18. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961; 4:561-71.
19. Lipman RS, Covi L, Shapiro AK. The Hopkins Symptom Checklist (HSCL): factors derived from the HSCL-90. J Affective Disord 1979; 1:9-24.
20. McLellan AT, Luborsky L, Griffith F, et al. New data from the Addiction Severity Index: reliability and validity in three centers. J Nerv Ment Dis 1985; 173:412-23.
21. McLellan AT, Luborsky L, Cacciola J, Griffith J, McGahan P, O'Brien CP. Guide to the Addiction Severity Index: background, administration, and field testing results. Rockville, Md.: National Institute on Drug Abuse, 1985. (DHHS publication no. (ADM) 85-1419.)
22. Alterman AI, Hayashida M, O'Brien CP. Treatment response and safety of ambulatory medical detoxification. J Stud Alcohol 1988; 49:160-6.
23. Rosen HS. Public finance. 2nd ed. Homewood, Ill.: Richard D. Irwin, 1988:247.
24. Gordis E, Sereny G. Controversy in approaches to alcoholism. In: Rosenoer VM, Rothschild MA, eds. Controversies in clinical care. New York: SP Medical and Scientific Books, 1981:37-55.
25. Armor DJ, Polich JM, Stambul H. Alcoholism and treatment. Santa Monica, Calif.: Rand, 1976:21.
26. Fuller RK, Lee KK, Gordis E. Validity of self-report in alcoholism research: results of a Veterans Administration Cooperative Study. Alcoholism (NY). 1988; 12:201-5.

MECHANISMS OF DISEASE

FRANKLIN H. EPSTEIN, M.D., *Editor*

TISSUE DESTRUCTION BY NEUTROPHILS

STEPHEN J. WEISS, M.D.

WITH increasing frequency, the human neutrophil is being implicated as a mediator of tissue-destructive events in inflammatory diseases ranging from rheumatoid arthritis and myocardial reperfusion injury to respiratory distress syndromes, blistering skin disorders, and ulcerative colitis.[1,2] In each of these diseases, as well as a variety of other acute inflammatory disorders, important components of these pathologic processes are being linked to the neutrophil's ability to release a complex assortment of agents that can destroy normal cells and dissolve connective tissues. Although these toxins normally defend the host against invading microbes, the neutrophil has little intrinsic ability to differentiate between foreign and host antigens and relies on other arms of the immune system (e.g., antibodies, complement, and cytokines) to select its targets. If normal host tissues are inappropriately identified as foreign or damaged structures, the appropriate receptors on the plasma membrane of the neutrophil will be engaged, eliciting the cell's destructive potential.

Predictably, as awareness of the neutrophil's ability to mediate acute inflammatory tissue damage has increased, so has the desire to develop therapeutic interventions that may attenuate the cell's unwanted destructive activities. However, this has proved to be an elusive goal, since the number of agents listed in the neutrophil's armamentarium continues to increase beyond the approximately 50 toxins that have already been identified.[1-3] According to popular convention, these are divided into two groups, which for the most part correspond to their localization to either of two sites in the cell: the plasma membrane or the intracellular granules. The plasma membrane of the triggered neutrophil is the site of an unusual enzyme, termed the NADPH oxidase, that underlies the cell's ability to generate a family of reactive oxidizing chemicals unique to mammalian biologic processes, whereas the granules contain microbicidal peptides, proteins, and enzymes. When the neutrophil is specifically triggered by any one of a number of proinflammatory signals, the oxidase begins to generate and release oxygen metabolites. Almost simultaneously, the granules fuse with the plasma membrane and discharge their contents both into the extracellular medium and into the phagocytic vacuole (i.e., the portion of plasma membrane that encircles a target small enough to be ingested).[1-3] Thus, in pathologic states, normal tissues rather than microbes bear the brunt of an attack heralded by the release of a complex mix of NADPH oxidase–derived oxygen metabolites and granule-based toxins that has been formulated over the course of millions of years of evolution.

In a review of the recent literature on the mechanisms of tissue damage mediated by the neutrophil, the distinct impression arises that oxygen metabolites are being consistently identified as the most destructive toxins released from the cell.[1-5] Nonetheless, dissenters have voiced concern that the case for neutrophil-derived oxidants may be less clear than has been suggested and that the role of proteolytic enzymes should be reevaluated. Although there is evidence to support either position, I believe that neither oxidants nor proteolytic enzymes alone completely explain the neutrophil's ability to damage tissues in vivo. Instead, I will review a series of findings from both the recent literature and studies predating the advent of modern biology (along with its technology) that appear to reinforce a single underlying theme of neutrophil function — i.e., that the neutrophil is specifically constructed to use both the NADPH oxidase system and the granule constituents in a cooperative and concerted manner, and it is an intermixing of these two components that allows the neutrophil to realize its ultimate destructive potential.

THE NADPH OXIDASE SYSTEM AND OXYGEN METABOLITES

The NADPH oxidase system is a membrane-associated enzyme complex that is widely perceived to participate directly in the generation of at least three oxy-

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gen metabolites: superoxide anion ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH\cdot$).[1,3] While the oxidase lies dormant in unstimulated neutrophils, triggered cells rapidly activate the enzyme system and begin to shuttle electrons from cytosolic NADPH to oxygen dissolved in the extracellular fluid. Under most conditions, one molecule of oxygen acts as an acceptor for a single, donated electron, resulting in the generation of one molecule of the superoxide anion (the dot in $O_2\cdot^-$ denotes the presence of an unpaired electron, which makes the product a free radical, whereas the additional negative charge donated by the electron gives the oxygen molecule a net charge of -1).

$$2O_2 + NADPH \xrightarrow[\text{oxidase}]{NADPH} 2O_2\cdot^- + NADP^+ + H^+$$

In turn, two molecules of $O_2\cdot^-$ interact spontaneously (dismutation reaction) to generate one molecule of H_2O_2.

$$2O_2\cdot^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Both $O_2\cdot^-$ and H_2O_2 can react with a number of important biologic substrates,[6,7] but intact neutrophils appear to be somewhat limited in their ability to use either metabolite alone to cause extracellular damage. First, the rate at which two molecules of superoxide interact at physiologic pH to form H_2O_2 is rapid. Thus, under most conditions the preferred substrate for one $O_2\cdot^-$ molecule is a second $O_2\cdot^-$ molecule, and few other substances can compete with the already fast spontaneous dismutation reaction.[6,7] Second, although H_2O_2 is a stable oxidant that can exert a number of damaging effects,[8] neutrophils themselves consume the bulk of this metabolite and only a small portion of the generated H_2O_2 can actually be detected in the extracellular pool.[9] As one might suspect, there are exceptions to these rules, but few studies have successfully demonstrated that human neutrophils use either $O_2\cdot^-$ or H_2O_2 alone to produce a toxic effect.[6,10,11]

The limitations placed on the ability of the neutrophil to use $O_2\cdot^-$ or H_2O_2 directly to mediate extracellular effects have fueled interest in the possibility that the cell uses these metabolites to generate a more powerful oxidant, $OH\cdot$ (hydroxyl radical).[1-6] In a number of cell-free model systems, it has been demonstrated that the $OH\cdot$ can be generated if $O_2\cdot^-$, H_2O_2, and a suitable transition metal catalyst (usually iron-based) are mixed. Given that neutrophils are able to act as a source of $O_2\cdot^-$ and H_2O_2, that a number of physiologic iron-containing molecules have been suggested as potential catalysts, and that the $OH\cdot$ is an extremely reactive and destructive oxidant, one can easily understand why this reaction scheme has received a great deal of attention. However, it is important to note that although it is often assumed that neutrophils can generate the $OH\cdot$ in the presence of a physiologic source of iron, they have never been shown to do so.[12] On the contrary, even if the neutrophil is triggered in the presence of high concentrations of an artificial iron chelate, the cell seems intent on preventing $OH\cdot$ generation. Apparently, this is accomplished by routing the generated H_2O_2 into alternate metabolic pathways at rates that limit the pool size available for the production of $OH\cdot$, and by releasing an iron-binding protein, lactoferrin, that sequesters available iron in a form that fails to catalyze $OH\cdot$ formation.[12] At present, I believe that only $O_2\cdot^-$ and H_2O_2 can be considered well-characterized oxygen metabolites generated by the NADPH oxidase system in the intact neutrophil.

The Bleaching Activity of the Human Neutrophil: A Cooperative Interaction between NADPH Oxidase and a Green Granule Enzyme

Because the bulk of the $O_2\cdot^-$ generated by the triggered neutrophil dismutates to H_2O_2 and this molecule in turn is rapidly catabolized, the identity of the intended end product of oxidative metabolism seems to depend on the fate of the consumed H_2O_2. The answer cannot be drawn from an analysis of the NADPH oxidase system alone, but can be obtained by a shift in focus to a second enzyme that is localized to the neutrophil granules. Originally purified by Agner in 1941 from purulent fluids of tubercular empyema, the enzyme was named verdoperoxidase to reflect both its green color and its ability to catalyze peroxidative reactions.[13] Indeed, it is verdoperoxidase (now known as myeloperoxidase) that gives pus and other purulent fluids their characteristic greenish hue. In view of this fact, it is not surprising that neutrophils contain large amounts of myeloperoxidase (up to 5 percent of the dry weight of the cell) and that triggered cells can release substantial amounts of the enzyme into extracellular fluids.[13]

Once discharged from the neutrophil, myeloperoxidase alone exerts little if any effect. However, in combination with H_2O_2, purified myeloperoxidase can oxidize the halides Cl^-, Br^-, or I^- (as well as the pseudohalide, thiocyanate [SCN^-]) to their corresponding hypohalous acids (HOX).[13]

$$H_2O_2 + X^- + H^+ \xrightarrow{\text{myeloperoxidase}} HOX + H_2O$$
$$(X^- = Cl^-, Br^-, I^-, SCN^-)$$

Because the plasma concentration of Cl^- is more than a thousand times that of the other halides, the H_2O_2–myeloperoxidase system probably uses Cl^- at most sites in vivo. (An interesting exception may be saliva, in which the ratio of Cl^- to SCN^- is much lower.[13]) The unusual ability of purified myeloperoxidase to oxidize Cl^- to HOCl (hypochlorous acid) was first proposed by Agner in 1958 but was not directly confirmed in a cell-free system until 1976.[13] Nonetheless, there was little interest in HOCl itself as an important product generated by the neutrophil, both because the biologic reactivity of this oxidant was not widely appreciated and because it was unclear wheth-

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er chlorinated oxidants actually constituted a major metabolic end product of the cell. Thus, few experiments were specifically designed to assess the ability of intact neutrophils to generate HOCl.[13] However, in the early 1980s, interest in HOCl as a direct-acting cytotoxin was rekindled, and a series of techniques were developed for determining the chlorinating potential of intact neutrophils.[14-16] For the first time, these studies revealed that human neutrophils could be triggered under a variety of experimental conditions to generate HOCl as the major product of oxidative metabolism. Quantitative analyses demonstrated that 10^6 maximally triggered neutrophils produced approximately 2×10^{-7} mol of HOCl during a two-hour incubation.[17] From a stoichiometric standpoint, the amount of HOCl detected accounted for almost all the H_2O_2 generated by the cell. Given the fact that HOCl is an extremely powerful oxidant that rapidly attacks a wide range of biologically relevant molecules (potential targets include amines, amino acids, thiols, thioethers, nucleotides, hemoproteins, and polyenoic acids),[17] the quantities of oxidant generated by the neutrophil are impressive. Indeed, at neutral pH, the 2×10^{-7} mol of HOCl generated by 10^6 neutrophils is enough to destroy as many as 150 million *Escherichia coli* organisms in milliseconds.[13,17] Even today, the concept that neutrophils purposefully generate such large quantities of so reactive an oxidant has an almost surrealistic quality — all the more so when one considers that HOCl is the sole active ingredient in household liquid bleach.

Because of the high reactivity of HOCl, it does not actually accumulate in biologic systems but instead almost instantaneously disappears in multiple reactions with available substrates.[17] Early interest focused on the possibility that HOCl might be consumed in reactions that yield additional, new oxidants. For example, reagent HOCl can react with H_2O_2 to form a reactive, electronically excited molecule known as singlet oxygen.[13] However, more recent studies have demonstrated that the H_2O_2–myeloperoxidase–Cl^- system generates only insubstantial amounts of this oxidant under physiologic conditions.[18] Nonetheless, HOCl does participate in the generation of a derivative group of oxidants known as the chloramines. In this rapid and spontaneous reaction, HOCl reacts with primary or secondary amines that either are released from the neutrophil itself or are present in the suspending medium, thus generating a complex family of nitrogen–chlorine (N-Cl) derivatives.[19-21]

$$R'RNH + HOCl \rightarrow R'RNCl + H_2O$$

Depending on the nature of the R group of the amine, the N–Cl oxidants may be either short-lived or long-lived. Indeed, some of these oxidants can be detected in the cell-free supernatants recovered from human neutrophils up to 16 hours after the cells have been triggered.[20] In general, chloramines are less powerful oxidants than HOCl itself, but they do retain two oxidizing equivalents and are able to chlorinate or oxidize a wide range of target molecules.[17]

In summary, neutrophils contain large amounts of an unusual chloride peroxidase that invests the cells with the ability to generate a family of highly reactive chlorinated oxidants. In the neutrophil, HOCl production is under the regulation of a complex, multicomponent system in which a latent enzyme (NADPH oxidase) must be specifically activated to fuel discharged myeloperoxidase with its required H_2O_2. Once this system is switched "on," HOCl is generated. As long as H_2O_2 is supplied (human neutrophils can generate H_2O_2 for up to three hours after specific triggering[22]), myeloperoxidase will continue to use plasma Cl^- to generate HOCl until the pool of oxidizable targets has been consumed. Only then will HOCl generation come to a halt, as the oxidant attacks and oxidatively autoinactivates myeloperoxidase itself.[17] That any mammalian cell has acquired the ability to generate such a powerful oxidant stands as testimony to the evolutionary pressures that have molded host defense mechanisms. However, this boon for microbicidal processes might be the bane of host tissues exposed to one of nature's most unusual toxins.

The in Vitro and in Vivo Toxicity of HOCl

Given the ability of neutrophils to generate large quantities of reactive chlorinated oxidants, it is not surprising that these cells are able to use these products to mediate extracellular cytotoxicity in vitro.[13,17,23] In most model systems, neutrophils are triggered adjacent to a target cell population and irreversible damage is monitored by measuring the release of radiolabeled, cytosolic markers from the lysed cells. HOCl can be implicated as the cytolytic mediator if damage can be prevented when HOCl generation is inhibited or the HOCl is preferentially intercepted (or as more commonly termed, scavenged) before it reacts with the target cell. Such scenarios can be duplicated in vitro by triggering neutrophils in the presence of the following substances: exogenous catalase, an enzyme that can "steal" and degrade H_2O_2 before it is metabolized by myeloperoxidase; azide, a heme-enzyme inhibitor that blocks myeloperoxidase activity; halide-free buffer, a medium in which neutrophils will generate H_2O_2 and release myeloperoxidase but will be unable to produce HOCl; or HOCl scavengers, compounds (e.g., methionine) that rapidly react with HOCl to yield nontoxic products (i.e., HOCl + methionine $\rightarrow$ $H_2O + Cl^-$ + methionine sulfoxide).[13,17] If cytotoxicity is ablated by these interventions, an HOCl-dependent effect is commonly assumed.[24] Although $O_2\cdot^-$ is not usually thought to be involved in the generation of HOCl (except as a precursor of H_2O_2), recent studies suggest that $O_2\cdot^-$ could actually double the amount of H_2O_2 available to the myeloperoxidase system by oxidizing physiologic substrates (e.g., ascorbate).[25] In addition, $O_2\cdot^-$

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may also have a direct role in regulating myeloperoxidase activity.[26] In either of these scenarios, superoxide dismutase, an enzyme that reduces the steady-state concentration of $O_2\cdot^-$ by catalyzing the dismutation of $O_2\cdot^-$ to H_2O_2, could inhibit HOCl generation.[25,26] Finally, the issue of whether the final mediator is HOCl or a derivative chloramine remains controversial, as does the identity of the biochemical processes underlying the development of the lethal event.[19] To date, most evidence suggests that HOCl is sufficiently reactive that it exerts its toxic effect by directly attacking membrane-associated targets, whereas less reactive chloramines, especially those with lipophilic characteristics, diffuse across the plasma membrane to attack cytosolic components.[27,28]

When these findings are taken together, it might seem that the in vitro "proof" of HOCl's role as the preeminent oxidative, proinflammatory mediator is complete. That is, HOCl is the most powerful oxidant generated in large quantities by the neutrophil, and this reactive metabolite can exert cytolytic effects against a wide range of mammalian targets. However, the very reactivity that lends HOCl its toxic qualities also places constraints on the oxidant's ability to cause specific damage in a complex, biologic medium. In vitro, neutrophil-mediated cytotoxicity is generally studied in simple, plasma-free buffers in order to maximize the number of productive collisions between HOCl and the intended target cell population. However, in the presence of plasma, HOCl will attack not only cellular targets but plasma constituents as well. Because reactive oxidants are not designed to act as specific toxins, they cannot selectively differentiate between nearby targets; a key thiol on a critical membrane transport protein will appear much the same to a molecule of HOCl as will a functionally silent thiol on a plasma protein. Thus, the more complex the suspending medium, the more likely that the oxidant's extracellular cytolytic potential will be dissipated by alternate substrates. Nonetheless, most investigators have felt secure in assuming that even in vivo, HOCl present in a sufficiently high local concentration would consume intervening targets and mediate extensive tissue damage. To my surprise, I have recently discovered not only that has this assumption been tested in a large patient population, but also that the results were far different from those that would have been predicted.

At the start of World War I, an alarming increase in the number of deaths due to infectious complications among wounded soldiers spurred a search for more effective disinfectants. Ideally, when applied to wounds, the selected agent would destroy microbes and microbial toxins without damaging normal tissues. In 1915, screening of more than 200 compounds for bactericidal activity in vitro led investigators to conclude presciently that the most promising microbicides were none other than HOCl and chloramines.[29,30] Thus, in their search for the ideal microbicide, they had unknowingly selected the very class of oxidants generated by neutrophils themselves. Moreover, in an unrecognized quest to outdo nature, they began perfusing a variety of wounds, ranging from deep flesh wounds, compound fractures, and suppurating joints to life-threatening gangrenous infections, with large volumes (1 to 2 liters per day) of buffered HOCl in high concentration (0.5 percent, or about 70 mM).[31,32] Regardless of the bactericidal potential of the infused HOCl, one would expect that these concentrations of oxidant would mediate extensive tissue damage. On the contrary, although the chlorinated solutions did exert a strong microbicidal effect in vivo, they did so without causing tissue damage or interfering with wound healing.[29-32] In vivo, the heightened sensitivity of the microbes to chlorinated oxidants, relative to the resistance of host tissues, was readily appreciated even in 1916: "Nothing in this experience has been more striking than the fact that while it is highly destructive to bacteria, it is nontoxic to the tissues."[33] This regimen, later known as the Carrel–Dakin technique, became the method of choice for treating infected wounds during World War I. Indeed, hospital ships were actually outfitted with large electrolytic cells to prepare HOCl from seawater.[30,31]

Despite the absence of tissue toxicity associated with the use of HOCl in the wounded, the oxidant's cytodestructive potential in vitro had already been recognized. Even 0.5 percent HOCl, the concentration normally used to treat patients, could solubilize skin or liver tissue in vitro.[34] (The corrosive tendencies of this oxidant were further highlighted in a macabre experiment in which tadpoles immersed in HOCl were "speedily reduced to a little heap of sand."[35]) However, if HOCl was added to slices of tissue that had been suspended in wound secretions, or if the tissues (or tadpoles) were treated with chloramines in place of HOCl, the solubilizing action of HOCl was lost. On the basis of these data, Dakin concluded that 0.5 percent HOCl was toxic to human tissues but *only* in a simple buffer system in vitro.[36-38] In a physiologic environment, even millimolar quantities of HOCl preferentially reacted with endogenous, amine-containing moieties to yield the derivative chloramines.[36] In turn, these newly generated chloramines exerted strong microbicidal activity but little if any ability to damage normal tissues.[37,38] Indeed, in 1917 Dakin and colleagues recommended that the HOCl solutions used for irrigating wounds be replaced with synthetic chloramines.[37,38] As predicted, clinical trials revealed that high concentrations of the synthetic chloramines (2 percent solutions, or about 90 mM) sterilized wounds as effectively as HOCl, without any signs of injury to host tissues.[36-38]

In the light of the above "new" information on the cytotoxic potential of chlorinated oxidants in vivo, can myeloperoxidase-generated HOCl still be considered a potent, direct-acting toxin in inflammatory diseases? Although it is not yet possible to provide a simple

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answer, the clinical data clearly demonstrate that HOCl, even in large quantities, is far less toxic than commonly assumed. The high reactivity of HOCl seems to ensure that its toxicity will be rapidly dissipated within a short distance of its site of generation in vivo. However, myeloperoxidase is a highly charged protein that, once released, can bind directly to host tissues.[13] In this manner, the localized production of HOCl could allow the neutrophil to mediate extracellular toxicity in vivo. For example, in rats the instillation of large concentrations of H_2O_2 and purified myeloperoxidase into the lungs or renal arteries has been shown to cause either severe pulmonary or glomerular damage, respectively.[39,40] These results suggest that the limitations that apply to HOCl-mediated damage in vivo might be circumvented if myeloperoxidase could first bind to its target. However, it is not clear whether these studies in animals provide a general mechanism for myeloperoxidase-mediated tissue damage in vivo or whether they reflect an unusual sensitivity of pulmonary and vascular tissues to chloramines. Inhalation of a volatile chloramine causes acute respiratory distress,[41] and as early as 1917 Dakin warned that despite their general lack of toxicity, neither HOCl nor chloramines should be injected intravenously since they acted as "endothelial poisons" that led to pericardial and pulmonary edema.[36] Thus, unlike most tissues, which successfully escape injury after exposure to chloramines,[36-38] the lungs and vascular bed appear to have an unexpected sensitivity to these oxidants. Given this information, my own enthusiasm for implicating HOCl alone as the primary key to the neutrophil's tissue-destructive potential has been blunted by a surfeit of 70-year-old clinical data.

The Proteolytic Enzymes of the Neutrophil

Although it is apparent that in a physiologic milieu, strong oxidants are short-lived, react but once, and are incapable of fastidiously selecting targets, an enzyme can remain active for exceptionally long periods, can repetitively catalyze a given reaction, and will ignore all targets save those dictated by the range of its substrate spectrum. Neutrophil granules contain a large family of over 20 enzymes,[42] but 3 proteolytic enzymes — the serine proteinase, elastase, and the two metalloproteinases, collagenase and gelatinase — seem to have the greatest potential to act as mediators of tissue destruction in immunologic injury. Each of these proteinases (i.e., enzymes that cleave peptide bonds in the body of the protein chain) has the distinction that they are able to attack key components of the extracellular matrix, which is composed of a complex mix of collagens, elastin, proteoglycans, and glycoproteins that lies under epithelia and surrounds connective-tissue cells.[43] Once thought to serve only as an extracellular scaffold that maintained tissue architecture, the matrix has been clearly shown to serve as an interactive substratum that regulates the shape, migration, growth, and differentiation of cells.[43] Indeed, of particular relevance to inflammatory tissue injury, the extracellular matrix also has an indispensable role in the orderly repair of damaged tissues.[44] Thus, if neutrophils are given the opportunity to mediate the dissolution of the extracellular matrix in vivo, then cell structure and function could suffer acute as well as irreparable damage.

Despite the destructive potential of proteinases, neutrophil oxidants remain the focus of most of the current literature as the final mediators of tissue damage, for at least three reasons. First, tissues in vivo are bathed in powerful plasma antiproteinases that can rapidly and irreversibly inhibit the neutrophil's serine proteinases.[45] Second, although neutrophil metalloproteinases are more resistant to circulating proteinase inhibitors, the enzymes are synthesized in a latent, inactive form, and establishing a physiologically relevant mechanism of activation has proved difficult.[46] Third, and perhaps most important, antioxidants exert strong antiinflammatory effects in a number of animal models of neutrophil-dependent tissue damage.[47-49] Together, these findings have led many to conclude that neutrophil proteinases do not have an important role in pathologic tissue damage, and neutrophil oxidants rather than proteinases are the single most important direct mediators of immune injury. However, given the fact that the rediscovered clinical studies performed with chlorinated oxidants demonstrated that HOCl and N-chloramines have only a limited ability to mediate toxicity in vivo, a more consistent interpretation of these experimental findings should be considered. As we will see, ample evidence already exists to support the conclusions that not only can neutrophil proteinases exert destructive effects in vivo, but also that their activities are unexpectedly linked to the cell's ability to generate HOCl.

The Tissue-Destructive Potential of Neutrophil Elastase

In many respects it is perplexing that neutrophil granules contain large quantities of an enzyme that can not only single-handedly degrade almost all components of the extracellular matrix but also cleave a variety of key plasma proteins (e.g., immunoglobulins, complement proteins, and clotting factors) and even attack intact cells.[42,50] Indeed, the ability of elastase to mediate extracellular damage has led to the suggestion that its physiologic action is purposely restricted to the phagocytic vacuole, where it participates in the destruction and digestion of ingested microorganisms.[50] In support of this premise is the fact that plasma and interstitial fluids are known to contain a series of powerful antiproteinases, including α_1-proteinase inhibitor (formerly termed α_1-antitrypsin), α_2-macroglobulin, and secretory leukoproteinase inhibitor, that can effectively regulate extracellular neutrophil elastase and prevent the enzyme from attacking extracellular substrates.[45,50] Among these three inhibitors, the host's primary defense against uncontrolled elastase-mediated damage is α_1-proteinase inhibitor, a 52-kd glycoprotein that

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irreversibly inhibits neutrophil elastase by forming an enzyme–inhibitor complex. Indeed, in apparent cognizance of the dangers associated with the extracellular release of elastase, the antiproteinase associates with the neutrophil enzyme at a rate that approaches the diffusion-controlled limit. In vivo, the calculated half-life of active elastase is only about 0.6 msec; by 3 msec all activity would be inhibited.[45] Given the effectiveness and multiplicity of the inhibitors that comprise the anti-elastase shield, it might seem reasonable to assume that neutrophil elastase would not have the opportunity to mediate extracellular tissue damage. However, a large body of data indicates that neutrophils not only can circumvent the entire antiproteinase shield but also are able to use their discharged elastase to attack and destroy host tissues.

Strong evidence supporting the ability of the neutrophil to penetrate the antiproteinase screen was first obtained from the demonstration that isolates of purulent fluids recovered from sites of inflammation contained free neutrophil enzymes capable of degrading a variety of native and denatured proteins at neutral pH in vitro.[51] The presence of free proteinases suggested that the antiproteinase shield had been subverted, and it was quickly established that the exudate fluids had lost their ability to inhibit exogenous neutrophil proteinases.[51,52] These observations led investigators to conclude that the attendant destruction of normal tissues at these sites was the result of the action of neutrophil proteinases in an inhibitor-free environment.[51,52]

The mechanism by which the proteinase inhibitors were inactivated remained unidentified for almost 10 years, until an interesting clue appeared from an unexpected quarter. During a study of the effect of chlorinated oxidants on plasma proteins in vivo, it was discovered serendipitously that the antiproteinase capacity of blood had decreased dramatically after treatment.[36] Together, these data provide all the information necessary to begin constructing an alternative model capable of resolving the relative roles of oxidants and serine proteinases in pathologic tissue damage. In this model, chlorinated oxidants do not independently mediate injurious effects but instead destroy the antiproteinase shield, allowing unregulated proteolytic enzymes to attack and degrade host tissues. Although the validity of this model has only recently been assessed, few investigators are aware of the fact that these studies on the perturbation of the proteinase–antiproteinase balance and the effects of chlorinated oxidants on plasma proteinase inhibitors were published independently at the turn of the century. In a fascinating series of experiments performed between 1888 and 1906, German and American scientists established the importance of neutrophil proteinases and plasma antiproteinases in the evolution of tissue damage in vivo.[51] Ten years later, Dakin's work with chlorinated oxidants and bactericidal mechanisms led to his discovery in 1916 that a plasma antiproteinase was sensitive to HOCl-mediated inactivation in vivo.[36] However, the potential biologic importance of these results was completely overlooked, and the data were largely forgotten. Doubtless, in 1916 no one realistically considered the possibility that neutrophils might generate chlorinated oxidants. Thus, interest in the relation between oxidants, proteinases, and antiproteinases lay dormant until the early 1980s.

By 1984, the tissue-destructive potential of purified neutrophil elastase both in vitro and in vivo had been clearly established and the apparent Achilles heel in the antiproteinase shield had been rediscovered. Structural and functional analyses of human α_1-proteinase inhibitor revealed the following: the antiproteinase contains a critical methionine (position 358) in its reactive center; the methionyl residue is sensitive to oxidation by either chemical reagents or triggered human neutrophils; oxidation of Met-358 causes a 2000-fold decrease in the rate of association between neutrophil elastase and the modified antiproteinase (thereby increasing the half-life of elastase in vivo 2000-fold, from 0.6 msec to 1.2 seconds); and oxidized α_1-proteinase inhibitor is unable to inhibit effectively the attack of purified neutrophil elastase against sensitive substrates.[45,50] Although one might assume that the neutrophil's ability to modify α_1-proteinase inhibitor ensures the cell's ability to mediate extracellular proteolysis, the relation between oxidative inactivation and elastase activity is more complex. In vivo, neutrophils are immersed in fluids containing huge excesses of α_1-proteinase inhibitor relative to their own elastase content. Indeed, 1 ml of plasma contains sufficient α_1-proteinase inhibitor (26 nmol per milliliter) to inhibit all the elastase contained in 500×10^6 neutrophils (assuming 1.5 μg of elastase per 10^6 cells). Thus, there is a race between the rate at which the neutrophil can oxidize the surrounding α_1-proteinase inhibitor and the rate at which native α_1-proteinase inhibitor can inhibit the released elastase (Fig. 1) — that is, if the triggered neutrophil is able to extend a zone of oxidizing equivalents into the α_1-proteinase inhibitor shield, a microenvironment could be generated in which released elastase would preferentially associate with host tissues before it collided with a native molecule of α_1-proteinase inhibitor. Once elastase is tissue bound, even native α_1-proteinase inhibitor cannot extricate or completely inhibit the active enzyme.[45,50] Alternatively, if the neutrophil is envisioned as being enveloped in a cloud of α_1-proteinase inhibitor that the cell can dissipate only inefficiently, then sufficient native antiproteinase would always be available in the cell's immediate vicinity to inhibit elastase rapidly, before it could bind to the surrounding tissues. (Oxidants cannot reactivate the inhibited enzyme.)

To determine whether neutrophils can or cannot attack extracellular tissues in a physiologic setting, an in vitro model of an inflammatory site was constructed in which neutrophils were triggered atop an insoluble matrix of connective-tissue macromolecules in the

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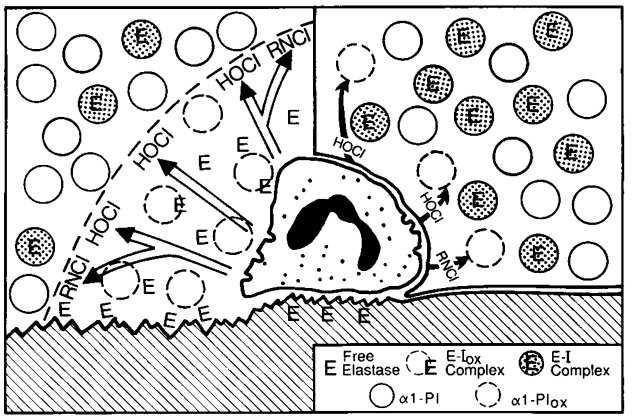

Figure 1. Interactions between Neutrophil Elastase, α_1-Proteinase Inhibitor (α1-PI), and Chlorinated Oxidants.

The left half of the figure shows neutrophil oxidants creating a zone of oxidized α_1-proteinase inhibitor that allows released elastase to attack and degrade tissues. Because oxidized α_1-proteinase inhibitor can inhibit elastase only inefficiently, free enzyme may also be detected (see text). The right half of the figure shows an alternative setting: only a small portion of the surrounding α_1-proteinase inhibitor is oxidized, thus ensuring the efficient regulation of the released elastase. In either setting proteolysis can occur to a limited degree subjacent to the neutrophil (see text for details).

In the inset, E denotes elastase, I inhibitor, and ox oxidized.

presence of purified α_1-proteinase inhibitor.[53] Under these conditions, neutrophils were able to generate and use both HOCl and N-chloramines to oxidize the surrounding α_1-proteinase inhibitor at a rate that permitted the discharged elastase to solubilize the matrix.[53,54] Although neutrophils rapidly inactivate α_1-proteinase inhibitor in vitro, it should be recalled that elastase released in vivo must still contend with both α_2-macroglobulin and secretory leukoproteinase inhibitor. α_2-Macroglobulin is a large (725 kd) broad-spectrum plasma antiproteinase that is able to inhibit almost all mammalian proteinases[42]; secretory leukoproteinase inhibitor is a small antiproteinase (14 kd) that is found in mucous secretions and interstitial fluids, where it can inhibit both free and tissue-bound elastase.[55] Interestingly, recent studies indicate that both α_2-macroglobulin (unpublished observations) and secretory leukoproteinase inhibitor can be oxidatively inactivated by triggered neutrophils.[56] Thus, all three components of the antielastase shield are sensitive to oxidative attack. Only if neutrophils are prevented from generating HOCl can the antiproteinases remain active, in which case they are able to prevent tissue damage almost completely.[53,54] Interestingly, even in the absence of oxidants, complete inhibition of proteolysis is never obtained.[53,54,57] Triggered neutrophils have the ability to create a subjacent sequestered environment in which antiproteinases are excluded and released proteinases can act in an unmolested fashion. However, from a quantitative standpoint, the amount of oxygen-independent proteolysis that occurs beneath the cell constitutes only a fraction of the neutrophil's destructive potential.[53,54] In a physiologic milieu, only the combined action of HOCl and elastase allows the cell to exert its maximal effect.

Taken together, these results demonstrate that the neutrophil could minimally attack all elastase-sensitive tissues within the protective zone of its oxidizing environment. However, if the oxidant load is large relative to the quantity of antiproteinases available at an inflamed site, then all the inhibitors could be inactivated and proteolytic damage would no longer be confined to the cell's immediate vicinity. Indeed, in vitro, free elastase can be detected in fluids recovered from mixtures of neutrophils triggered in the presence of serum or epithelial lining fluid.[58] In vivo, the depletion of an inflammatory focus of all its antiproteinases would depend on both the number of neutrophils and the volume of the inflammatory exudate. As demonstrated by Opie, when an inflammatory stimulant (turpentine) was injected into the pleural cavity, where large amounts of fluids could collect, the antiproteinase shield was not penetrated by the infiltrating neutrophils, free neutrophil proteinases could not be detected, and the inflamed pleura healed normally.[59] In contrast, when an identical quantity of turpentine was injected subcutaneously, in which case tissue tension limited the volume of the exudate, the antiproteinase shield was destroyed, active neutrophil proteinases accumulated, and in confirmation of the importance of the extracellular matrix, the wound healed with a scar.[52,59] Given the fact that a large interstitial inflammatory site contains approximately 0.1 ml of fluid and 25×10^6 neutrophils, and that millimolar concentrations of HOCl (i.e., >100 nmol per 0.1 ml) are needed to inactivate the antiproteinases in plasma,[60] the enormous quantities of available HOCl (up to 2500 to 5000 nmol per 25×10^6 cells) are easily in excess of the amounts required. Indeed, even larger volumes of antiproteinases can be consumed if a chronic inflammatory stimulus induces successive waves of neutrophils to infiltrate the affected site.[58,59] Thus, in pathologic states, neutrophils could cooperatively use chlorinated oxidants to penetrate multiple antiproteinase barriers, thereby allowing the released elastase to attack all susceptible tissues within the inflammatory focus.[1-3]

The Activation of Latent Metalloproteinases: An Unexpected Link to Oxidative Metabolism

The inclusion of the collagen-degrading metalloproteinases collagenase and gelatinase in the arsenal of the neutrophil would seem to provide additional support for the premise that enzymes have strategic roles as key mediators of pathologic tissue damage.[43] Neutrophil collagenase can cleave each of the interstitial collagens (i.e., type I, II, and III collagens) at a single, specific locus, whereas gelatinase has been reported to degrade native type V, type XI, and possibly type IV collagens.[46] The distinctive pattern of the distribution of these collagens in the interstitium and in basement membranes underlines the vulner-

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ability of all host tissues to proteolytic attack by these metalloenzymes.[43]

Despite the pathologic potential of these collagenolytic enzymes, interest in the metalloproteinases has been tempered by the fact the enzymes are synthesized and released in latent, inactive forms.[46] Until recently, little was known about the ability of intact neutrophils to activate either of the metalloenzymes. Instead, efforts focused on attempts to identify potential activation schemes with purified collagenases in cell-free model systems. Neutrophil collagenase, like the closely related but distinct tissue collagenases found in other cell types (e.g., macrophages, fibroblasts, and endothelial cells), is a proenzyme that undergoes proteolytic activation when it is treated in vitro with trypsin.[46] Although neutrophil elastase or cathepsin G (another neutrophil serine proteinase) may be considered as likely endogenous homologues of trypsin, the purified serine proteinases destroy rather than activate latent collagenase.[46] To date, a neutrophil proteinase capable of proteolytically activating collagenase has not been identified. However, in addition to proteolytic activation (and at first glance, of no apparent physiologic relevance), latent collagenase can also be activated by an array of chemical reagents ranging from alkylating agents and organomercurials to heavy metals.[46] Although none of these reagents are likely to serve as endogenous activators, each of these compounds can react with thiol groups. This shared chemical characteristic led to the suggestion that endogenously derived thiol-reactive agents may have a similar ability to regulate collagenase activity.[61]

Given the fact that oxygen metabolites, particularly chlorinated oxidants, undergo facile reactions with thiols, the possibility was examined that intact neutrophils may employ these reactive species as collagenase activators. This hypothesis was first tested by comparing the ability of neutrophils from normal subjects and neutrophils from patients with chronic granulomatous disease (CGD neutrophils) to activate discharged collagenase.[61] Like normal neutrophils, CGD neutrophils contain a full complement of granule proteinases, but the cells have a defective NADPH oxidase and do not generate substantial amounts of $O_2^{\cdot -}$, H_2O_2, or chlorinated oxidants.[3] As predicted, both normal and CGD neutrophils discharged comparable amounts of collagenase extracellularly after the addition of a triggering agent.[61] However, unlike the normal cells, the CGD neutrophils failed to activate their released collagenase, and all of the enzyme remained in its latent state. Thus, the ability to generate oxygen metabolites was an absolute requirement for the successful activation of collagenase by intact neutrophils under these conditions. Further studies revealed that normal neutrophils were likewise unable to activate released collagenase if the cells were prevented from generating HOCl.[61] Remarkably, latent collagenase could even be specifically activated ex situ if reagent HOCl was simply added directly to the cell-free supernatant.[61] In this unusual scenario, a powerful oxidant heretofore only associated with unregulated, destructive events actually participated in the specific activation of a latent enzyme.

Not unexpectedly, a similar role for oxidative activation was extended to neutrophil gelatinase.[62] Despite the fact that this latent metalloproteinase is structurally distinct from collagenase and possesses a completely different substrate spectrum, endogenously generated HOCl activated gelatinase in an intact cell system, and reagent HOCl directly activated purified gelatinase in a cell-free system.[62] The precise molecular mechanism by which HOCl activates the latent enzymes is unknown, but the data are most consistent with a thiol-driven intramolecular perturbation that unmasks the active sites of the metalloenzymes.[46] In any case, the activities of both neutrophil collagenase and gelatinase are tightly linked to the myeloperoxidase system and HOCl generation. In many respects, the design of this collagenolytic system resembles that of a binary weapon: neither HOCl alone nor latent collagenases alone can specifically cleave collagen substrates, but if the oxidants and metalloproteinases intermix, collagen degradation is catalytically effected.[61-63]

Once the collagenases are activated, the ability of the antiproteinase screen to regulate these enzymes is unclear. α_2-Macroglobulin and a second, more specific antiproteinase, the tissue inhibitor of metalloproteinases (TIMP), are powerful regulators of other tissue collagenases, but these inhibitors appear to be less effective against the neutrophil enzymes.[46] Regardless of the ability of the inhibitors to regulate the neutrophil collagenases, α_2-macroglobulin can be inactivated by oxidants whereas TIMP can be destroyed by neutrophil elastase.[46,64] Another plasma collagenase inhibitor capable of more effectively inhibiting neutrophil collagenase has also been described, but the antiproteinase contains one or two critical thiol groups that can be oxidized easily.[46] These data suggest that human neutrophils have the ability not only to activate but also to express their collagenolytic potential in vivo. Thus, like the regulation of neutrophil elastase, the expression of the collagenolytic enzymes is also tightly linked to the generation of HOCl. However, whereas antiproteinases seem designed to "fail" in the face of HOCl attack in order to allow elastase to attack host tissues, the latent collagenases are specifically constructed so that they can transform the oxidizing potential of HOCl into the enzyme-catalyzed degradation of interstitial, pericellular, and basement-membrane-associated collagens.

Evidence for Neutrophil-Mediated Tissue Destruction in Vivo

In contrast to the ability of human neutrophils to use chlorinated oxidants to regulate the expression of destructive proteinases in vitro, direct proof that these processes catalyze injury in vivo remains elusive. Studies of immune-mediated tissue damage in animal models have clearly established a pathogenic role for oxygen metabolites,[1,5,47-49] but the possibility that the oxidants do not act as direct toxins and instead trigger

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proteolytic destruction has not been widely considered. In part, this reticence may be explained by the fact that specific proteinase inhibitors that are able to act in an oxidizing milieu in vivo are only now being developed. However, even with these inhibitors in hand, increasing concern has been focused on an apparent lack of homology between the properties of human neutrophils and the neutrophils of animals commonly used in experimental models. For example, neutrophils from rats and sheep contain only small amounts of elastase whereas neutrophils from rabbits rely predominantly on acid rather than neutral proteinases.[65-67] As one might expect, differences between the cells in their proteinase profiles are linked to important variations in their antiproteinase screens as well. α_1-Proteinase inhibitor from sheep and rabbits is highly resistant to oxidative inactivation and, at least in the case of sheep, does not contain a methionyl residue at its reactive center.[66,67] At present, due caution must be exercised in extending to humans insights obtained in animals.

In the absence of either a large body of applicable animal studies or clinically approved proteinase inhibitors that could be tested in humans, what evidence can be marshaled to support an in vivo role for neutrophil oxidants and proteinases in acute inflammatory disease states? As discussed above, the in vitro findings indicate that active neutrophil proteinases and oxidatively inactivated antiproteinases should be present at sites of acute injury. Indeed, a review of the recent literature reveals that active neutrophil elastase, collagenase, and myeloperoxidase as well as inactivated α_1-proteinase inhibitor can be detected in fluids (and in some cases, tissues) recovered from areas of inflammation in diseases ranging from idiopathic pulmonary fibrosis to rheumatoid arthritis and the adult respiratory distress syndrome.[68-73] Strong support for the presence of an oxidative component in these events lies in the fact that large amounts of the dysfunctional α_1-proteinase inhibitor recovered from these sites have been shown to have undergone oxidative modification.[45,74] Because free neutrophil elastase and oxidized α_1-proteinase inhibitor are often detected together in the same fluids, it is reasonable to assume that chlorinated oxidants are responsible for the inactivation of the antiproteinase. However, it should be noted that other inflammatory-cell populations can oxidatively inactivate α_1-proteinase inhibitor by means of processes independent of myeloperoxidase. For example, human eosinophils contain a structurally distinct lysosomal peroxidase (termed eosinophil peroxidase) that preferentially uses the relatively small amounts of bromide found in plasma (about 50 to 100 μM Br^-, versus 100 mM Cl^-) to generate the powerful oxidants hypobromous acid (HOBr) and singlet oxygen.[74,75] In addition, even peroxidase-deficient cell populations such as human alveolar macrophages (and perhaps neutrophils from subjects with hereditary myeloperoxidase deficiency) can generate an uncharacterized oxygen metabolite that is able to oxidize thioethers and inactivate α_1-proteinase inhibitor.[13,76] Despite the fact that eosinophils and macrophages contain little if any neutrophil elastase, these cells could play an accessory part in elastase-dependent proteolysis by oxidatively depleting the antielastase shield.

In addition to oxidized α_1-proteinase inhibitor in fluids recovered from sites of inflammation, an appreciable fraction of the inactive antiproteinase also appears to have been proteolyzed.[58,73] Neutrophil elastase itself can cleave oxidized α_1-proteinase inhibitor,[58] but triggered neutrophils also release and express an unusual metalloproteinase activity that directly inactivates native α_1-proteinase inhibitor by hydrolyzing the antiproteinase at a primary cleavage site located between Phe-352 and Leu-353.[77] Like the collagenolytic enzymes (neither of which have been reported to hydrolyze Phe–Leu sequences), the metalloproteinase that cleaved α_1-proteinase inhibitor also proved to be a latent enzyme whose activity was directly linked to that of HOCl.[77] In apparent defiance of Occam's razor, neutrophils therefore use HOCl to inactivate α_1-proteinase inhibitor by two independent but linked processes, through both the direct oxidation of Met-358 and the release of an oxidatively activated α_1-proteinase inhibitor–cleaving metalloproteinase. Surprisingly, more recent studies have revealed that both neutrophil collagenase and gelatinase have the unexpected ability to inactivate α_1-proteinase inhibitor by cleaving at the identical Phe–Leu sequence (unpublished data). Apparently, the substrate spectrum of the neutrophil metalloproteinases extends beyond collagenous macromolecules, and the pathologic effect of the oxidatively activated enzymes may be broader than originally thought. In any case, the presence of both oxidized and proteolyzed α_1-proteinase inhibitor at sites of inflammation is entirely consistent with both the neutrophil's repertoire of offensive capabilities and the cell's demonstrated ability to express elastase and collagenase activities in vivo. Taken together, the in vivo findings closely parallel those delineated in vitro and strongly suggest that in disease states, an oxidizing environment is created that allows the unregulated expression of the neutrophil's tissue-damaging proteinases.

Summary

From a historical perspective the neutrophil's ability to damage tissues has been credited to its ability either to release toxic granule components or to generate reactive oxygen metabolites. However, as we have seen, powerful oxidants are by nature short-lived and nonspecific, and proteinases are held in check by either their own latency or a highly effective antiproteinase screen. Acting alone, either set of weapons would allow the neutrophil to exert only highly localized effects, but if relatively small amounts of HOCl and proteinases are simply combined, the cell can subvert all the intrinsic and host-erected barriers that have been designed to protect host tissues from injury. By oxidatively inactivating a series of key proteinase inhibitors and simultaneously activating latent protein-

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ases, neutrophils can create an environment in which elastase, collagenase, and gelatinase are able to exert destructive effects more efficiently and with greater specificity than could even enormous doses of oxidants alone. Although the regulation of only three proteinases has been reviewed in this perspective, it seems likely that similar regulatory processes apply to other enzymes (and possibly other populations of immune cells). For example, plasminogen activator inhibitor-1 and α_1-antichymotrypsin — antiproteinases directed against neutrophil plasminogen activator and cathepsin G, respectively — are also sensitive to attack by neutrophils. Plasminogen activator inhibitor-1 is sensitive to inactivation by chlorinated oxidants[78]; α_1-antichymotrypsin is sensitive to proteolytic attack by both neutrophil elastase[45] and collagenases (unpublished observation). In turn, the unregulated enzymes can attack additional antiproteinases (e.g., α_2-plasmin inhibitor or C1 esterase inhibitor[79]), whose destruction would serve to extend and intensify the inflammatory response. According to this construct, neutrophils use chlorinated oxidants to initiate a cascade of proteolytic effects that damage all host tissues within the cell's reach (Fig. 2). Although other oxidants might take the place of HOCl,[13,76] the neutrophil's enormous content of myeloperoxidase seems to destine the normal cell to use chlorinated oxidants as its primary oxygen metabolite.

In overview, it seems that oxidants, proteinases, and antiproteinases paradoxically interact to allow the neutrophil to maximize its ability to damage host tissues. That is, oxidants and proteinases are released into an extracellular environment in which there is little or no superoxide dismutase or catalase to regulate the concentration of oxygen metabolites,[4] and proteinases and antiproteinases are designed in an apparently perilous fashion that allows oxidants to turn proteinases on and antiproteinases off. However, in terms of host defense, this design would allow the neutrophil to exert its proteolytic potential extracellularly in order to traverse connective-tissue barriers, to dissolve infected tissues in an attempt to dislodge sequestered microbes, or to participate in abscess formation.[46] Indeed, given the tissue-destructive potential of the neutrophil's weapons, the compartmentalization of enzymes and oxidants in separate locales that are only combined after the cell is specifically triggered appears to be the safest means of packaging toxins in relatively innocuous forms.

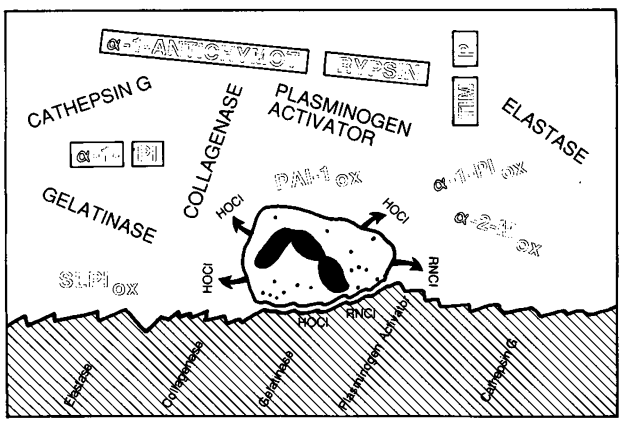

Figure 2. Oxidative Regulation of Neutrophil Proteinases.

Triggered neutrophils use chlorinated oxidants to inactivate antiproteinases (α_1-proteinase inhibitor [α-1-PI], α_2-macroglobulin [α-2-M], secretory leukoproteinase inhibitor [SLPI], and plasminogen activator inhibitor-1 [PAI-1]) and to activate latent metalloproteinases (collagenase and gelatinase). In turn, unregulated neutrophil elastase can hydrolyze both the tissue inhibitor of metalloproteinases (TIMP) and α_1-antichymotrypsin, while neutrophil metalloproteinases can attack α_1-proteinase inhibitor. In this environment, neutrophil elastase, cathepsin G, plasminogen activator, collagenase, and gelatinase can degrade host tissues in an unmolested fashion. ox denotes oxidized.

In a physiologic inflammatory response, neutrophils do not continuously degrade host tissues in an uninterrupted manner. Normally, the influx of neutrophils, along with their subsequent triggering, is carefully regulated and ceases when the initiating antigen is destroyed.[23] After the oxidizing environment has dissipated, the antiproteinase screen of the inflamed site can be reconstituted by diffusion of inhibitors from the plasma bed or by locally synthesized antiproteinases,[80] thereby inhibiting the released enzymes. If, however, inflammatory stimuli are chronically directed against host tissues or are not properly down regulated, neutrophils activate agents that can penetrate all of the host's defenses. As exemplified in inflammatory disease states, the host is ill-prepared to protect itself from this onslaught. The development of synthetic inhibitors directed against myeloperoxidase[81,82] or proteolytic enzymes,[83] the availability of large quantities of recombinant antiproteinases,[84] and the design of mutant antiproteinases that resist oxidative attack[85,86] may succeed in attenuating inflammatory damage if host regulatory mechanisms fail. Nevertheless, it should be stressed that the examples presented in this perspective may be just that — specific examples of a more general principle; additional important interactions between oxidants, proteinases, and other cellular constituents may well be discovered. Experimental attempts to elucidate these events should not only provide insights into acute and chronic pathologic tissue damage, but also lead to the identification of important new targets of pharmacologic intervention.

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References

1. Malech HL, Gallin JI. Neutrophils in human diseases. N Engl J Med 1987; 317:687-94.
2. Henson PM, Johnston RB Jr. Tissue injury in inflammation: oxidants, proteinases, and cationic proteins. J Clin Invest 1987; 79:669-74.
3. Lehrer RI, Ganz T, Selsted ME, Babior BM, Curnutte JT. Neutrophils and host defense. Ann Intern Med 1988; 109:127-42.
4. Cross CE, Halliwell B, Borish ET, et al. Oxygen radicals and human disease. Ann Intern Med 1987; 107:526-45.
5. Halliwell B, ed. Oxygen radicals and tissue injury: proceedings of a Brook Lodge symposium, Augusta, Michigan, U.S.A., April 27 to 29, 1987. Bethesda, Md.: Federation of American Societies for Experimental Biology, 1988:1-143.
6. Weiss SJ. Oxygen, ischemia and inflammation. Acta Physiol Scand [Suppl] 1986; 548:9-37.

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7. Fridovich I. Biological effects of superoxide radical. Arch Biochem Biophys 1986; 247:1-11.
8. Hyslop PA, Hinshaw DB, Halsey WA Jr, et al. Mechanisms of oxidant-mediated cell injury: the glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. J Biol Chem 1988; 263:1665-75.
9. Test ST, Weiss SJ. Quantitative and temporal characterization of the extracellular H_2O_2 pool generated by human neutrophils. J Biol Chem 1984; 259:399-405.
10. Weiss SJ. The role of superoxide in the destruction of erythrocyte targets by human neutrophils. J Biol Chem 1980; 255:9912-7.
11. Weiss SJ, Young J, LoBuglio AF, Slivka A, Nimeh NF. Role of hydrogen peroxide in neutrophil-mediated destruction of cultured endothelial cells. J Clin Invest 1981; 68:714-21.
12. Britigan BE, Cohen MS, Rosen GM. Hydroxyl radical formation in neutrophils. N Engl J Med 1988; 318:858-9.
13. Klebanoff SJ. Phagocytic cells: products of oxygen metabolism. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: basic principles and clinical correlates. New York: Raven Press, 1988:391-444.
14. Weiss SJ, Klein R, Slivka A, Wei M. Chlorination of taurine by human neutrophils: evidence for hypochlorous acid generation. J Clin Invest 1982; 70:598-607.
15. Thomas EL, Grisham MB, Jefferson MM. Myeloperoxidase-dependent effect of amines on functions of isolated neutrophils. J Clin Invest 1983; 72:441-54.
16. Foote CS, Goyne TE, Lehrer RI. Assessment of chlorination by human neutrophils. Nature 1983; 301:715-6.
17. Test ST, Weiss SJ. The generation of utilization of chlorinated oxidants by human neutrophils. Adv Free Radical Biol Med 1986; 2:91-116.
18. Kanofsky JR, Wright J, Miles-Richardson GE, Tauber AI. Biochemical requirements for singlet oxygen production by purified human myeloperoxidase. J Clin Invest 1984; 74:1489-95.
19. Weiss SJ, Lampert MB, Test ST. Long-lived oxidants generated by human neutrophils: characterization and bioactivity. Science 1983; 222:625-8.
20. Test ST, Lampert MB, Ossanna PJ, Thoene JG, Weiss SJ. Generation of nitrogen-chlorine oxidants by human phagocytes. J Clin Invest 1984; 74:1341-9.
21. Grisham MB, Jefferson MM, Melton DF, Thomas EL. Chlorination of endogenous amines by isolated neutrophils: ammonia-dependent bactericidal, cytotoxic, and cytolytic activities of the chloramines. J Biol Chem 1984; 259:10404-13.
22. Nathan CF. Neutrophil activation on biological surfaces: massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. J Clin Invest 1987; 80:1550-60.
23. Clark RA. Extracellular effects of the myeloperoxidase-hydrogen peroxide-halide system. Adv Inflamm Res 1983; 5:107-46.
24. Weiss SJ, Slivka A. Monocyte and granulocyte-mediated tumor cell destruction: a role for the hydrogen peroxide-myeloperoxidase-chloride system. J Clin Invest 1982; 69:255-62.
25. Thomas EL, Learn DB, Jefferson MM, Weatherred W. Superoxide-dependent oxidation of extracellular reducing agents by isolated neutrophils. J Biol Chem 1988; 263:2178-86.
26. Kettle AJ, Winterbourn CC. Superoxide modulates the activity of myeloperoxidase and optimizes the production of hypochlorous acid. Biochem J 1988; 252:529-36.
27. Albrich JM, Gilbaugh JH III, Callahan KB, Hurst JK. Effects of the putative neutrophil-generated toxin, hypochlorous acid, on membrane permeability and transport systems of *Escherichia coli*. J Clin Invest 1986; 78: 177-84.
28. Grisham MB, Jefferson MM, Thomas EL. Role of monochloramine in the oxidation of erythrocyte hemoglobin by stimulated neutrophils. J Biol Chem 1984; 259:6757-65.
29. Dakin HD. On the use of certain antiseptic substances in the treatment of infected wounds. Br Med J 1915; 2:318-20.
30. Dakin HD, Dunham EK. A handbook of antiseptics. New York: Macmillan, 1918.
31. Carrel A, Dehelly G. The treatment of infected wounds. New York: Paul B. Hoebber, 1917.
32. Smith JL, Drennan AM, Rettie T, Campbell W. Experimental observations on the antiseptic action of hypochlorous acid and its application to wound treatment. Br Med J 1915; 2:129-36.
33. Hypochlorous acid in surgery. Br J Med 1916; 1:171-2.
34. Bashford EF. Sterilisation by Dakin's solution and the occurrence of secondary haemorrhage. Lancet 1917; 2:595-7.
35. Taylor HD, Austin JH. The solvent action of antiseptics on necrotic tissue. J Exp Med 1918; 27:155-64.
36. Dakin HD. The behaviour of hypochlorites on intravenous injection and their action on blood serum. Br Med J 1916; 1:852-4.
37. Dakin HD, Cohen JB, Kenyon J. Studies in antiseptics (II): on chloramine: its preparation, properties, and use. Br Med J 1916; 1:160-2.
38. Dakin HD, Lee WE, Sweet JE, Hendrix BM, Le Conte RG. A report of the use of dichloramin-T (toluene-parasulphondi-chloramin) in the treatment of infected wounds. JAMA 1917; 69:27-30.
39. Johnson KJ, Fantone JC III, Kaplan J, Ward PA. In vivo damage of rat lungs by oxygen metabolites. J Clin Invest 1981; 67:983-93.
40. Johnson RJ, Couser WG, Chi EY, Adler S, Klebanoff SJ. New mechanism for glomerular injury: myeloperoxidase-hydrogen peroxide-halide system. J Clin Invest 1987; 79:1379-87.
41. Gapany-Gapanavicius M, Molho M, Tirosh M. Chloramine-induced pneumonitis from mixing household cleaning agents. Br Med J 1982; 285: 1086.
42. Henson PM, Henson JE, Fitlschen C, Kimani G, Bratton DL, Riches DWH. Phagocytic cells: degranulation and secretion. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: basic principles and clinical correlates. New York: Raven Press, 1988:363-80.
43. Hay ED, ed. Cell biology of the extracellular matrix. New York: Plenum Press, 1981.
44. Vracko R. Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. Am J Pathol 1974; 77:314-46.
45. Travis J, Salvesen GS. Human plasma proteinase inhibitors. Annu Rev Biochem 1983; 52:655-709.
46. Weiss SJ, Peppin GJ. Collagenolytic metalloenzymes of the human neutrophil: characteristics, regulation and potential function in vivo. Biochem Pharmacol 1986; 35:3189-97.
47. Schalkwijk J, van den Berg WB, van de Putte LBA, Joosten LAB, van den Bersselaar L. Cationization of catalase, peroxidase, and superoxide dismutase: effect of improved intraarticular retention on experimental arthritis in mice. J Clin Invest 1985; 76:198-205.
48. Rehan A, Wiggins RC, Kunkel RG, Till GO, Johnson KJ. Glomerular injury and proteinuria in rats after intrarenal injection of cobra venom factor: evidence for the role of neutrophil-derived oxygen free radicals. Am J Pathol 1986; 123:57-66.
49. Hatherill JR, Till GO, Bruner LH, Ward PA. Thermal injury, intravascular hemolysis, and toxic oxygen products. J Clin Invest 1986; 78:629-36.
50. Janoff A. Elastase in tissue injury. Annu Rev Med 1985; 36:207-16.
51. Opie EL. Intracellular digestion: the enzymes and anti-enzymes concerned. Physiol Rev 1922; 2:552-85.
52. *Idem*. Solution of tissue with abscess. J Exp Med 1906; 8:536-41.
53. Weiss SJ, Regiani S. Neutrophils degrade subendothelial matrices in the presence of alpha-1-proteinase inhibitor: cooperative use of lysosomal proteinases and oxygen metabolites. J Clin Invest 1984; 73:1297-303.
54. Weiss SJ, Curnutte JT, Regiani S. Neutrophil-mediated solubilization of the subendothelial matrix: oxidative and nonoxidative mechanisms of proteolysis used by normal and chronic granulomatous disease phagocytes. J Immunol 1986; 136:636-41.
55. Bruch M, Bieth JG. Influence of elastin on the inhibition of leucocyte elastase by α_1-proteinase inhibitor and bronchial inhibitor: potent inhibition of elastin-bound elastase by bronchial inhibitor. Biochem J 1986; 238:269-73.
56. Kramps JA, Van Twisk C, Dijkman JH. Oxidative inactivation of bronchial antileukoprotease by triggered polymorphonuclear leukocytes. Am Rev Respir Dis 1987; 135:290. abstract.
57. Campbell EJ, Campbell MA. Pericellular proteolysis by neutrophils in the presence of proteinase inhibitors: effects of substrate opsonization. J Cell Biol 1988; 106:667-76.
58. Ossanna PJ, Test ST, Matheson NR, Regiani S, Weiss SJ. Oxidative regulation of neutrophil elastase-alpha-1-proteinase inhibitor interactions. J Clin Invest 1986; 77:1939-51.
59. Opie EL. The transformation of sero-fibrinous into purulent pleurisy. J Exp Med 1907; 9:414-27.
60. Wasil M, Halliwell B, Hutchison DC, Baum H. The antioxidant action of human extracellular fluids: effect of human serum and its protein components on the inactivation of alpha-1-antiproteinase by hypochlorous acid and by hydrogen peroxide. Biochem J 1987; 243:219-23.
61. Weiss SJ, Peppin G, Ortiz X, Ragsdale C, Test ST. Oxidative autoactivation of latent collagenase by human neutrophils. Science 1985; 227: 747-9.
62. Peppin GJ, Weiss SJ. Activation of the endogenous metalloproteinase, gelatinase, by triggered human neutrophils. Proc Natl Acad Sci USA 1986; 83:4322-6.
63. Shah SV, Baricos WH, Basci A. Degradation of human glomerular basement membrane by stimulated neutrophils: activation of a metalloproteinase(s) by reactive oxygen metabolites. J Clin Invest 1987; 79:25-31.
64. Okada Y, Watanabe S, Nakanishi I, et al. Inactivation of tissue inhibitor of metalloproteinases by neutrophil elastase and other serine proteinases. FEBS Lett 1988; 229:157-60.
65. Virca GD, Metz G, Schnebli HP. Similarities between human and rat leukocyte elastase and cathepsin G. Eur J Biochem 1984; 144:1-9.
66. Schraufstatter IU, Revak SD, Cochrane CG. Proteases and oxidants in experimental pulmonary inflammatory injury. J Clin Invest 1984; 73: 1175-84.
67. Sinha U, Sinha S, Janoff A. Characterization of sheep alpha-1-proteinase inhibitor: important differences from the human protein. Am Rev Respir Dis 1988; 137:558-63.
68. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG. Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. J Clin Invest 1987; 79:1665-73.

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69. Velvart M, Fehr K. Degradation in vivo of articular cartilage in rheumatoid arthritis and juvenile chronic arthritis by cathepsin G and elastase from polymorphonuclear leukocytes. Rheumatol Int 1987; 7:195-202.
70. Weiland JE, Garcia JG, Davis WB, Gadek JE. Neutrophil collagenase in rheumatoid interstitial lung disease. J Appl Physiol 1987; 62:628-33.
71. Edwards SW, Hughes V, Barlow J, Bucknall R. Immunological detection of myeloperoxidase in synovial fluid from patients with rheumatoid arthritis. Biochem J 1988; 250:81-5.
72. Damiano VV, Tsang A, Kucich U, et al. Immunolocalization of elastase in human emphysematous lungs. J Clin Invest 1986; 78:482-93.
73. Cochrane CG, Spragg R, Revak SD. Pathogenesis of the adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 1983; 71:754-61.
74. Weiss SJ, Test ST, Eckmann CM, Roos D, Regiani S. Brominating oxidants generated by human eosinophils. Science 1986; 234:200-3.
75. Kanofsky JR, Hoogland H, Wever R, Weiss SJ. Singlet oxygen production by human eosinophils. J Biol Chem 1988; 263:9692-6.
76. Hubbard RC, Ogushi F, Fells GA, et al. Oxidants spontaneously released by alveolar macrophages of cigarette smokers can inactivate the active site of α 1-antitrypsin, rendering it ineffective as an inhibitor of neutrophil elastase. J Clin Invest 1987; 80:1289-95.
77. Desrochers PE, Weiss SJ. Proteolytic inactivation of alpha-1-proteinase inhibitor by a neutrophil metalloproteinase. J Clin Invest 1988; 81:1646-50.
78. Lawrence DA, Loskutoff DJ. Inactivation of plasminogen activator inhibitor by oxidants. Biochemistry 1986; 25:6351-5.
79. Brower MS, Harpel PC. Proteolytic cleavage and inactivation of alpha 2-plasmin inhibitor and C1 inactivator by human polymorphonuclear leukocyte elastase. J Biol Chem 1982; 257:9849-54.
80. Barbey-Morel C, Pierce JA, Campbell EJ, Perlmutter DH. Lipopolysaccharide modulates the expression of alpha 1 proteinase inhibitor and other serine proteinase inhibitors in human monocytes and macrophages. J Exp Med 1987; 166:1041-54.
81. Stendahl O, Molin L, Dahlgren C. The inhibition of polymorphonuclear leukocyte cytotoxicity by dapsone: a possible mechanism in the treatment of dermatitis herpetiformis. J Clin Invest 1978; 62:214-20.
82. Cuperus RA, Hoogland H, Wever R, Muijsers AO. The effect of D-penicillamine on myeloperoxidase: formation of compound III and inhibition of the chlorinating activity. Biochim Biophys Acta 1987; 124-31.
83. Doherty JB, Ashe BM, Argenbright LW, et al. Cephalosporin antibiotics can be modified to inhibit human leukocyte elastase. Nature 1986; 322:192-4.
84. Thompson RC, Ohlsson K. Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. Proc Natl Acad Sci USA 1986; 83:6692-6.
85. Rosenberg S, Barr PJ, Najarian RC, Hallewell RA. Synthesis in yeast of a functional oxidation-resistant mutant of human alpha-antitrypsin. Nature 1984; 312:77-80.
86. Courtney M, Jallat S, Tessier L-H, Benavente A, Crystal RG, Lecocq J-P. Synthesis in *E. coli* of α_1-antitrypsin variants of therapeutic potential for emphysema and thrombosis. Nature 1985; 313:149-51.

MEDICAL INTELLIGENCE

A CHILD WITH PHENOTYPIC LARON DWARFISM AND NORMAL SOMATOMEDIN LEVELS

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THE regulation of somatic growth is complex and requires a number of hormones, including growth hormone, somatomedin C (insulin-like growth factor I), thyroid hormones, insulin, and sex steroids. A new development in the area of human growth hormone is the discovery of circulating growth hormone–binding proteins,[1-3] one of which appears to be a fragment of the growth hormone receptor.[4,5] The precise role of the binding proteins in the regulation of growth is unknown, but they may be important, directly or indirectly, in growth, since the principal binding protein is absent in patients with Laron dwarfism[6,7] and significantly decreased in African pygmies.[8] It is unclear whether the binding protein simply reflects growth hormone receptors in tissues or whether it is actively involved in growth promotion.

We recently observed a 4½-year-old child with severe growth retardation of unknown cause who had no growth hormone–binding protein activity in plasma. Phenotypically, this child resembled a Laron dwarf, but his biochemical profile differed from that for classic Laron dwarfism, in which somatomedin C levels are low, in that he had consistently normal somatomedin C levels. He had massive elevations of basal and stimulated levels of plasma growth hormone and resistance to endogenous and exogenous growth hormone, but his endocrine function was otherwise normal. His endogenous growth hormone was found to be normal on the basis of physicochemical and receptor-binding criteria. We report this unusual syndrome because it may suggest new insights into the potential importance of the growth hormone–binding protein.

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CASE REPORT

The patient was a boy who had been born to a 35-year-old Sardinian woman after an uncomplicated term pregnancy. At birth he measured 48.5 cm and weighed 3.15 kg. There was no family history of endocrine disease or short stature; his father, mother, and sister were of normal stature. There was no consanguinity between the parents. Both parents had normal basal and stimulated levels of plasma growth hormone as well as normal levels of somatomedin C.

The patient's early development was soon recognized as abnormal because of failure to thrive, short stature, and microphallus. When first seen at the age of two months, he was 50 cm tall and weighed 3.45 kg; both measurements were below the third percentile for his age. With the exception of the short stature, small genitalia, and mild facial hypoplasia, he was normal physically. Specifically, no skeletal abnormalities were present, body proportions were normal, and the size and shape of the sella turcica were unremarkable. The blood-cell count was normal, as were blood chemistry values, with the exception of glucose, which was repeatedly low (<2.2 mmol per liter [<40 mg per deciliter]). Serum levels of thyroxine (121 nmol per liter [9.4 μg per deciliter]), triiodothyronine (2.76 nmol per liter [180 ng per deciliter]), and thyrotropin (1.8 mU per liter) were all within normal ranges.

Fasting growth hormone levels, determined on several occasions, fluctuated between 2.02 and 3.04 nmol per liter (44 and 66 ng per

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