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Original Contribution

ROLE OF O_2^- IN NEUTROPHIL RECRUITMENT INTO SITES OF DERMAL AND PULMONARY VASCULITIS

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Abstract—Using analogous models of acute dermal vasculitis and alveolitis in rats, we have examined the role of oxygen-derived metabolities in the tissue damage associated with neutrophil influx into sites of immune complex deposition. In the lung, as previously reported, catalase and deferoxamine are highly protective, while superoxide dismutase (SOD) has a transient protective effect. The xanthine oxidase inhibitors, allopurinol, and lodoxamide, are also protective. In the skin, neither catalase (which has been covalently linked to the antibody) nor deferoxamine is protective, suggesting that H_2O_2 and iron are not absolutely required for the development of dermal vasculitis. In the skin, SOD, as well as the inhibitors of xanthine oxidase, have protective effects. These data suggest that the neutrophil-mediated pathways of immune complex injury in the dermal and pulmonary microvascular compartments are fundamentally different.

As a measurement of neutrophil accumulation, measurements of myeloperoxidase in tissue extracts have been employed. In both the lung and skin, the protective effects of SOD and the xanthine oxidase inhibitors are paralleled by reductions in neutrophil influx into sites of injury. In contrast, catalase and deferoxamine have no effect on neutrophil accumulation.

These data suggest that vascular beds in rat skin and lung are fundamentally different with respect to mechanisms of acute immune complex mediated injury. The data also provide evidence that O_2^- contributes significantly to the accumulation of neutrophils.

Keywords—Immune complexes, Vasculitis, Superoxide anion, Neutrophils, Xanthine oxidase, Free radicals

INTRODUCTION

It has been recognized for several years that oxygen derived free radicals and their metabolities are important mediators of microvascular injury.[1] In 1981, Granger et al. proposed a mechanism to account for the apparent role of superoxide anion in postischemic injury of the feline intestine.[2] Under conditions of ischemia or hypoxia, ATP-depleted cells exhibit increased permeability to extracellular calcium. A cytosolic calcium-dependent protease (calpain) is assumed to become activated, converting intracellular xanthine dehydrogenase into xanthine oxidase. In turn, xanthine oxidase catalyzes the univalent reduction of molecular oxygen resulting in superoxide anion formation.[3] The catabolic breakdown of ATP that occurs during this low-energy state provides abundant xanthine and hypoxanthine which are necessary substrates for xanthine oxidase. Although enzyme conversion times vary, it appears that nearly all rat tissues exhibit the generation of xanthine oxidase from xanthine dehydrogenase in response to global ischemia.[4,5]

Concomitant to investigations regarding the role of oxygen-derived metabolities in ischemia-reperfusion injury, considerable attention has been directed toward elucidating mechanisms of oxidant-mediated injury in acute inflammation. Activated neutrophils, as well as other inflammatory cells, possess a plasma membrane-associated NADPH oxidase that reduces molecular oxygen to superoxide anion.[6] Superoxide anion gives rise to several oxygen-derived species that have been

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shown to participate in proinflammatory processes (ref. 7; reviewed).

In 1980, Petrone et al.[8] and Perez et al.[9] described an additional potential role for O_2^- in acute inflammation. These investigators independently described the in vitro generation of a superoxide anion-dependent chemotactic factor in plasma. This chemotactic factor appears to consist of a lipid-albumin complex that attracts neutrophils without triggering their activation. It has been suggested that this factor may augment acute inflammatory responses by amplifying neutrophil recruitment. More recently, other investigators have shown that exposure of serum to oxygen radical generating systems can lead to formation of chemotactically active derivatives of C5 both through changes in conformation of the intact molecule or through its cleavage.[10,11] Delineation of the mechanisms of complement-independent neutrophil recruitment has received relatively little attention in immune complex vasculitis despite the observation that complement depletion does not completely abrogate vascular injury or neutrophil influx.[12,13]

Despite the clearcut potential for interface between ischemia-reperfusion and inflammation-associated microvascular injury, the relative balance between these processes in various pathophysiologic circumstances is unclear. While activation of xanthine oxidase seems to be associated with ischemia-reperfusion and neutrophil-derived (NADPH oxidase) O_2^- is associated with acute inflammation, these processes are not necessarily mutually exclusive. These issues are further clouded by apparent intrinsic differences in susceptibility of endothelial cells from different sources to oxidant-mediated damage. For instance, Varani et al.[14] have shown that monolayers of rat pulmonary artery endothelial cells are susceptible to neutrophil-derived oxidants and that the intrinsic susceptibility of these cells can be altered by various inflammatory mediators. Smedly et al.[15] have shown that monolayers of human microvascular endothelial cells from omental fat are resistant to neutrophil-derived oxidants, while umbilical vein endothelial cells are susceptible to physiologically relevant concentrations of H_2O_2. We have systematically compared, in the same animals, the susceptibilities of the pulmonary and dermal microvascular beds to injury induced by the influx of neutrophils following deposition of immune complexes. Experimental evidence indicates that these microvascular beds exhibit markedly different patterns of susceptibility to products of neutrophil mediated injury. Quantitative measurements of neutrophil influx suggest that these models neutrophil recruitment is in part related to the generation of O_2^-.

MATERIALS AND METHODS

Materials

Lodoxamide tromethamine was from The Upjohn Company, Kalamazoo, MI. Allopurinol, catalase, superoxide dismutase, hydrogen peroxide, ferritin, dextran blue and phenol red were purchased from Sigma Chemical Co., St. Louis, MO. Bovine serum albumin (fraction V, low endotoxin (<0.012 ng/mg), fatty acid free) was from ICN Immunobiologicals, Lisle, Il. Deferoxamine mesylate was purchased from CIBA-GEIGY, Summit, NJ.

Animal models

Male Long-Evans specific pathogen-free rats (300–400 g; Charles River Breeding Laboratories, Inc., Wilmington, MA) were used for all studies. Intraperitoneal injections of ketamine (2.5–5.0 mg/100 g body wt) and sodium pentobarbital (5 mg/100 g body wt) were given for sedation and anesthesia.

Under anesthesia, reversed dermal Arthus reactions and immune complex lung injury were induced in the same rats by intradermal injection of anti-bovine serum albumin (BSA) (250 μg; 50 μl) and intratracheal instillation of anti-BSA (1800 μg; 200 μl). The antibody was prepared from hyperimmune rabbit serum by isolating the IgG fraction by means of saturated ammonium sulfate (50%) precipitation and diethylaminoethyl-cellulose ion exchange chromatography as previously described.[12,16] Immediately after administration of antibody, the rats received intravenous injections of BSA (10 mg in 1.0 ml saline), which also contained an aliquot (800,000 cpm) of radioactive indicator (^{125}I-labeled nonspecific IgG) for quantitation of tissue injury.

At specified time points, the rats were sacrificed and tissue injury was quantitated by determination of a permeability index. As described previously, the permeability indices in these models equals the amount of radioactivity present within standard full-thickness skin sites or whole, saline-perfused lungs compared with the radioactivity within 1.0 ml of caval blood.[17]

Antioxidant interventions

Catalase (active or inactive) was covalently linked to IgG anti-BSA (6.5 units/μg antibody; see below), and as such, administered at the same time that immune complex injury was initiated. Intraperitoneal superoxide dismutase (3650 units/mg) was administered in equal 2.5 mg injections given 2 h before and 2 h after induction of injury. Deferoxamine was infused intra-

venously in two 5 mg doses; 30 min prior to initiation of injury at time zero. Intraperitoneal lodoxamide (1 mg/kg) was administered 10 min prior to initiation of injury and at 30, 60, and 120 min after induction of injury. Allopurinol (50 mg/kg in 0.1 N NaOH) was infused intravenously 1 h prior to initiation of injury. For all interventions, control rats received the appropriate vehicle or inactive catalase: IgG anti-BSA according to the above administration schedules.

Covalent cross-linking of IgG anti-BSA and catalase

Affinity purified IgG anti-BSA was linked to catalase (active or inactive) using glutaraldehyde as modified from Avrameas and Ternynck.[18] Catalase (10 mg) was dissolved in 0.2 ml 0.1 M phosphate buffer (pH 6.8) containing 1.25% glutaraldehyde (Electron Microscopy Services, Fort Washington, PA). The solution was allowed to stand overnight at room temperature and then passed through a Sephadex G-25 column (45 × 0.9 cm) equilibrated with 0.15 M NaCl. The void volume containing activated catalase (brown color) was collected and concentrated to 1.6 ml with an Amicon PM10 membrane. One ml of 0.15 M NaCl containing anti-BSA (5.4 mg) was added to this solution, followed by the addition of 0.1 ml of 1 M carbonate–bicarbonate buffer (pH 9.5). After 24 h at 4°C, 0.1 ml of 0.2 M lysine was added. Following exhaustive dialysis against normal saline the reaction mixture was characterized.

Measurement of O_2^-

Superoxide generation was measured by superoxide dismutase-inhibitable reduction of ferricytochrome *c* according to Babior et al.[19] Aliquotes of cells were incubated in duplicate samples woth phrobol myristate acetate (Consolidated Midland Corporation, Brewster, New York), FMLP (Sigma) or immune complexes (IgG anti-BSA:BSA) in the presence of 80 μM ferricytochrome *c* (horse heart, grade III, Sigma) with or without 10 μg/ml of superoxide dismutase (2700 units/mg; Sigma) in 1 ml of HBSS for 30 min at 37°C. As previously described, the concentration of antibody was determined by quantitative precipitin reactions.[20] Complexes were prepared with a constant antigen/antibody ratio (wt/wt) of 1:5. Neutrophils used in the in vitro functional assays were obtained from glycogen-elicited peritoneal exudates 4 h after the intraperitoneal instillation of 1.0% glycogen into adult rats.[21] The cell suspensions contained >94% neutrophils and were finally suspended in Hanks' balanced salt solution (HBSS), pH 7.35.

Tissue myeloperoxidase activity

Whole lungs or skin plugs were homogenized with a Polytron (Beckman) homogenizer (4 × 10 s at a setting of 4) using 6 ml of homogenization buffer. The homogenization buffer (50 mM phosphate, pH 6.0) contained 0.5% hexadecyltrimethyl ammonium bromide (Sigma) and 5 mM EDTA. Homogenized samples were then sonicated (3 × 10 s at a setting of 5) and centrifuged (3000 × g; 30 min) at 4°C. Myeloperoxidase activity in supernatants was assayed by measuring the change in A_{460} resulting from decomposition of H_2O_2 in the presence of o-dianisidine (Sigma).[22] For all experiments, at least 3 different volumes (10–150 μl) of each supernatant were assayed in order to generate a linear myeloperoxidase activity curve.

Tissue myeloperoxidase activity as a measure of neutrophil recruitment

In order to assess the use of tissue myeloperoxidase activity as a measure of neutrophil influx, known concentrations of glycogen-elicited neutrophils were added to normal rat lungs or skin plugs. These tissues were homogenized and assayed for myeloperoxidase activity as described above.

RESULTS

Characterization of IgG anti-BSA: catalase conjugates

To ensure spacial proximity of catalase to sites of immune complex formation, IgG anti-BSA and catalase were covalently linked using the two step glutaraldehyde coupling method described by Avrameas and Ternynck.[18] The catalase containing reaction mixture (as determined by brown color) eluting from the Sephadex G-200 void volume was chromatographed on Sephacryl S-300 which had been calibrated for molecular weight determination. As shown in Figure 1, there was a catalase-containing peak with a molecular weight of approximately 360,000 as well as catalase-containing material in the void volume. The molecular weight of 360,000 approximates the sum of IgG plus catalase (1:1 molar ratio). The 23–25 ml fractions were pooled and assayed for catalase activity and for anti-BSA activity. Equivalent concentrations (based on catalase absorbance maximum, A_{406}) of conjugated and native catalase were assayed for their capacities to degrade reagent H_2O_2 using the Thurman assay.[23] Conjugated catalase retained 84% of its native activity (data not shown). To verify the presence of anti-BSA in the catalase conjugates, a qualitative immunoprecipitation

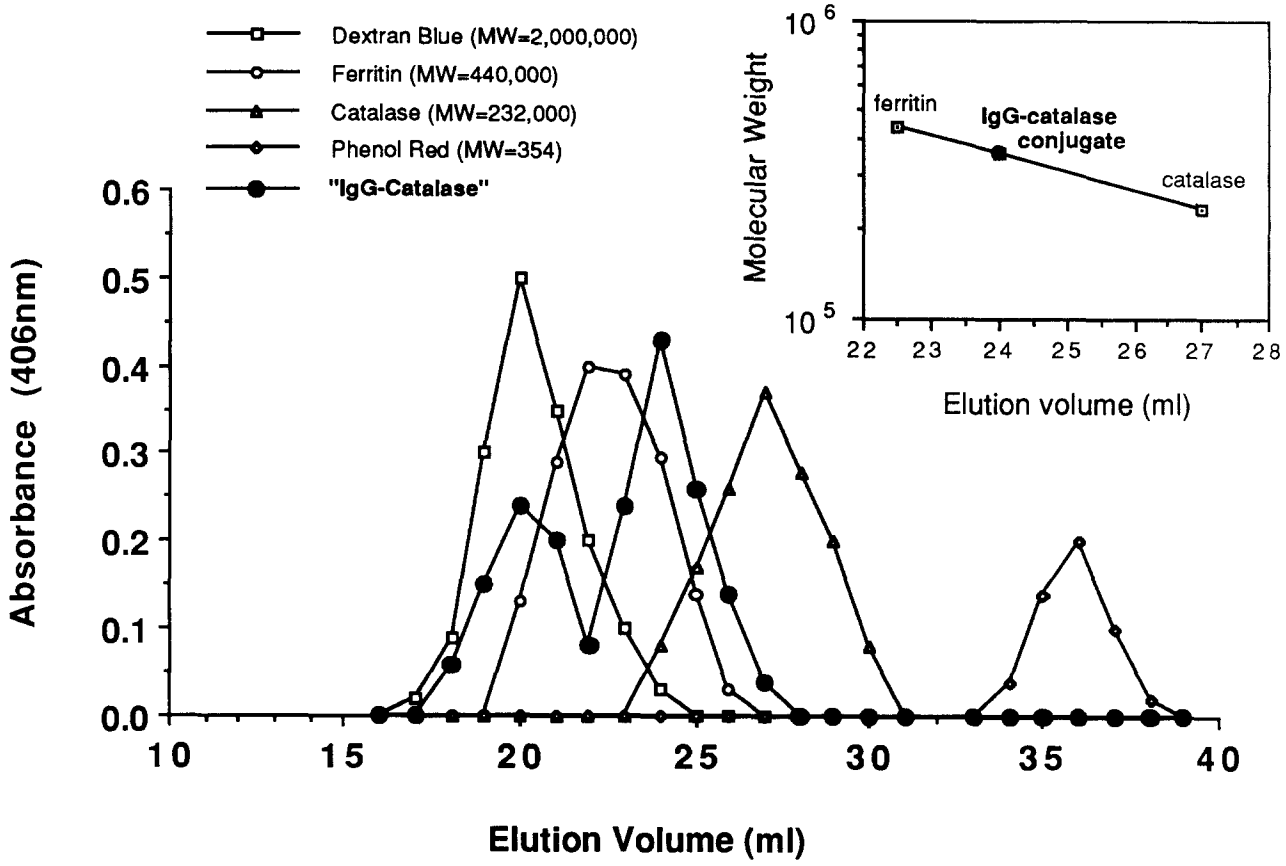

Fig. 1. Molecular weight determination of catalase linked to IgG anti-BSA using a Sephacryl S-300 column. Column: 0.9 × 38 cm; Buffer: 0.15 M sodium phosphate, pH 7.4; Rate: 0.6 ml/min.; Fraction size: 1 ml.

curve was constructed (Fig. 2). IgG anti-BSA: catalase conjugates exhibited an equivalence point and zones of antibody and antigen excess that were similar to the native antibody (not shown). Identically prepared conjugates containing IgG anti-BSA plus catalase that had previously been inactivated by alkylation[24] exhibited an immunoprecipitation curve similar to that obtained for IgG anti-BSA: catalase conjugates (Fig. 2). This conjugation procedure provided an antibody preparation containing approximately 6.5 units of catalase/μg IgG anti-BSA.

Effect of antibody conjugated catalase on development of lung and dermal vasculitis

Immune complex dermal vasculitis and alveolitis were induced using conjugates of IgG anti-BSA and inactivated catalase. Intradermal injections of conjugate resulted in permeability indices slightly exceeding half of the index values achieved with equal concentrations of unconjugated anti-BSA (Table 1). When dermal vasculitis was induced using anti-BSA linked to enzymatically active catalase, there was no reduction in vascular permeability compared to sites that received anti-BSA conjugated to inactive catalase. In marked contrast, there was a 64% reduction in lung injury in rats that received intratracheal anti-BSA conjugated to active catalase compared to rats that received intratracheal anti-BSA conjugated to inactive catalase (Table 1).

Effect of superoxide dismutase, deferoxamine, allopurinol, and lodoxamide on development of lung and dermal vasculitis

Treatment of rats with SOD revealed differing patterns of susceptibility of pulmonary and dermal vessels to injury associated with immune complex deposition. It should be noted that permeability indices at 1.5 hours were lower than the corresponding indices observed after 3 h. This is consistent with the expected time-dependent increase in tissue damage. When SOD-treated rats were sacrificed 1.5 hours after induction of injury there was a 68% reduction in dermal vascular injury and a 43% reduction in lung injury (Table 2, A). When identically treated rats were sacrificed at

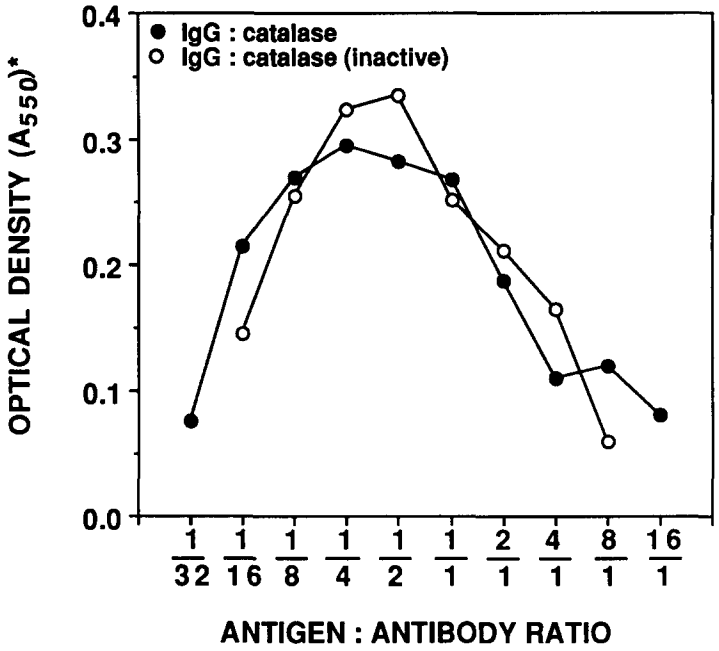

Fig. 2. Incubation of BSA with IgG conjugated to either active catalase or alkylated catalase (inactive) yields nearly superimpossible precipitation curves. Antigen-antibody equivalence (wt/wt) was determined as previously detailed (20).

3 h, there was a 50% reduction in dermal vascular injury but the protective effect in the lung had been lost (Table 2, B).

Treatment of rats with the iron chelator, deferoxamine, also revealed differences in the susceptibilities of the pulmonary and dermal microvascular beds to injury. In contrast to rats treated with catalase or SOD, parenteral deferoxiamine administration resulted in a 67% reduction in pulmonary vascular leakage but no effect on dermal vascular injury at 3.0 h (Table 2, C).

The antioxidant actions of catalase and SOD are predicated on the interception of oxygen-derived species *after* they have been formed. Likewise, deferoxamine is thought to abort the formation of one oxidant species (HO·) from another (H_2O_2) through its capacity to chelate ferrous iron. Because of the putative role of xanthine oxidase (XO) in the pathogenesis of ischemia-reperfusion injury and the possibility that this enzyme system may contribute to the oxidant burden in vasculitis, we examined the effects of two inhibitors of xanthine oxidase, allopurinol and iodoxamide, on the development of immune complex alveolitis and dermal vasculitis. The effects of allopurinol and lodoxamide treatments on the development of vasculitis generally paralleled those seen in rats treated with SOD. Allopurinol and lodoxamide diminished dermal vascular

Table 1. Effects of Catalase on Immune Complex Induced Tissue Injury*

	Dermal Vasculitis Model		Alveolitis Model	
Intervention	*n*	Permeability Index ($x \pm$ SEM)	*n*	Permeability Index ($x \pm$ SEM)
Saline	6	0.12 ± .04	6	0.09 ± .08
IgG†	6	0.58 ± .12	5	0.83 ± .18
IgG conjugated to inactive catalase‡	8	0.38 ± 0.9	7	0.66 ± .12
		NSt		$p < .05$§
IgG conjugated to active catalase‡	8	0.43 ± .10	8	0.39 ± .06

*All rats received BSA (10 mg, intravenous) at time zero.
†250 μg anti-BSA (intradermal); 1800 μg intratracheal
‡250 μg anti-BSA conjugated to 1584 units catalase (intradermal); 1800 μg anti-BSA conjugated to 11880 units catalase (intratracheal).
§One-way analysis of variance.

Table 2. Effect of Protective Interventions on Development of Lung and Dermal Immune Complex Vasculitis*

Experiment	Time of Reaction	Intravenous Injection	Intervention†	Permeability Indices ($\bar{x} \pm$ SEM) Lung	Skin
A.	1.5 Hours				
		Saline	—	.09 ± .12 (n = 6)	.06 ± .06 (n = 4)
		BSA	—	.49 ± .08 (n = 8)	.46 ± .07 (n = 9)
				$p < .05$‡	$p < .05$‡
		BSA	SOD	.23 ± .07 (n = 10)	.19 ± .04 (n = 10)
B.	3.0 Hours				
		Saline	—	—	.13 ± .04 (n = 5)
		BSA	—	.76 ± .14 (n = 12)	.69 ± .05 (n = 9)
				NS‡	$p < .05$‡
		BSA	SOD	.62 ± .11 (n = 11)	.41 ± .06 (n = 13)
C.	3.0 Hours				
		Saline	—	0.26 ± .04 (n = 6)	0.26 ± .13 (n = 6)
		BSA	—	0.87 ± .09 (n = 6)	0.85 ± .10 (n = 14)
				$p < .05$‡	NS‡
		BSA	Deferoxamine	0.41 ± .09 (n = 8)	0.88 ± .11 (n = 10)
D.	3.0 Hours				
		Saline	—	0.18 ± .04 (n = 6)	0.08 ± .01 (n = 5)
		BSA	—	0.67 ± .04 (n = 8)	0.90 ± .04 (n = 8)
				$p < .05$‡	$p < .05$‡
		BSA	Allopurinol	0.46 ± .06 (n = 8)	0.59 ± .05 (n = 13)
E.	3.0 Hours				
		Saline	—	0.16 ± 0.3 (n = 5)	0.11 ± .03 (n = 6)
		BSA	—	0.67 ± .07 (n = 6)	0.60 ± .05 (n = 12)
				$p < .05$‡	$p < .05$‡
		BSA	Iodoxamide	0.50 ± 0.03 (n = 6)	0.24 ± 0.08 (n = 14)

*Dermal and alveolar vasculitis were induced using 250 μg anti-BSA and 1800 μg anti-BSA as outlined in Table 1.
†Treatments with SOD, deferoxiamine, allopurinol, and lodoxamide are detailed in the Materials and Methods section.
‡One-way analysis of variance within respective groups.

injury by 38% and 73%, respectively (Table 2, D, E). Allopurinol-treated rats exhibited a 43% reduction in lung injury while lodoxamide-treated rats exhibited a 33% reduction in lung injury. The ability of these inhibitors to suppress tissue damage triggered by immune complex deposition suggests that NADPH oxidase (neutrophil-derived) O_2^- is not the sole precursor of oxygen-derived species involved in the pathogenesis of dermal vasculitis. Allopurinol and lodoxamide concentrations up to 10^{-4} M did not reduce O_2^- production by glycogen-elicited rat neutrophils (2×10^6/ml) stimulated with IgG-BSA immune complexes (10 μg antibody/ml), phorbol myristate acetate (20 ng/ml), or FMLP (10^{-5} M) (data not shown).

Relationship between tissue myeloperoxidase activity and neutrophil recruitment

As shown in Figure 3, a nearly linear relationship between tissue myeloperoxidase and number of added neutrophils could be constructed by utilizing an appropriate volume of (20–50 μl) of homogenized tissue supernatant in the o-dianisidine assay. It should be noted that in different experiments the selected volumes of supernatant varied but that they were constant within individual experiments. By increasing the volume of tissue supernatant to 150 μl myeloperoxidase activity from as few as $5–10 \times 10^6$ neutrophils per skin plug could be distinguished from background (data now shown). Despite this limitation in sensitivity, and some potential limitations in specificity, tissue myeloperoxidase activity measurements appear to provide an objective means of quantitating neutrophil influx into sites of inflammation.

Role of oxidant products in neutrophil recruitment

Assessment of neutrophil accumulation was accomplished by measuring myeloperoxidase activity in skin and lung extracts obtained from sites of immune complex-induced injury. Treatment of rats with SOD resulted in a 49% reduction at 1.5 h and a 40% reduction at 3.0 h in neutrophil influx into skin sites (Table 3). Allopurinol and lodoxamide treatments also resulted

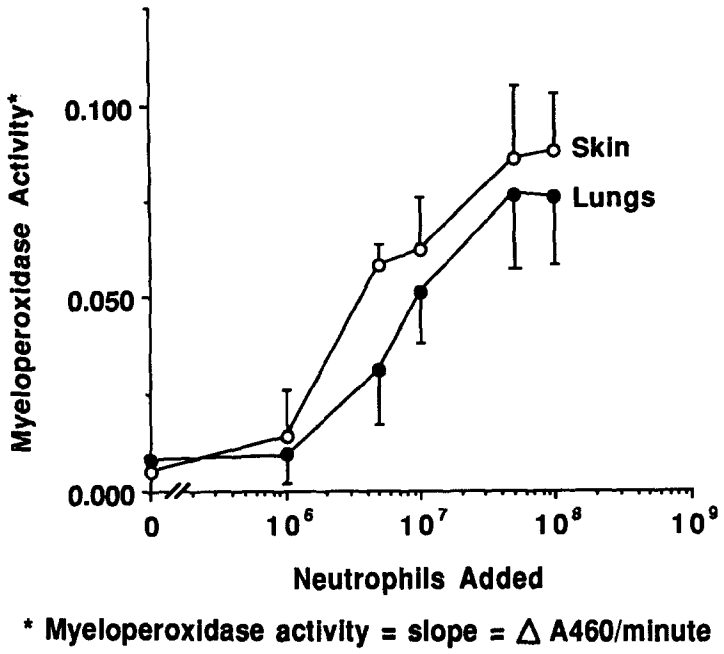

Fig. 3. Relationship between number of added glycogen-elicited neutrophils and tissue myeloperoxidase activity. Supernatants (25 μl and 50 μl, respectively) from homogenized lungs and skin plugs were assayed for myeloperoxidase activity as described in the Materials and Methods. Change in A460 was monitored for at least 3 min to ensure linearity. Data are expressed as means ± standard deviations (n = 4 for each point except 0 added neutrophils where n = 2).

Table 3. Neutrophil Influx into Sites of Immune Complex Injury

	Myeloperoxidase Activity†	
	Time	
Intervention*	1.5 Hours	3.0 Hours
Skin		
Positive controls	1.0 ± .08	1.0 ± .13
Negative controls	0.0 ± .01	0.0 ± .03
Catalase	—	1.12 ± .13
SOD	.51 ± .08‡	.60 ± .09‡
Deferoxamine	—	.96 ± .11
Allopurinol	—	.64 ± .04‡
Lodoxamide	—	.61 ± .09‡
Lung		
Positive controls	1.0 ± .09	1.0 ± .12
Negative controls	0.0 ± .03	0.0 ± .02
Catalase	—	1.15 ± .07
SOD	.73 ± .06‡	.99 ± .10
Deferoxamine	—	.87 ± .05
Allopurinol	—	.71 ± .06‡
Lodoxamide	—	.63 ± .09‡

*Immune complex alveolitis and dermal vasculitis were induced as described in Tables 1 and 2. Antioxidant intervention proptocols were also carried out as described in Tables 1 and 2.

†Myeloperoxidase activities are normalized within experiments such that all data are expressed relative to positive control values (1.0) and negative control values (0). Each normalized MPO value represents the mean ± SD of 7 to 18 tissues samples. In all experiments positive and negative control were run in triplicate in order to allow intraexperimental normalization of MPO activities. Myeloperoxidase activities were derived from Δ O.D. (A460) per minute resulting from decomposition of H_2O_2 in the presence of o-dianisidine.[22]

‡$p < .05$ versus positive controls; one-way analysis of variance.

in reductions (36% and 39%, respectively) in intradermal neutrophil influx. Catalase and deferoxamine did not diminish the recruitment of neutrophils into dermal vasculitis sites. Pretreatment of rats with SOD diminished neutrophil influx into the lungs at 1.5 h, but the effect was absent by 3.0 h. Allopurinol and lodoxamide treatments reduced neutrophil influx into the lungs by 29% and 37%, respectively (Table 3). Interventions (catalase, deferoxamine) that are highly protective against immune complex-induced alveolitis (Tables 1 and 2) had no effect on neutrophil accumulation indicating that the protective effects of these agents cannot be related to a failure to recruit neutrophils into sites of immune complex deposition. These data are consistent with the hypothesis that O_2^- plays an early role in neutrophil recruitment. The data also suggest that products (O_2^-) produced by xanthine oxidase may play a role in neutrophil influx into sites of immune complex vasculitis.

DISCUSSION

These studies provide direct evidence that the dermal and pulmonary microvascular beds of rats differ in the mechanisms through which acute neutrophil-mediated immune complex injury evolves. These studies also provide quantitative in vivo data that support a role for oxygen metabolite-derived products (O_2^-) in

neutrophil recruitment into sites of immune complex deposition. Reduced neutrophil influx into sites of dermal and pulmonary vasculitis in rats treated with allopurinol and lodoxamide suggests that metabolities derived from xanthine oxidase may contribute to neutrophil recruitment. This latter observation is consistent with the hypothesis that the formation of xanthine oxidase leads to the generation of O_2^- which in turn may amplify the influx of neutrophils.

It has previously been shown that intratracheal installation of catalase suppresses the development of immune complex alveolitis.[25,26] Surprisingly, whether given parenterally or intradermally with the eliciting antibody, catalase does not suppress injury resulting from the reversed dermal Arthus reaction.[27] McCormick et al. have speculated that lack of diffusability due either to the molecular weight (232,000) or positive charge of catalase might explain this disparity.[27] In the present study, we covalently cross-linked catalase and anti-BSA to ensure spacial proximity of immune complex deposits and active catalase. As shown in Table 1, the presence of inactive catalase linked to IgG anti-BSA substantially reduces the phlogistic potential of anti-BSA compared to equivalent concentrations of nonconjugated anti-BSA. Despite this, the ability of inactive catalase: IgG anti-BSA conjugates to form immune complexes and elicit tissue injury confirms the ability of this reagent to participate in the development of vasculitis. As in previous studies,[13,25-27] catalase attenuates lung injury but not dermal injury, suggesting that there is either an intrinsic difference in susceptibility between these two microvascular beds or that differing mediator pathways are preeminent.

Parenteral treatment of rats with SOD also suggests that the lungs and skin differ in their patterns of susceptibility to oxidants. It appears that the early development of injury (1.5 h) is heavily dependent on O_2^- generation, but that as the process evolves, the net effect of O_2^- becomes less prominent. The differing susceptibilities of lung and skin to intervention with deferoxamine are even more striking. Johnson et al. have previously shown that iron chelators exert a marked suppressive effect on the development of immune complex-mediated alveolitis.[25,26] Our data differ somewhat from a previous study in which systemic deferoxamine treatment reduced immune complex-mediated dermal vascular leakage by about 35%.[28] The explanation for this difference is unclear but could be due to differences in the time elapsed between induction of injury and measurement of dermal vascular permeability or due to intrinsic differences in the phlogistic characteristics of the anti-BSA preparation administered. Despite quantitative differences, these studies consistently support the premise that lung and dermal microvascular beds differ with respect to the protective effects of deferoxamine.

Systemic treatment of rats with allopurinol and lodoxamide also resulted in the suppression of both dermal and pulmonary immune complex vasculitis. These data, coupled with the observation that allopurinol and lodoxamide (up to 10^{-4} M) do not reduce neutrophil-derived O_2^- production in vitro, are consistent with the observation that xanthine oxidase plays a role in the pathogenesis of vasculitis. However, the data must be interpreted with caution because of the reported oxygen radical scavenging effects attributed to allopurinol and lodoxamide.[29,30]

Potential explanations for regional differences in microvascular susceptibility to various oxidants are speculative. First, dermal and alveolar microvascular endothelial cells may differ in their intrinsic susceptibility to oxygen metabolite-mediated injury. The observation by Varani et al.[14] that rat pulmonary artery endothelial cells are susceptible to lysis by neutrophil-derived oxidants coupled with the observation by Smedly et al.[15] that endothelial cells obtained from omental fat and umbilical veins differ in susceptibility to oxidants provides strong precedent for regional variation in endothelial cell phenotype. Aside from potential intrinsic differences in endothelia, there are also major anatomic and physiologic differences between the alveolar capillary and dermal vascular microenvironments. For instance, the pulmonary alveolar compartment maintains a higher pO_2 than does the dermis.[31] In vitro studies by Edwards et al. suggest that rat neutrophils exhibit a relatively high apparent Km (15–30 μM) for O_2 binding[32] and that tissue pO_2 may be a limiting factor in vivo during oxygen-radical-mediated tissue injury. It is possible that the role xanthine oxidase plays in the pathogenesis of vasculitis is related to the rapidity and efficiency with which xanthine dehydrogenase is converted to xanthine oxidase. Studies by Engerson et al.[5] suggest that rat tissues vary in their rates of conversion of xanthine dehydrogenase to xanthine oxidase during global ischemia and studies by Craddock et al.[33] suggest that intravascular aggregation of neutrophils may actually lead to localized microvascular ischemia. It is entirely possible, but unproven, that microvascular ischemia could lead to the local conversion of xanthine dehydrogenase to xanthine oxidase. Finally, there are major differences between lung and skin with respect to the densities and types of resident cells that secrete modulatory products. For instance, there is emerging evidence that alveolar macrophage products can modulate acute alveolitis.[34,35] Conversely, there is recent evidence that mast cell products such as histamine can augment xanthine oxidase activity.[36]

The neutrophil-trafficking studies provide quantitative data relating to cell recruitment in the presence of various anti-oxidant interventions. Superoxide dismutase-mediated reductions in neutrophil influx would be predicted in view of the reported in vitro actions of O_2^- in the generation of a chemotactic lipid from arachidonate.[8,9] More recent reports suggest that H_2O_2 can induce chemotactic activity via conformational changes and proteolytic changes in C5.[10,11] The observation that catalase has no net effect on neutrophil influx suggests that C5-hydrogen peroxide interactions are quantitatively less important in neutrophil recruitment than O_2^- mediated mechanisms. It is possible that the contribution of C5-hydrogen peroxide reaction products to the total chemotactic load is below the sensitivity provided by tissue myeloperoxidase measurements of neutrophil influx. It should be emphasized that these data do not obviate the chemotactic activities attributed to other mediators such as C5a generated via the complement cascade. While the present studies do not address the mechanisms involved in O_2^--mediated neutrophil influx, they support the hypothesis that oxidants play a role. The capacity of xanthine oxidase inhibitors to suppress the development of immune complex vasculitis and to diminish neutrophil influx into inflammatory sites suggests that xanthine oxidase-derived O_2^- may also play a role. Absolutely specific xanthine oxidase inhibitors will be required to definitively address this issue.

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ABBREVIATIONS

SOD—superoxide dismutase
NADPH—nicotinamide adenine dinucleotide phosphate hydride
ATP—adenosine triphosphate
BSA—bovine serum albumin
IgG—immunoglobulin G
HBSS—Hanks' balanced salt solution
EDTA—ethylene diamine tetraacetic acid
FMLP—*N*-formyl methionyl leucyl phenylalanine
SEM—standard error of mean