–80.
115. M.N. Sela, M. Lahav and I. Ginsburg (1977) Effect of leukocyte hydrolases on bacteria IX. The release of lipoteichoic acid from group A streptococci and from *Streptococcus mutans* by leukocyte extracts and by lysozyme, relation to tissue damage in inflammatory sites. *Inflammation*, **2,** 151–164.
116. D. Horn and A. Tomasz (1979) Release of lipoteichoic acid from *Streptococcus sanguis*. Stimulation of release during penicillin treatment. *Journal of Bacteriology*, **137,** 1180–1184.
117. Nealon, M.J., E.H. Beachey, H.S. Courtney and A. Simpson (1986) Release of fibronectin-lipoteichoic acid complexes from group A streptococci with penicillin. *Infection and Immunity*, **51,** 529–533.
118. M. Sela and E. Katchalski (1959) Biological properties of poly alpha amino acids. *Advances in Protein Chemistry*, **14,** 391–478.
119. P.P. Lambert, P. Bergmann and R. Beauwens, eds. (1983) *The pathogenicity of cationic proteins*. Raven Press, New York.
120. S.J. Ackerman, D.A. Loegering, P. Venge, I. Olsson, J.B. Harley, A.S. Fauci and G.J. Gleich (1983) Distinctive cationic proteins of the human eosinophil granule: major basic protein, osinophilic cationic protein and eosinophil-derived neurotoxin. *Journal of Immunology*, **131,** 2982–29997.
121. R.I. Lehrer, T. Ganz, M.E. Selsted, B.M. Babior and J.T. Curnutte (1988) Neutrophils and host defense. *Annals of Internal Medicine*, **109,** 127–142.
122. G.J. Gleich, E.A. Ottesen, K.M. Leiferman and S.J. Ackerman (1989) Eosinophils and human disease. *International Archives of Allergy and Applied Immunology*, **88,** 59–62.
123. G.J. Gleich (1990) Eosinophilic granule basic proteins. *Clinical Immunology News*, **10,** 15–18.
124. J.K. Spitznagel (1990) Antibiotic proteins of human neutrophils. *Journal of Clinical Investigation*, **86,** 1381–1386.
125. B.L. Kagen, T. Ganz, and R.I. Lehrer (1994) Defensins: a family of antimicrobial and cytotoxic peptides. *Toxicology*, **87,** 131–149.
126. J.E. Gabay and R.P. Almeida (1993) Antobiotic peptide and serine protease homologs in human polymorphonuclear leukocytes, defensins and azurocidin. *Current Opinion in Immunology*, **5,** 97–102.
127. A.K. Lichtenstein, T. Ganz, M.E. Selsted and R.I. Lehrer (1986) *In vitro* tumor cells cytolysis by peptide defensins of human and rabbit granulocytes. *Blood*, **68,** 1407–1410.
128. D.M.L. Morgan, J. Clover and J.D. Pearson (1988) Effects of synthetic polycations on leucine

incorporation, lactate dehydrogenase release and morphology of human umbilical vein endothelial cells. *Journal of Cell Science*, **91,** 231–238.

129. P.E.M. Van Kent, C. Dekker, L. Mosterd, L. Van Den Bersselaar and W.B. Van Den Berg (1989) Allergic arthritis induced by cationic proteins, role of molecular weight. *Immunology*, **67,** 447–452.
130. D.G. Okrent, A.K. Lichtenstein and T. Ganz (1990) Direct cytotoxicity of polymorphonuclear leukocyte granule protein to human lung-derived cells and endothelial cells. *American Review of Respiratory Disease*, **141,** 179–185.
131. S.R. Batsford (1991) Cationic antigens as mediators of inflammation. *Acta Pathologica Microbiologica et Immunologica Scandinavica*, **99,** 1–9.
132. I. Ginsburg, M.N. Sela, A. Morag, Z. Ravid, Z. Duchan, M. Ferne, S. Rabinovitz-Bergner, P. Page-Thomas, P. Davies, J. Nichols, J. Humes and R. Bonney (1981) The role of leukocyte factors and cationic polyelectrolytes in the phagocytosis of group A streptococci and *Candida albicans* by neutrophils macrophages, fibroblasts and epithelial cells, modulation by anionic polyelectrolytes in relation to the pathogenesis of chronic inflammation. *Inflammation*, **5,** 289–312.
133. I. Ginsburg, S.E.G. Fligeil, R.G. Kunkell, B.L. Riser and J. Varani (1987) Phagocytosis of *Candida albicans* enhances malignant behavior of murine tumor cells. *Science*, **238,** 1573–1575.
134. I. Ginsburg, R. Borinski, M. Sadovnic, Y. Elkam and K. Raisford (1987) Poly-L-histidine, a potent stimulator of superoxide generation in human blood leukocytes. *Inflammation*, **11,** 253–277.
135. I. Ginsburg, R. Borinski, D. Malamud, F. Struckmayer and V. Klimetzek (1985) Chemiluminescence and superoxide generation by leukocytes stimulated by polyelectrolytes opsonized bacteria, role of polyarginine, polylysine, polyhistidine cytochalasins and inflammatory exudates as modulators of the oxygen burst. *Inflammation*, **9,** 245–271.
136. I. Ginsburg (1989) Cationic polyelectrolytes, potent opsonic agents which activate the respiratory burst in leukocytes. *Free Radical and Research Communications*, **8,** 11–26.
137. J.N. Moy, G.J. Gleich and L.L. Thomas (1990) Noncytotoxic activation of neutrophils by eosinophil granule major basic protein: effect on superoxide anion generation and lysosomal enzyme release. *Journal of Immunology*, **145,** 2626–2632.
138. R.S. Oseas, J. Allen, H.H. Yang, R.L. Baehner and L.A. Boxer (1981) Rabbit cationic protein enhances leukocyte adhesiveness. *Immunology*, **33,** 523–526.
139. J.S. Warren, P.A. Ward, K.J. Johnson and I. Ginsburg (1987) Modulation of acute immune-complex-mediated tissue injury by the presence of polyanionic substances. *American Journal of Pathology*, **128,** 67–77.
140. J.R. Geffner, A.S. Trevani, M. Schatner, E. Malchiodi, D.H. Lopez, M. Lazzari, M.A. Isturiz, M.A. (1993) Activation of human neutrophils and monocytes induced by immune complexes prepared with cationized antibodies and antigens. *Clinical Immunology and Immunopathology*, **69,** 9–15.
141. J.M. Weiler, R.R. Edens and G.J. Gleich (1992) Eosinophil granule cationic poteins regulate complement. I. Activity on the alternative pathway. *Journal of Immunology*, **149,** 643–648.
142. L. Meedham, P.G. Hallewell, T.J. Williams and J.L. Gordon (1989) Endothelial function responses and increased vascular permeability induced by polycations. *Laboratory Investigations*, **59,** 538–548.
143. L.L. Thomas, L.M. Zheutilin and G.J. Gleich (1989) Pharmacological control of human basophil histamine release stimulated by eosinophil granule major basic proteins. *Immunology*, **66,** 611–615.
144. D.N. Shapiro, J. Varani and I. Ginsburg (1989) Activation of murine cell hybridoma by cationized bacteria. *Immunology*, **67,** 478–483.
145. I. Ginsburg (1988) The biochemistry of bacteriolysis, paradoxes, facts and myths. *Microbiological Sciences*, **5,** 137–142.
146. A.K. Lichtenstein, T. Ganz, T.M. Nguyen, M.E. Selsted and R.I. Lehrer (1988) Mechanism of target cell cytolysis by peptide defensins, target cell metabolic activities, possibly involving endocytosis, are crucial for expression of cytotoxicity. *Journal of Immunology*, **140,** 2686–2696.
147. B.L. Kagan, M.E. Selsted, T. Ganz and R.I. Leprer (1990) Antimicrobial defensin peptide form voltage-dependent ion-permeable channels in planar bilayer membranes. *Proceeding of the National Academy of Sciences of the United States of America*, **87,** 210–214.
148. A. Lichtenstein (1991) Mechanism of mammalian cell lysis mediated by peptide defensins, evidence for an initial alteration of the plasma membrane. *Journal of Clinical Investigation*, **88,** 93–100.
149. R.L. Barker, R.H. Gindel, G.J. Gleich, J.L. Checkel, D.A. Loegering, L.R. Pease and K.L. Hamann (1993) Acidic polyamino acids inhibit human eosinophil granules major basic protein toxicity, evidence of a functional role for ProMBP. *Journal of Clinical Investigation*, **88,** 798–805.
150. A.K. Lichtenstein, T. Ganz, M. Selsted and R.I. Lehrer (1988) Synergistic cytolysis mediated by hydrogen peroxide combined with peptide defensins. *Cellular Immunology*, **114,** 104–116.
151. M. Yazdanbakish, P.C. Tai, C.J. Spry, G.J. Gleich and D. Roos (1987) Synergism between eosinophil cationic protein and oxygen metabolites in killing of Schistosomula of *Schistosoma mansoni*. *Journal of Immunology*, **138,** 3443–3447.

152. S.J. Klebanoff (1968) Myeloperoxidase-halide-hydrogen peroxide antibacterial system. *Journal of Bacteriology*, **95,** 2131–2138.
153. S.J. Klebanoff, A.M. Waltersdorph and H. Rosen (1984) An antimicrobial activity of myeloperoxidase. *Methods in Enzymology*, **105,** 399–403.
154. R.J. Johnson, W.G. Couser, E.Y. Chi, S. Adler and S.J. Klebanoff (1987) New mechanism for glomerular injury. Myeloperoxidase-hydrogen peroxide-halide system. *Journal of Clinical Investigation*, **79,** 1379–1387.
155. R.J. Johnson, S.J. Guggenheim, S.J. Klebanoff, R.F. Ochi, A. Wass, P. Baker, M. Schulze and W.G. Courser (1988) Morphological correlates of glomerular oxidant injury induced by the myeloperoxidase-hydrogen peroxide-halide system of the neutrophil. *Laboratory Investigations*, **5,** 294–301.
156. S.J. Weiss, J. Young, A.F. Lobuglio, A. Slivka and N.F. Nimen, (1981) Role of H_2O_2 in neutrophil-mediated destruction of cultured endothelial cells. *Journal of Clinical Investigation*, **68,** 714–721.
157. A. Ferrante, I.C. Kowanko and E.J. Bates (1992) Mechanisms of host tissue damage by cytokine-activated neutrophils. In *Immunology Series* (eds. R.G. Coffey and M. Dekker), Vol. 5, pp. 499–521.
158. J.M. Agosti, L.C. Altman, H.A. Garrison, D.A. Loegering, G.J. Gleich and S.J. Klebanoff (1987) The injurious effect of eosinophil peroxidase, hydrogen peroxide and halide on pneumocytes *in vitro*. *Journal of Allergy and Clinical Immunology*, **79,** 496–504.
159. S. Motojima, E. Frigas, D.A. Loegering and G.J. Gleich (1989) Toxicity of eosinophil cationic proteins for guinea pig tracheal eothelium *in vitro*. *American Review of Respiratory Disease* **139,** 801–806.
160. C.H. Ayars, L.C. Altman, M.N. Mcmanus, J.M. Agosti, C. Baker, D.L. Luchtel, D.A. Loegering and G.J. Gleich (1989) Injurious effect of the beosinophil peroxide-hydrogen peroxide-halide system and maJor basic protein on human nasal epithelium *in vitro*. *American Review of Respiratory Disease*, **140,** 125–131.
161. S.J. Klebanoff, J.B. Agisti, A. Jorg and A.M. Waltersdorph, (1989) Comparative toxicity of the horse eosinophil peroxidase-H_2O_2-halide system and granule basic isoproteins. *Journal of Immunology*, **143,** 239–244.
162. A. Slungaard and J.R. Mahoney (1991) Bromide-dependent toxicity of eosinophil peroxidase for endothelium and isolated working rat hearts, a model for eosinophilic endocarditis. *Journal of Experimental Medicine*, **173,** 117–126.
163. M.N. Sela, I. Ofek, M. Lahav and I. Ginsburg (1970) The effect of leukocyte hydrolases on bacteria XI. Lysis by leukocyte extracts and by myelopoeroxidase of a *Staphylococcus auerus* mutant which is deficient teichoic acid and the inhibition of bacteriolysis by lipoteichoic acid. *Proceeding of the Society for Experimental Biology and Medicine*, **159,** 125–130.
164. A. Cantin and D.E. Woods (1993) Protection by antibiotics against myeloperoxidase-dependent cytotoxicity of lung epithelial cells *in vitro*. *Journal of Clinical Investigation*, **91,** 38–45.
165. I.C. Kowanko, E.J. Bates and A. Ferrante (1989) Mechanisms of human neutrophil-mediated cartilage damage *in vitro*: role of lysosomal enzymes, hydrogen peroxide, and hypochlorous acid. *Immunology and Cell Biology*, **67,** 321–329.
166. M. Katrantzis, M.S. Baker, C.J. Handley and D.A. Lowther (1991) The oxidant hypochlotite (OCl^-) a product of the myeloperoxidase system degrades artucular cartilage proteoglycan aggregate. *Free Radicals in Biology and Medicine*, **10,** 101–109.
167. S. Yoshikawa, S.G. Kayes and J.C. Parker (1993) Eosinophils increase lung microvascular permeability via the peroxidase-hydrogen peroxide-halide system. Bronchoconstriction and vasoconstriction unaffected by eosinophil peroxidase inhibition. *American Review of Respiratory Disease*, **147,** 914–920.
168. J.M. Davies, D.A. Horwitz and K.J.A. Davies (1993) Potential role of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. *Free Radicals in Biology and Medicine*, **15,** 637–643.
169. H.P. Friedl, G.O. Till, U.S. Ryan and P.A. Ward (1989) Mediator-induced activation of xantin oxidase in endothelial cells. *FASEB Journal*, **3,** 2512–2518.
170. D.J. Kusner and C.H. King (1989) Protease modulation of neutrophil superoxide response. *Journal of Immunology*, **143,** 1696–1702.
171. S.B. Taubman and R.B. Cigen (1975) Cell-detaching activity mediated by enzyme(s) obtained from human leukocyte granules. *Laboratory Investigations*, **32,** 555–560.
172. J.M. Harlan, P.D. Killen, L.A. Harker, G.E. Striker, D. Wright (1981) Neutrophil-mediated endothelial injury *in vitro*, mechanisms of detachment. *Journal of Clinical Investigation*, **687,** 1394–1403.
173. J.M. Harlan, B.R. Schwartz, M.A. Reidy, S.M. Schwartz, H.D. Ochs and L.A. Harker (1985) Activated neutrophils disrupt endothelial monolayer integrity by an oxygen radical independent mechanism. *Laboratory Investigations*, **52,** 141–150.
174. I. Ginsburg, D.F. Gibbs and J. Varani (1989) Interaction of mammalian cells with polymorphonuclear leukocytes, reactive sensitivity to monolayer disruption and killing. *Inflammation*, **13,** 529–542.

175. P.E. Starke and J.L. Farber (1985) Endogenous defenses against the cytotoxicity of hydrogen peroxide in cultured rat hepatocytes. *Journal of Biological Chemistry*, **260,** 86–92.
176. W.F. Westlin and M.A. Gimbrone (1993) Neutrophil-mediated damage to human vascular endothelium, role of cytokine activation. *American Journal of Pathology*, **142,** 117–128.
177. T. Giraldi, C. Nisi and G. Sava (1977) Lysosomal enzyme inhibitors and anti-metastic activity in the mouse. *European Journal of Cancer*, **13,** 1321–1323.
178. J. Varani, I. Ginsburg, L. Schuger, D.F. Gibbs, J. Bromberg, K.J. Johnson, U.S. Ryan and P.A. Ward (1989) Endothelial cell killing by neutrophils. Synergistic interaction of oxygen products and proteases. *American Journal of Pathology*, **135,** 435–438.
179. D.F. Gibbs, J. Varani and K.J. Johnson (1993) The cooperative interaction of oxidants and proteinases in endothelial cell injury by rat neutrophils. *Federation of American Societies for Experimental Biology*, Abstract No. 4130.
180. A. Janoff (1985) Elastase in tissue injury. *Annual Reviews of Medicine*, **36,** 207–216.
181. B R. Baird, J.C. Cheronis, R.A. Sandhaus, E.M. Berger, C.W. White and J.E. Repine (1986) Oxygen metabolites and neutrophil elastase synergistically cause edematous injury in isolated rat lungs. *Journal of Applied Physiology*, **61,** 2224–2229.
182. J.S. Bartholomew and D.A. Lowther (1987) Receptor-mediated binding of leukocyte elastase by chondrocytes. *Arthritis and Rheumatism*, **30,** 431–438.
183. S.E. McGowan and J.J. Murry (1987) Direct effect of neutrophil oxidants on elastase-induced extracellular matrix proteolysis. *American Review of Respiratory Disease*, **135,** 1286–1293.
184. T.C. Rodell, J.C. Cheronis and J.E. Repine (1988) Endothelial cell xantine oxidase-derived toxic oxygen metabolites contribute to acute lung injury from neutrophil elastase. *Chest*, **93,** 146s.
185. R.J. Mcdonald, L.U. Bruckka, and J.E. Repine (1989) Neutrophil elastase augments acute edematous injury in isolated rat lungs perfused with neutrophil cytoplasts. *American Review of Respiratory Disease*, **140,** 1825–1827.
186. S.J. Klebanoff, M.G. Kinsella and T.N. Wight (1993) Degradation of endothelial matrix heparan sulfate proteoglycan by elastase and the myeloperoxidase-H_2O_2-chloride system. *American Journal of Pathology*, **193,** 907–917.
187. M.S. Palmgren, R.D. deShazo, R.M. Carter, M.L. Zimmy, S.V. Shah (1992) Mechanisms of neutrophil damage to human alveolar extracellular matrix, the role of serine metalloproteases. *Allergy and Clinical Immunology*, **89,** 905–915.
188. S. Pontremoli, E. Melloni, M. Michetti, O. Sacco, B. Sparatore, F. Salamino and G. Damiani (1986) Cytolystic effects of neutrophils, role for a membrane-bound neutrophil proteinase. *Proceeding of the National Academy of Sciences of the United States of America*, **83,** 1685–1689.
189. D.G. Adams, W.J. Johnson, E. Fiorito and C.F. Nathan (1981) Hydrogen peroxide and cytolitic factor can interact synergistically in effecting cytolysis of neophastic targets. *Journal of Immunology*, **127,** 1973–1977.
190. H. Hurkhardt, M. Schwingel, H. Menninger, H.W. McCartney and H. Tschsche (1986) Oxygen radicals as effectors of cartilage destruction. Direct degradative effects of matrix components and direct action via activation of latent collagenase from polymorphonuclear leukocytes. *Arthritis and Rheumatism*, **29,** 379–387.
191. V.B. Anthony, C. Owen and D. English, D. (1988) Polymorphonuclear leukocyte cytoplasts mediate acute lung injury. *Journal of Applied Physiology*, **65,** 706–713.
191a. H. Abe, K. Okajima, H. Okabe, K. Takatsuki and B.B. Binder (1994) Granulocyte proteases and hydrogen peroxide synergistically inactivate thrombomodulin of endothelial cells in vitro. *Journal of Laboratory Clinical Medicine*, **123,** 874–881.
192. G.J. Peppin and S.J., Weiss (1986) Activation of endogenous metalloprotease, gelatinase by triggered human neutrophils. *Proceeding of the National Academy of Sciences of the United States of America*, **83,** 4322–4326.
193. S.J. Weiss, G. Peppin, X. Oritz, C. Ragsdale and S.T. Test (1986) Oxidative autoactivation of latent collagenase by human neutrophils. *Science*, **227,** 747–749.
193a. M. Michaelis, M.C. Visser and C.C. Winterbourn (1992) Different effects of hypochlorous acid on human neutrophil metalloproteases: Activation of collagenase and inactivation of collagenase and gelatinase. *Archives of Biochemistry and biophysics*, **292,** 555–562.
193b. C. Capodici, G. Muthukumaran, M.A. Amoruso and R.A. Berg. (1992) Activation of neutrophil collagenase by cathepsin G. *Inflammation* **13,** 245–258.
194. S.V. Shah, W.H. Baricos and A. Basci (1987) Degradation of human glomerular basement membrane by stimulated neutrophils, activation of metalloproteinase(s) by reactive oxygen metabolites. *Journal of Clinical Investigation*, **79,** 25–31.
195. S.J. Weiss and G. Peppin (1986) Collagenolytic metalloenzymes of the human neutrophil, characteristics, regulation and potential function *in vivo*. *Biochemical Pharmacology*, **35,** 189–197.

196. Z. Wert, C.L. Mainardi, C.A. Vater and E.D. Harris (1977) Endogenous activation of latent collagenase by rheumatoid synovial cells-evidence for the role of plasminogen activator. *New England Journal of Medicine*, **269**. 1017–1023.
197. E.A. Dennis, S.G. Rhee, M.M. Billah and Y.A. Hannun (1991) Role of phospholipases in generating second messengers in signal transduction. *FASEB Journal*, **5**, 2068–2077.
198. P. Vadas and W. Pruzanski (1986) Role of secretory phospholipase A_2 in the pathobiology of disease. *Laboratory Investigation*, **55**, 391–404.
199. W. Prizanski and P. Vadas (1990) Soluble phospholipase A_2 in human pathology, clinical laboratory interface. In *Biochemistry, molecular biology and physiology of phospholipase A_2*, and its regulatory factors (ed. A.B. Mukhergee), Plenum Press, New York, pp. 239–251.
200. W. Prizanski and P. Vadas (1991) Phospholipase A_2-mediator between proximal and distal effectors in inflammation. *Immunology Today*, **12**, 143–146.
201. J.S. Bomalski, P. Lawton and J.L. Browing (1991) Human extracellular recombinant phospholipase A_2 induces an inflammatory response in rabbit joints. *Journal of Immunology*, **146**, 3904–3910.
202. B.J. Mormann, C.K. Huang, W.M. Mackin and E.L. Becker (1984) Receptor-mediated activation of phospholipase A_2 in rabbit neutrophil plasma membrane. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 767–770.
203. S.P. Kennedy and E.L. Becker (1987) Ectophospholipase A_2 activity of the rabbit peritoneal neutrophil. *International Archive of Allergy and Applied Immunology*, **83**, 238–246.
204. M. Victor, J. Weiss, M.S. Klempner and P. Elsbach (1981) Phospholipase A_2 activity in the plasma membrane of human polymorphonuclear leukocytes. *Federation of European Biochemical Societies*, **136**, 298–300.
205. L.M. Henderson, J.B. Chappell and O.T.G. Jones (1989) Superoxide generation is inhibited by phospholipase A_2 inhibitors. Role of phospholipase A_2 in the activation of NADPH oxidase. *Biochemical Journal*, **264**, 249–255.
206. F. Watson, J.J. Robinson and S.W. Edwards (1992) Sequential phospholipase activation in the stimulation of the neutrophil NADPH oxidase. *FEMS Microbiology Immunology*, **105**, 239–248.
207. J.R. Forehand, R.B. Johnston and J.S. Bomalski (1993) Phospholipase A_2 activity in human neutrophils. Stimulation by lipopolysaccharide and possible involvement in priming for an enhanced respiratory burst. *Journal of Immunology*, **151**, 4918–4925.
208. W. Engelberger, D. Bitter-Suermann and U. Hadding (1987) Influence of lysophospholipids and PAF on the oxidative burst. *International Journal of Immunopharmacology*, **9**, 275–282.
209. I. Ginsburg, P.A. Ward and J. Varani (1989) Lysophosphatidies enhance superoxide responses in stimulated human neutrophils. *Inflammation*, **13**, 163–174, (1989).
210. K.S. Kilgore and B.R. Lucchesi (1993) Reperfusion injury after myocardial infarction: The role of free radicals and the inflammatory response. *Clinical Biochemistry*, **26**, 359–370.
211. H.M. Piper (ed.) (1990) *Patophysiology of severe ischemic myocardial injury*. Kluwer Academic Publisher, Dodrecht/Boston/London.
212. I. Ginsburg, R. Kohen and M. Ligumsky (1994) Ethanol synergizes with hydrogen peroxide, peroxyl radical and trypsin to kill epithelial cells in culture. *Free Radicals in Biology and Medicine*, **16**, 263–269.
213. S.J. Klebanoff (1980) Oxygen metabolism and the toxic properties of phagocytes. *Annals of Internal Medicine*, **93**, 480–489.
214. R. Dean and J.L. Virelizier (1983) Interferon as macrophage activating factors. I. Enhancement of cytotoxic activity in the absence of other soluble signals. *Clinical and Experimental Immunology*, **51**, 5011–511.
215. M.R. Shalaby, B.B. Aggrawal, R. Rinderknecht, L.P. Svedersky, B.S. Finkie and M.A. Palladino Jr. (1985) Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumor necrosis factor. *Journal of Immunology*, **135**, 2069–2073.
216. M. Freund and E. Pick (1985) The mechanism of action of lymphokines. VIII. Lymphokine-enhanced spontaneous hydrogen peroxide production by macrophages. *Immunology*, **54**, 35–45.
217. E.J. Wing, N.M. Ampel, A. Waheed and R.K. Shadduck (1985) Macrophage colony stimulating factor (M-CSF) enhances the capacity of murine macrophages to secrete oxygen products. *Journal of Immunology*, **135**, 2052–2056.
218. M. Tsujimoto, S. Yokota, J. Viclek and G. Weissman (1986) Tumor necrosis factor provokes superoxide anion generation from neutrophils. *Biochemical and Biophysical Research Communications*, **137**, 1094–1099.
219. S.J. Klebanoff, M.A. Vadas, J.N. Harlan, L.H. Sparks, G.R. Gamble, J.M. Agosti and A.M. Watersdorph (1986) Stimulation of neutrophils by tumor necrosis factor. *Journal of Immunology*, **136**, 4220–4225.
220. I.S. Figari, N.A. Mori, M.A. Palladino Jr. (1987) Regulation of neutrophil migration and superoxide

production by recombinant tumor necrosis factor α and β: Comparison to recombinant interferon-γ and interleukin-1a. *Blood*, **70,** 979–984.

221. A. Ferrante, M. Nandoskar, A. Walz, D.H.B. Goh and I.C. Kowanko (1988) Effects of tumor factor alpha and interleukin-1 alpha and beta on neutrophil migration, respiratory burst and degranulation. *International Archives of Allergy and Applied Immunology*, **86,** 82–91.
222. J. Varani, M.J. Bendelew, D.E. Sealy, S.L. Kunkel, D.E. Gannon, U.S. Ryan and P.A. Ward (1988) Tumor necrosis factor enhances susceptibility of vascular endothelial cells to neutrophil-mediated killing. *Laboratory Investigations*, **59,** 292–295.
223. J.S. Warren, S.L. Kunkel, T.W. Cunningham, K.L. Johnson and P.A. Ward (1988) Macrophage derived cytokines amplify immune complex-triggered O_2 responses by rat alveolar macrophages. *American Journal Pathology*, **130,** 489–495.
224. A. Ferrante (1989) Tumor necrosis factor alpha potentiates neutrophil antimicrobial activity: increased fungicidal activity against *Torulopsis glabarata*/*Candida albicans* and associated increases in oxygen radical production and lysosomal enzyme release. *Infection Immunity*, **57,** 2115–2122.
225. S.S. Kaplan, R.E. Basford, E.J. Wing and R.K. Shadduck (1989) The effect of recombinant human granulocyte macrophage cology-stimulating factor on neutrophil activation in patients with refractory carcinoma. *Blood*, **73,** 636–638.
226. A. Kharazmi, H. Nielsen, C. Rechitzer and K. Bendzen (1989) Interleukin 6 primes human neutrophils and monocyte oxidative burst response. *Immunology Letters*, **21,** 177–184.
227. J.B. Klein, J.A. Scherzer and K.R. McLeish (1991) Interferon-gamma enhances superoxide production in HL-60 cells stimulated with multiple agonists. *Journal of Interferon Research*, **11,** 69–74.
228. E. Kownatzki, M. Neumann and S. Unrich (1989) Stimulation of human neutrophils granulocytes by monocyte-derived cytokines. *Agents and actions*, **26,** 180–182.
229. J.L. Mege, J.G. Cambronero, T.F.P. Molski, E.L. Becker and R.I. Sha'afi (1989) Effect of granulocyte-macrophage colony-stimulating factor on superoxide production in cytoplasts and intact human neutrophils, role of protein kinase and G-proteins. *Journal of Leukocyte Biology*, **46,** 161–168.
230. C.F. Nathan (1989) Respiratory burst in adherent human neutrophils, triggering by colony-stimulating factor GSF-GM and GSF-G. *Blood*, **73,** 301–306.
231. R. Sullivan, J.P. Predette, M. Socinski, A. Elias and K. Antman (1989) Enhancement of superoxide anion release by granulocytes harvested from patients receiving granulocyte-macrophage colony-stimulating factor. *British Journal of Hematology*, **71,** 475–479.
232. L. Borish, R. Rosenbaum, L. Albury and S. Clark (1989) Activation of neutrophils by recombinant interleukin 6. *Cellular Immunology*, **121,** 281–289.
233. A. Melingaard, G.M. Vercelloti, G. Walker, R.D. Nelson and H.S. Jacob (1990) Tumor necrosis factor cachectin stimulates eosinophil oxidant production and toxicity towards human endothelium. *Journal of Experimental Medicine*, **171,** 2025–2041.
234. L.M. Kumaratilake, A. Ferrante, E.J. Bates, I.C. Kowenko (1990) Augmentation of the human monocyte/macrophage chemiluminescence response during short-term exposure to interferon-gamma and tumor necrosis factor. *Clinical and Experimental Immunology*, **80,** 257–262.
235. M.A. West (1990) Role of cytokines in leukocyte activation, phagocytic cells. *Current Topics in Membranes and Transport*, **35,** 537–570.
236. J.A. Badwey, J. Bing, P.G. Heyworth and J.M. Robinson (1991) Products of inflammatory cells synergistically enhance superoxide production by phagocytic cells. In *Cell-cell interaction in the release of inflammatory mediators* (eds. P.Y.K. Wong and C.N. Serhan), Plenum Press, New York, pp. 19–33.
237. K.J. Balazowich, H.I. Almeida and L.A. Boxer (1991) Recombinant human GM-CSF and GM-CSF prime human neutrophils for superoxide production through different signal transduction mechanisms. *Journal of Laboratory and Clinical Medicine*, **118,** 576–584.
238. A. Kapp, A. Komann and E. Schopf (1991) Effect of tumor necrosis factor alpha *in vivo* on human granulocyte oxidative metabolism. *Archives for Dermatological Research*, **283,** 362–365.
239. J.B. Klein, J.A. Scherzer and K.R. McLeish (1991) Interferon-γ enhances superoxide production by HL-60 cells stimulated by multiple agonists. *Journal of Interferon Research*, **11,** 69–74.
240. S.D. Tannenberg (1991) The effect of inflammatory mediators on neutrophil functions. In *New aspects of human polymorphonuclear leukocytes* (eds. W.H. Horol and P.J. Schollmeyer), Plenum Press, New York, pp. 75–92.
241. A. Ferrante (1992) Activation of neutrophils by interleukins-1 and 2 and tumor necrosis factors. In *Granulocyte responses to cytokines, basic and clinical research* (ed. R.G. Coffey), Marcel Dekker, New York, pp. 417–436.
242. A. Yuo, S. Kitagawa, K. Motoyoshi, E. Azuma, M. Saito and F. Takaku (1992) Rapid priming of human monocytes by human hematopoietic growth factor: granulocyte-macrophage colony-stimulating factor (CSF), macrophage-CSF, and interleukin-3 selectively enhance superoxide release triggered by receptor-mediated agonists. *Blood*, **79,** 1553–1557.

243. A. Khwaja, J.E. Carver and D.C. Linch (1992) Interaction of granulocyte-macrophage colony-stimulating factor (CSF), granulocyte CSF, and tumor necrosis factor in priming of the neutrophil respiratory burst. *Blood*, **79,** 745–753.
244. J.G. Williams, G.J. Jutkovich, G.B. Hahnel amd R.W. Mailer (1992) Macrophage priming by interferon gamma: a selective process with potential harmful effects. *Journal Leukocyte Biology*, **52,** 579–584.
245. C. Baglioni (1992) Mechanisms of cytotoxicity, cytolysis and growth stimulation by TNF. In *Tumor necrosis factor: the molecules and their emerging role in medicine* (ed. B. Beutler), Raven Press, New York, pp. 425–438.
246. J. Gomez-Cambronero and R.I. Sha'afi (1992) Granulocyte-macrophage colony-stimulating factor and neutrophil, mechanisms of action. In *Cell-cell interaction in the release of inflammatory mediators* (eds. P.Y.K. Wong and C.N. Serhan), Plenum Press, New York, pp. 35–71.
247. S.D. Tannenberg, D.E. Fey and M.J. Lieser (1993) Oxidative priming of neutrophils by interferon-γ. *Journal of Leukocyte Biology*, **53,** 301–308.
248. J.D. Ogle, J.G. Noel, R.M. Sramkoski, C.K. Ogle and J.W. Alexander (1992) Effects of combination of tumor necrosis factor alpha and chemotactic peptide f-MET-LEU-PHE on phagocytosis of opsonized microspheres by human neutrophils. *Inflammation*, **16,** 57–68.
249. Y. Hatsura, S. Tsuru, M. Noritake, H. Kayashima, N. Shimomiya, M. Shinomiya and M. Rokutanda (1993) Granulocyte colony-stimulating factor enhances the extracellular emission of reactive oxygen from neutrophils stimulated with formylmethionyl eucylphenylalanine. *Cellular Immunology*, **148,** 10–17.
250. A. Wozniak, W.H. Betts, G.A. Murohy and M. Rocicinski (1993) Interleukin-8 prime human neutrophils for enhanced superoxide anion production. *Immunology*, **79,** 608–615.
251. S.W. Edward, J.E. Say and V. Hughes (1988) Gamma interferon enhances the killing of *Staphylococcus aureus* by human neutrophils. *Journal of General Microbiology*, **134,** 37–42.
252. I.C. Kowanko, A. Ferrante, D.P. Harvey and K.L. Carman (1991) Granulocyte-macrophage colony-stimulating factor augments neutrophil killing of *Torulopsis glabrata* and stimulates neutrophil respiratory burst and degranulation. *Clinical and Experimental Immunology*, **83,** 225–230.
253. E. Roilides, K. Uhlig, D. Venzon, P.A. Pizzo and T.J. Walsh (1993) Enhancement of oxidative response and damage caused by human neutrophils to *Asppergillus fumigatus hyphae* by granulocyte colony-stimulating factor and gamma interferon. *Infection Immunity*, **61,** 1185–1193.
254. B.H. Kushner and N.K.V. Cheung (1989) GM-CSF enhances 3F8 monoclonal antibody-dependent cellular cytotoxicity against human melanoma and neuroblastoma. *Blood*, **73,** 1936–1941.
255. L. Baud, H. Affres, J. Perez and R. Ardiallou (1990) Reduction in tumor necrosis factor and cytotoxicity by hydrogen peroxide. *Journal of Immunology*, **166,** 556–560.
256. S.L. Abramson and J.I. Gallin (1991) Il-4 inhibits superoxide production by human mononuclear phagocytes. *Journal of Immunology*, **244,** 625–630.
257. R.H. Simon, P.D. DeHart and D.M. Nadeau (1989) Resistance of rat pulmonary alveolar endothelial cells to neutrophil- and oxidant-induced injury. *American Journal of Respiratory Cellular and Molecular Biology*, **1,** 221–229.
258. F. Dallegri, A. Ballestrero, L. Ottonelloand and F. Patrone (1989) Platelets as inhibitory cells in neutrophil-mediated cytolysis. *Journal of Laboratory and Clinical Medicine*, **114,** 502–509.
259. E.M. Berger, C.J. Beehler, R.N. Harada and J.E. Repine (1990) Human phagocytic cells as oxygen metabolite scavengers. *Inflammation*, **14,** 613–619.
260. M.D. Scott, B.H. Lubin, L.X. Zuo and F.A. Kuypres (1991) Erythrocyte defense against hydrogen peroxide: Preeminent importance of catalase. *Journal of Laboratory and Clinical Medicine*, **188,** 7–16.
261. M. Chevion (1988) A site-specific mechanism for free radical induced biological damage: the essential role of redox-active transition metals. *Free Radical Biology and Medicine*, **5,** 27–37.
262. J.A. Scott, A.J. Fischman, C.J. Homcy, J.T. Fallon, B.A. Khaw, C.A. Peto and C.A. Rabito (1990) Morphologic and functional correlates of plasma membrane injury during oxidant exposure. *Free Radicals in Biology and Medicine*, **6,** 361–367.
263. C. Nathan, S.S. Silverstein, L.H. Brukner and Z.A. Cohn (1979) Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. *Journal of Experimental Medicine*, **149,** 100–113.
264. J. Martin (1984) Neutrophils kill pulmonary endothelial cells of a hydrogen peroxide-dependent pathway. *American Review of Respiratory Disease*, **130,** 209–213.
265. E.L. Becker (1988) The cytotoxic action of neutrophils on mammalian cells *in vitro*. *Progress in Allergy*, **40,** 183–208.
266. J. Varani, S.E.G. Fligiel, G.O. Till, R.G. Kunkel, W.S. Ryan and P.A. Ward (1985) Pulmonary endothelial cell killing by human neutrophils. Possible involvement of hydroxyl radical. *Laboratory Investigations*, **53,** 656–663.

267. D.E. Gannon, J. Varani, S.H. Phan, J.H. Ward, J. Kaplan, G.O. Till, R.H. Simon and P.A. Ward (1987) Source of iron in neutrophil-mediated killing of endothelial cells. *Laboratory Investigations*, **57**, 34–44.
268. A. Puppo and B. Halliwell (1988) Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. *Biochemical Journal*, **249**, 185–190.
269. L.E. Gerschenson and R.J. Rottelo (1992) Apoptosis, a different type of cell death. *FASEB Journal*, **6**, 2450–2455.
270. T.M. Buttke and P.A. Sandstrom (1994) Oxidative stress as a mediator of apoptosis. *Immunology Today*, **15**, 7–10.
271. M.K. Cathcart, A.K. McNally, D.W. Morel and G.M. Chislom (1989) Superoxide anion participation in human monocyte-mediated oxidation of low density lipoprotein in conversion of low- density lipoproteins to cytotoxins. *Journal of Immunology*, **142**, 1963–1969.
272. J.M. McCord and I. Fridovich (1988) Superoxide dismutase, the first twenty years (1969–1988). *Free Radicals in Biology and Medicine*, **5**, 363–369.
273. L. Flore (1988) Superoxide dismutase for therapeutic use: clinical experience, dead ends and hopes. *Molecular and Cellular Biochemistry*, **84**, 123–131.
274. R.A. Greenwald (1990) Superoxide dismutase and catalase as therapeutic agents for human disease. A critical review. *Free Radicals in Biology and Medicine*, **8**, 201–209.
275. A.R. Gross (1990) Inhibitors of leukocyte superoxide generating oxidase: mechanisms of action and methods for their evaluation. *Free Radicals in Biology and Medicine*, **8**, 71–93.
276. B. Halliwell, J.M.C. Gutteridge and C.E. Cross (1992) Free radicals, antioxidants, and human disease: Where are we now? *Journal of Laboratory and Clinical Medicine*, **119**, 598–620.
277. C.A. Rice-Evans and A.T. Diplock (1993) Current status of antioxidant therapy. *Free Radical in Biology and Medicine*, **15**, 77–96.
278. R.C. Rose and A.M. Bode (1993) Biology of free radical scavengers: an evaluation of ascorbate. *FASEB Journal*, **7**, 1135–1142.
279. E.D. Harris (1992) Regulation of antioxidant enzymes. *FASEB Journal*, **6**, 2675–2683.
280. J. Chwalwijk, W.B. Van Der Berg, L. Van Der Pute, L.A.B. Joosten and L. Van Bersselaar (1985) Cationization of catalase, peroxidase and superoxide dismutase. Effect of improved intraarticular retention on experimental arthritis in mice. *Journal of Clinical Investigation*, **76**, 198–205.
281. D.F. Gibbs, J. Varani and I. Ginsburg (1989) Formation and use of poly-L-histidine catalase complexes: protection from hydrogen peroxide-mediated injury. *Inflammation*, **13**, 465–474.
282. R. Kohen, A. Kakunda and A. Rubinstein (1992) The role of cationized catalase and cationized glucose oxidase in mucosal oxidative damage induced in the rat Jejunum. *Journal of Biological Chemistry*, **267**, 21349–21354.
283. A. Rubinstein, A. Kakunda and R. Kohen (1993) Protection of the rat Jejunal mucosa against oxidative injury by cationized superoxide dismutase. *Journal of Pharmaceutical Sciences*, **82**, 1285–1287.
284. A.K. Nussier and T.R. Billar (1993) Inflammation, immunoregulation, and inducible nitric oxide sythase. *Journal of Leukocyte Biology*, **54**, 171–178.
285. P.L. Feldman, O.W. Griffith and D.J. Stuehr (1993) The surprising life of nitric oxide. *Chemical and Engineering News*, **20**, 26–38.
286. C. Cazevieille, A. Muller, F. Meynier and C. Bonne (1993) Superoxide and nitric oxide cooperation in hypoxia/reoxygenation-induced neuron injury. *Free Radicals in Biology and Medicine*, **14**, 389–395.
287. J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall and B.A. Freeman (1990) Apparent hydroxyl radical production by peroxinitrite: implications for endothelial injury from nitric oxide and superoxide. *Proceeding of the National Academy of Sciences of the United States of America*, **87**, 1620–1624.
288. G.M. Rubanyi, E.H. Ho, E.H. Cantor, W.C. Lumma and B.L.M. Parker (1991) Cytoprotective function of nitric oxide: inactivation of superoxide radical induced by human leukocytes. *Biochemical and Biophysical Research Communications*, **181**, 1392–1397.
289. I. Ginsburg (in press, 1994) Can hemolytic streptococci be considered "forefathers" of modern phagocytes? Both cell types freely migrate in tissues and kill targets by a "cross-talk" among their secreted agonists. *Comparative Biochemistry and Physiology*.

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