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Review Article

OXYGEN RADICALS, INFLAMMATION, AND TISSUE INJURY

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Abstract—Inflammatory reactions often result in the activation and recruitment of phagocytic cells (e.g., neutrophils and/or tissue macrophages) whose products result in injury to the tissue. In killing of endothelial cells by activated neutrophils as well as in lung injury produced by either activated neutrophils or activated macrophages there is evidence that H_2O_2 and iron play a role. HO· may be a key oxygen product related to the process of injury. Endothelial cells in some vascular compartments may be susceptible to neutrophil mediated injury in a manner that is independent of oxygen radicals. On the basis of in vitro observations, a synergy exits between platelets and neutrophils, resulting in enhanced oxygen radical formation by the latter. Finally, the cytokines, interleukin 1 and tumor necrosis factor, released from macrophages have both direct stimulatory effects on oxygen radical formation in neutrophils and can "prime" macrophages for enhanced oxygen radical responses to other agonists. Cytokines may also alter endothelial cells rendering them more susceptible to oxygen radical mediated injury by neutrophils. This suggests a complex network of interactions between phagocytic cells and peptide mediators, the result of which is acute, oxygen radical mediated tissue injury.

Keywords—Phagocytic cells, Neutrophils, Macrophages, Monocytes, Platelets, Tissue injury, Free radical

INTRODUCTION

There is abundant evidence that oxygen products of NADPH oxidase contained within phagocytic cells (neutrophils, monocytes, eosinophils, macrophages) play an important role in the injury associated with the triggering of an inflammatory process.[1,2] There is also emerging evidence that, in some cases, a linkage exists between the oxygen products of activated phagocytic cells and the xanthine dehydrogenase/oxidase pathway, as suggested by recent experiments in ischemia-reperfusion injury of myocardium or small intestine.[3] In this situation, it appears that products (perhaps chemotactic lipids) resulting from exposure of plasma to xanthine oxidase products bring about recruitment of neutrophils whose toxic oxygen products are most directly linked to tissue injury. Petrone et al.[4] and Perez et al.[5] have demonstrated generation of a chemotactic lipid from arachidonic acid following exposure to xanthine/xanthine oxidase, an oxygen radical-generating system that is suppressible with superoxide dismutase (see below).

With respect to toxic species of oxygen derived from inflammatory cells, it appears that $O_2^{\cdot-}$ has little direct toxicity for cells or tissues. However, as indicated above, there is an accumulating body of evidence that $O_2^{\cdot-}$ may contribute to the ultimate pathogenesis of injury following mobilization and activation of inflammatory cells. This may be related to transport of $O_2^{\cdot-}$ across anionic channels of the cell membrane, resulting in intracellular localization where reduction of ferritin associated Fe(III) to Fe(II) occurs, with resultant Fenton products (HO·) formed from H_2O_2. Recently it has been demonstrated that neutrophil mediated killing of endothelial cells is iron-dependent, with the source of the iron being the target (endothelial) cell, not the neutro-

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phil.[6] In parallel studies, it has been also shown that neutrophil mediated killing of endothelial cells is H_2O_2 and iron dependent as demonstrated by the ability of catalase and deferroxamine to protect the cells from injury.[7] While superoxide dismutase has limited protective effects against injury of endothelial cells produced either by H_2O_2 or by activated neutrophils, the ability of dimethylthiourea and dimethylsulfoxide to protect against target cell injury is consistent with role of HO·.

It is possible that target cells such as endothelial cells may also provide a source of O_2^-, perhaps in a manner analogous to hepatocytes undergoing H_2O_2 mediated injury.[8] In this latter case, cell injury has an absolute requirement for iron which is derived from the hepatocytes[9] and it has been shown that the hepatocyte serves as a source of O_2^-. Accordingly, it is postulated that within the hepatocyte O_2^- reduces Fe(III) to Fe(II), causing its release from ferritin. As the hepatocyte then is exposed to H_2O_2 from an extrinsic source, HO· is generated resulting in cytotoxicity. Thus, in this complex scheme of interaction both products from the target cells as well as from effector cells (such as phagocytic cells) are involved in the process leading to cell injury. With respect to neutrophil-endothelial interactions which result in cytotoxic effects on the endothelial cell, a similar story seems to be unfolding.

Endothelial cell killing by phorbol ester activated neutrophils is catalase sensitive and iron-dependent.[7] Iron appears to be contributed by the endothelial cells and not by the neutrophil since pretreatment of endothelial cells but not neutrophils with the iron chelator, deferroxamine, will prevent the killing of endothelial cells.[6] The protective effects of catalase but not SOD could suggest that if O_2^- is required for the killing process, it is not physically accessible to interception of SOD, either because of intracellular synthesis within the endothelial cell or because of intimate physical contact between the neutrophil and the endothelial cell. There is abundant evidence that endothelial cells contain xanthine oxidase which could serve as the source of O_2^-.[10,11] It is, of course, possible that the requirement for iron in H_2O_2 mediated killing of endothelial cells could be related to the formation of iron centered (ferryl and perferryl) radicals but the bulk of the evidence seems to suggest that the requirement of iron is more likely related to the formation of a Fenton reaction product such as HO·. There is relatively little evidence that hypochlorous acid (HOCl), a product of Cl^-, H_2O_2 and neutrophil derived myeloperoxidase (MPO), is involved in the cytotoxic reaction produced by activated neutrophils. In fact, inactivation of MPO accentuates the ability of neutrophils to kill endothelial cells.[12] It has recently been suggested that MPO may function as a scavenger for HO·,[13] which would provide an alternative explanation for why chemical inactivation of MPO accentuates endothelial cell injury. There is no doubt that generation of HOCl is inimical for tissues. This product is highly reactive and is cytotoxic for tumors and other cells.[14-16] It can bring out activation of collagenase and gelatinase which reside within granules of neutrophils, resulting in active enzyme species.[17,18]

Finally, the role of singlet oxygen ($^1O_2$) in tissue related injury is unknown. It has been predicted but not directly demonstrated that $^1O_2$ should be produced in biological systems. Singlet oxygen is formed as a product of several reactions[19] including a reaction between HOCl and H_2O_2.[20] The chemiluminescent response of activated phagocytes is thought to be mediated, at least in part, by singlet oxygen.[2] The chemical origin of the chemiluminescent reaction is unclear and there appears to be no specific scavenger or enzyme available to probe its role in biological systems, in contrast to O_2^- and H_2O_2. In order to understand more clearly the role of phagocytic cells and their products in tissue injury associated with the recruitment of inflammatory cells, we will briefly discuss the role of neutrophils, platelets, macrophages and cytokines in oxygen radical mediated tissue injury.

NEUTROPHILS

Neutrophils have long been known to be an important source of tissue-destructive mediators involved in the inflammatory response. In IgG-immune complex mediated vasculitis in the rat, neutrophil depletion or complement depletion virtually abolishes all parameters (edema, vascular permeability, hemorrhage) of vascular damage.[21] With respect to complement activation products, C5 deficient mice are protected against IgG immune complex induced injury and in these animals very few neutrophils accumulate at sites of immune complex deposition.[22] The complement requirement seems to be linked to generation of the chemotactic peptide, C5a, which is linked to the recruitment of neutrophils in these acute inflammatory reactions. There is as yet no direct evidence that the complement derived membrane attack complex (C5–9) participates in events leading to vascular injury, although the evidence is currently incomplete.

Complement-mediated and neutrophil-dependent microvascular injury in the lung has an absolute requirement for C3 as well as C5[22,23] but, again, whether components distal to C5 are involved in the pathogenesis of the vascular injury is not known. What can be said on the basis of recent studies is that C3 activation products are per se insufficient to produce lung in-

jury.[24] With respect to complement and neutrophil mediated injury of the lung, the question also remains open as to whether or not C5–9 participates in the injury, whether via cytotoxic or noncytotoxic events. C5–9 has been shown to cause O_2^- production in neutrophils[25] and to open a calcium channel in the membrane of platelets.[26] Although each of these events is related to noncytotoxic functions of C5–9, either could have phlogistic effects.

Both granule related products (proteases, peptides, etc.) and cell membrane associated products can be linked to the process of tissue injury following recruitment of phagocytic cells, especially neutrophils. IgG immune complex triggered pulmonary alveolitis and dermal vasculitis are well established models of neutrophil-mediated injury. While in both models there is evidence for the role of oxygen products from the neutrophil, there are some puzzling differences. In the case of injury to the lung, it appears that immune complex deposition leads to complement activation, generation of C5a, and recuitment of neutrophils from the vascular compartment. Interventions with SOD produce brief (1–2 h) protection from the injury, and during this period there is evidence of impairment in the recruitment of neutrophils.[27] With time, however, the protective effects of SOD are lost, and neutrophils accumulate in the tissue in large numbers. The transient effect of SOD may be due to increasing amounts of C5a being generated in the locales of deposits of immune complexes surpassing the ability of SOD to suppress generation of chemotactic lipid. Catalase provides consistent and sustained protection against immune complex induced lung injury, while not seeming to interfere with the influx of neutrophils.[28] As mentioned above, the protective effects of deferroxamine as well as dimethylthiourea and dimethylsulfoxide suggest that $HO\cdot$ is the most likely candidate for the tissue damaging effects of the neutrophil.

It seems probable that proteases released from the neutrophil, perhaps in concert with oxygen products, cause release of epithelial and endothelial cells from basement membranes resulting in permeability defects.[29] In addition, hydrolysis of basement membranes and other matrix products almost surely occurs, but evidence for these protease-induced events is at best indirect. There is good evidence for synergy between oxygen products of the neutrophil and granule-derived proteases, an example being the greatly enhanced proteolysis of vascular basement membrane exposed to H_2O_2 followed by addition of leukocytic protease or trypsin.[30]

In contrast to the well defined oxygen radical mediated damage of the lung microvasculature and alveolar compartment of IgG immune complexes described above, a different picture of the pathogenesis of vasculitis has emerged when the same immune complexes are deposited in vessels of the rat dermis. As referred to above, SOD has transient protective effects but catalase and deferroxamine are not protective.[31,32] On the other hand, these injurious inflammatory reactions have an absolute dependency on complement and neutrophil participation.[21] These observations suggest that perhaps the endothelial cells in the skin are resistant to oxygen radical mediated damage even though products from the recruited neutrophils are toxic to these cells. Such a possibility would be analogous to a recent report indicating that, while endothelial cells from human foreskin or unbilical vein are both killed by the presence of activated neutrophils, injury to the latter is via toxic oxygen products from the neutrophil while injury to the former is independent of oxygen products but apparently linked to a toxic factor in neutrophil granules. This factor may be linked to the catalytic activity or the cationic charge of elastase.[33]

The observations described above suggest a considerable complexity in neutrophil mediated damage of endothelial cells, due both to the differences in susceptibility of endothelial cells (based on their geographic location) to oxidant mediated damage and to diverse toxic products from the neutrophil.

PLATELETS

There is little question that platelets often accumulate in vascular lumens during inflammatory reactions and that their repertoire of phlogistic products is extremely broad. Recently some evidence has accumulated that the presence of platelets together with neutrophils leads to an accentuated O_2^- response of latter.[34] The enhanced O_2^- response of the neutrophil occurs even with platelets that have been pretreated with cyclooxygenase or lipoxygenase inhibitors and the enhancement in the O_2^- response can be reproduced by lysates of normal platelets or by secretion products of platelets treated with thrombin or immune complexes. The platelet related factor has a low molecular weight and has recently been shown to be related to the effects of ATP and ADP released from stimulated platelets.[35]

To what extent synergy between the platelet and the neutrophil is biologically significant remains to be determined. However, two experimental models of complement and neutrophil mediated acute lung injury have been investigated. Microvascular injury of the lung occurs after intravenous infusion of the cobra venom factor or following acute thermal trauma of the skin, in both models the injury to the microvascular endothelial cells injury being due to toxic oxygen products

from activated neutrophils (see above). When rats are platelet-depleted (using antibody) prior to induction of acute lung injury, there is a marked attenuation in the degree of increased vascular permeability.[36] Although it is not possible to prove the mechanism by which the availability of platelets leads to intensified injury of lung microvascular endothelial cells, the speculation that this is linked to platelet-neutrophil synergy of the type described above seems reasonable.

MACROPHAGES

Most organs contain either fixed or mobile macrophages that have substantial capacity to produce oxygen products from activated NADPH oxidase. The very small amounts of MPO in macrophages suggest that, in the absence of neutrophils, halide derivatives of H_2O_2 would be present in very small amounts. Using a variety of agonists, macrophages do not show the same pattern of O_2^- responses when compared to neutrophils. For instance, while IgG immune complexes stimulate O_2^- and H_2O_2 formation from both neutrophils and macrophages, IgA immune complexes are only stimulatory to macrophages.[37] Taking advantage of this observation, IgA and IgG immune complex lung injury models have been induced in rats. The acute lung injury resulting from IgA immune complex deposition is independent of the requirement for neutrophils (although the complement system is requisite for the injury).[38] Not surprisingly, in this model, bronchoalveolar lavage (BAL) results in the retrieval of few neutrophils, but an increased number of alveolar macrophages. These macrophages exhibit increased "basal" production of O_2^- and enhanced O_2^- production in the presence of a macrophage agonist. These cells can therefore be considered to be "primed" for enhanced oxygen radical responses. The ability of SOD, catalase, desferroxamine, DMTU and DMSO to protect against lung injury have indicated the role for toxic oxygen products (possibly HO·) in this model. Thus, it would appear that acute organ or tissue injury can be produced exclusively by oxygen products of activated tissue macrophages, the details of the pathogenic mechanism being similar to the story with the neutrophil as described above in then IgG immune complex model of acute lung injury.

Recent studies to assess qualitative or quantitative differences between interstitial and intraalveolar macrophages of the lung have revealed that macrophages from these two compartments are virtually indistinguishable with respect to morphological features, nonspecific esterase activity, phagocytic ability and generation of oxygen products.[39] It is of course difficult to assess the extent to which cells from each of these two compartments have been engaged in phlogistic responses in the lung. Whether rapid mobilization of interstitial macrophages into the intraalveolar compartment also occurs is not known. The demonstration that blood monocytes have greatly diminished O_2^- responses as compared to lung macrophages suggests that if blood monocytes are recruited into the lung, they must undergo some type of "maturation" in order to produce substantial oxygen radical responses in the lung.[39]

CYTOKINES AND OXYGEN RADICAL FORMATION

The demonstrated ability of macrophages to produce oxygen radicals is paralleled by their release of cytokines following stimulation with a variety of agonists. An especially potent agonist for macrophages is bacterial lipopolysaccharide. This bacterial product is especially effective in terms of its ability to cause macrophages to release the cytokines interleukin (IL-1) and tumor necrosis factor (TNF). These cytokines affect many different cells and have a variety of proinflammatory effects.[40] Of particular interest is the evidence that IL-1 and TNF can directly initiate oxidant production by phagocytes.[41,42] It has also been demonstrated that contact of macrophages with very low concentrations (e.g., $<10^{-9}$M) of these cytokines can "prime" macrophages but not neutrophils for enhanced O_2^- responses following addition of agonists such as IgG immune complexes.[43] Thus, TNF and IL-1 can affect macrophages by at least two different mechanisms to bring about enhanced oxygen radical responses. Recently, another mechanism has been discovered wherein cytokines can enhance oxygen radical mediated damage. In a time-dependent and dose-dependent manner, contact of endothelial cells in vitro with either IL-1 or TNF causes some type of change in the endothelial cell so as to enhance its susceptibility to oxygen radical mediated damage by activated neutrophils.[44] Damage to the endothelial cells occurs by phorbal activated neutrophils in an H_2O_2 and iron-dependent manner, similar to what has been described above. The ability of cytokines to alter the susceptibility of endothelial cells to injury is related to some type of noncytotoxic modification of the endothelial cells. The replating assay for IL-1 or TNF treated endothelial cells is a highly sensitive assay for evidence of damaged endothelial cells. No hint of injury to the endothelial cells by their prior contact with IL-1 or TNF has been found. As earlier reported by Pober et al.[45] endothelial cells so treated demonstrate enhanced adhesive interactions with neutrophils, a change that would increase the efficiency of cytotoxic interactions between endothelial cells and neutrophils. Whether there

are additional mechanisms underlying this phenomenon remains to be seen, but this evidence underscores the important role of macrophages and their products in oxygen radical produced tissue injury, and the findings suggest that in inflammatory reactions in which both neutrophils and macrophages are engaged, products of the macrophage can cause positive reinforcement of neutrophil mediated tissue injury.

SUMMARY

Inflammatory reactions involving activation of phagocytic cells often result in generation of a variety of toxic oxygen products which can directly or indirectly lead to tissue injury. Identification of the toxic oxygen species responsible for cell or tissue injury is difficult, but the theme often emerges that H_2O_2 and iron are key elements involved in events leading to injury, although myeloperoxidase and halide dependent derivatives of H_2O_2 also play important roles, perhaps more related to degradation of matrix products of connective tissue. The evolving evidence for a synergy between proteases and oxygen products is now emerging. Of considerable interest is the recognition of synergistic interactions between neutrophils and platelets and between macrophages and neutrophils, as related to in vitro generation of oxygen radicals as well in vivo reactions that involve a role for these cells in oxygen radical mediated injury. A better understanding of these interactions should lead to more effective therapeutic interventions in human diseases where related mechanisms are at play.

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