ROLE OF OXYGEN REACTIVE SPECIES IN <u>SCHISTOSOMA MANSONI</u>
EGG-INDUCED GRANULOMATOUS INFLAMMATION

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The role of oxygen reactive species in granulomatous hypersensitivity was explored using a model of pulmonary granulomas induced by intravenous injection of eggs from the parasite <u>Schistosoma mansoni</u>. Macrophages from sychronously developing lesions spontaneously released significant quantities of superoxide anion (26 nmoles/10^6/2h) by 8 days of development. In contrast, the non-T cell foreign body (Sephadex bead) granuloma macrophages produced only (2 nmoles/10^6/2h) small quantities.
    Daily administration of the oxygen scavenger, α-tocopherol, by either oral or parenteral routes caused up to 60% suppression of granuloma size. Moreover, parenteral administration of specific inactivators of O_2 and H_2O_2, superoxide dismutase and catalase respectively, resulted in a 30 to 40% reduction in granuloma size. These data suggest that oxygen reactive species take part in the generation of hypersensitivity granulomas.

Numerous studies have accumulated supporting the role of leukocyte-generated oxygen metabolites in microbial resistance and acute inflammation (1,2). While the bulk of evidence is derived from <u>in vitro</u> experiments, there are several studies that demonstrate an amelioration of tissue destruction and inflammation by employing inhibitors of oxygen metabolites <u>in vivo</u>. For example, parenteral administration of catalase or superoxide dismutase, which respectively catalyze the breakdown of hydrogen peroxide and superoxide anion, have been shown to inhibit immune complex-induced lung damage (3), carrageenan induced footpad edema (4), adjuvant induced arthritis and kaolin-induced granulomas (5).

    In a recent report we demonstrated that macrophages isolated from hepatic granulomas formed during experimental murine schistosomasis mansoni spontaneously produced significant quantities of superoxide anion during the vigorous unmodulated phase of the disease (6). In the present study, we further characterize the contribution of oxygen metabolites to the generation and mainte-nance of the hypersensitivity granulomas induced by <u>S. mansoni</u> eggs. By

induction of synchronous pulmonary egg granulomas we have demonstrated the time

course of O_2^- production by macrophages isolated from these lesions. Further-

more we show significant inhibition of granuloma size by the in vivo admini-

stration of superoxide dismutase or catalase. Likewise, the antioxidant,

vitamin E, given orally or parenterally resulted in notable inhibition of

granuloma formation. These results suggest that oxygen metabolites contribute

to the maintenance of hypersensitivity granulomatous reactions.

MATERIALS AND METHODS

Animals - Female, CBA mice obtained from the Jackson Laboratories (Bar
Harbor, Maine) were maintained under pathogen-free conditions and given mouse
chow and water ad libitum.
Induction of synchronous pulmonary granulomas and isolated of macrophages.
Synchronous pulmonary granulomas were induced by tail vein injection of 3000 S.
mansoni eggs. The latter were harvested aseptically from the livers mice
infected with 200-300 cercariae of the Puerto Rican strain of Schistosoma
mansoni as previously described (6). The pulmonary lesions were isolated at
designated times after embolization and macrophages were collected as pre-
viously described (7). Briefly, the granulomatous lungs were excised then
chopped in ice cold culture medium with a Waring blender. The granulomas were
collected on stainless steel screens then subjected to collagenase digestion
(Sigma Chemical Co., St. Louis, MO, Type IV from horseheart) to disperse cells.
Macrophages were allowed to adhere overnight then monolayers were washed free
of nonadherent cells. Culture medium was RPMI-1640 (Grand Island Biological
Co., Grand Island, N.Y.) with 10% fetal bovine serum (KC Biological, Inc.,
Lenexa, Kansas) and 10 μg/ml gentamicin (Schering Pharmaceutical Corp., Kenel-
worth, N.J.).
Determination of macrophage superoxide anion production. Superoxide was
measured by reduction of ferricytochrome c (Sigma Chemical Co., St. Louis, MO)
in Hanks' balanced salts solution as described by Johnston et al. (8). A two
hour incubation was employed and the total amount of superoxide dismutase
inhibitable O_2^- produced was normalized to cell number by direct counting of
macrophages adhering to the 35 mm culture dishes (Corning Glass Works, Corning,
N.Y.). Both phorbol myristate acetate (2 μg/dish) and opsonized zymosan (100
μg/dish) were used to measure elicited O_2.
Administration of oxygen metabolite inhibitors. Both catalase and super-
oxide dismutase (Sigma) were given intraperitoneally at the doses indicated in
the text. Vitamin E, D-α-tocopherol (Sigma), was given i.p. or by feeding tube
diluted in peanut oil. Doses of peanut oil provided a control.
Granuloma measurement. Granuloma area was determined by histological
examination of tissue sections of lungs harvested at 8 days post egg emboliza-
tion using an Omicron-Alpha image analyzer (Bausch and Lomb, Rochester, N.Y.)
as previously described (9). At least 20 granulomas were measured from each
mouse lung with a minimum of five mice per group. The Student's t-test was
used to compare control and treated groups and p > .05 was considered not
significant. In general, differences in granuloma size of less than 20% were
not significant.

RESULTS

Production of superoxide anion by macrophage from synchronously develop-

ing granulomas. The macrophage is thought to be a key effector cell in the

generation and maintenance of granulomas (10). Therefore, we initially wished

TABLE I

Source of Granuloma Macrophages	Granuloma Area $(\times 10^{-3} \mu m^2)$	nmoles of $O_2^-/10^6$ cells/2 hr in presence of:		
		HBSS [*]	PMA	ZYM
4 day egg	9.6 ± 0.7	0.0 ± 0	54.3 ± 7.8	81.3 ± 3.3
8 day egg	19.7 ± 1.5	26.2 ± 5.2	118.4 ± 16	119.8 ± 42
16 day egg	39.5 ± 2.2	13.3 ± 9.2	115.2 ± 19	149.1 ± 21
32 day egg	22.5 ± 2.0	13.6 ± 8.6	96.1 ± 15	171.5 ± 16
8 day Sephadex bead	7.2 ± 0.4	2.0 ± 1.34	105 ± 16	N.D.

[*] Mean ± SEM of 4-7 experiments. Each experiment was performed in duplicate or triplicate. N.D. not determined.

to establish the capacity of granuloma macrophages to produce O_2^- and to determine if this could be related to the time course of granuloma development. As shown in Table 1, granuloma macrophages from early 4d lesions showed no spontaneous O_2^- production. Subsequent to this time spontaneous O_2^- production could be detected, the greatest quantity being observed at 8 days when granuloma development was in its most accelerated phase. In contrast, macrophages from the non-T cell-mediated Sephadex bead granuloma produced low levels of O_2^- at day 8. These differences could be largely overcome by providing a maximal stimulus with PMA or zymosan which elicited a large burst of O_2^- from all granuloma macrophages (Table 1). However, macrophages from 4d lesions tended to produce lesser amounts.

Effect of α-tocopherol on schistosome egg granuloma formation. Vitamin E, α-tocopherol, has well known antioxidant activity (11). Thus, it was of interest to determine if this compound would affect granuloma development. Figure 1 shows the effect of daily oral administration of graded doses of Vitamin E. A maximum of 39% suppression of granuloma formation was obtained with 100 units of oral vitamin E per day. In contrast peanut oil did not significantly inhibit granuloma development. Interestingly, when given by intraperitoneal route, vitamin E caused a 60% suppression of granuloma formation.
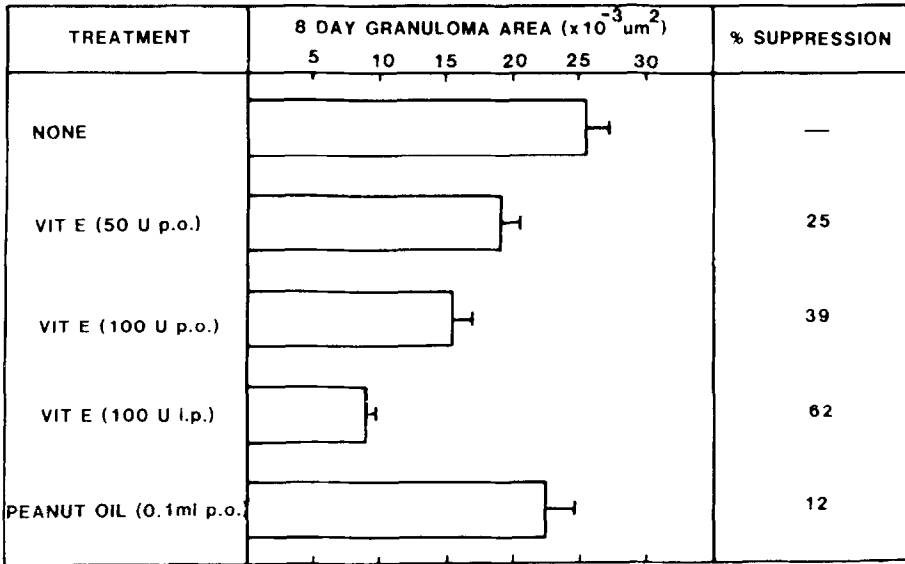
Figure 1.   Effect of α-tocopherol (vitamin E) on hypersensitivity granuloma
                formation. Bars represent the mean + SE. Five mice per group. A
                minimum of 20 granulomas were measured from each lung. Mice were
                given daily 0.1 ml doses of vitamin E dissolved in peanut oil by the
                indicated routes.

Effect of catalase and superoxide dismutase on schistosome egg granuloma

formation. The above studies suggested that oxygen metabolites may contribute

to hypersensitivity granuloma formation. Therefore, we next parenterally

administered bovine superoxide dismutase or catalase, specific inactivators of

O_2^- and H_2O_2 respectively, to help further identify the metabolites involved.

As shown in Figure 2 catalase caused a dose-dependent suppression of granuloma

formation. A 30% suppression was achieved with a 5 mg/day dose. Likewise, SOD

caused a 40% suppression of the 8 day lesion. The control protein, bovine

serum albumin did not significantly suppress the lesion at comparable doses.

Interestingly, SOD and catalase given in combination did not suppress beyond

40% (data not shown). These results suggested that both H_2O_2 and O_2^- contribute

to the granulomatous inflammatory response.

DISCUSSION

    The capacity of leukocytes to produce a variety of oxygen radicals in

vitro is well established, but their precise function in vivo is not fully

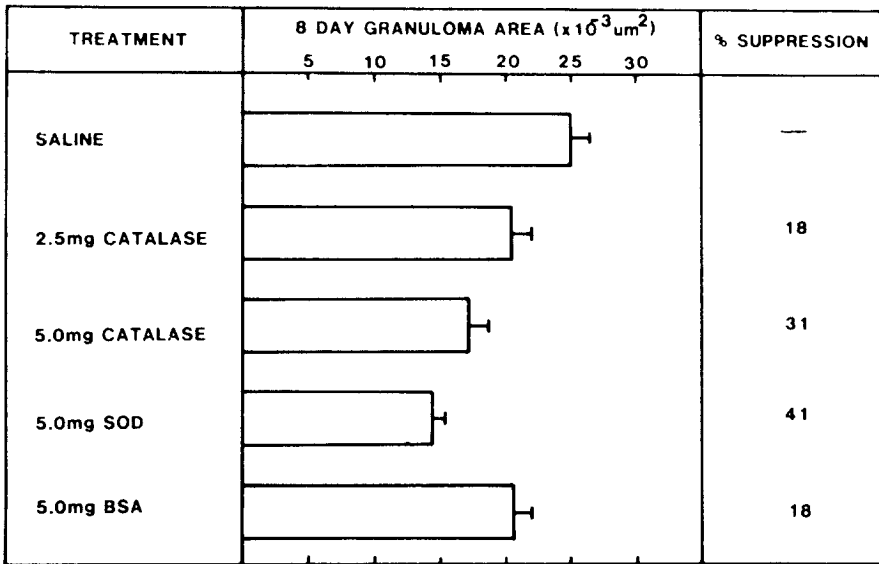| TREATMENT	8 DAY GRANULOMA AREA $(\times 10^3 um^2)$	% SUPPRESSION
SALINE		—
2.5mg CATALASE		18
5.0mg CATALASE		31
5.0mg SOD		41
5.0mg BSA		18

Figure 2.   Effect of catalase and superoxide dismutase on hypersensitivity
              granuloma formation. Bars represent the mean + SE. Five mice per
              group. A minimum of 20 granulomas were measured from each lung.
              Mice were given daily intraperitoneal injections of the enzymes
              dissolved in saline.

understood. It is known that rare individuals with genetic defects affecting
their ability to generate these products have clearly impaired microbicidal
capacity (12). Furthermore, animal studies suggest that oxygen radicals are
important in cell and tissue injury (1,3). Attempts to demonstrate the phlo-
gistic activity of oxygen radicals have primarily utilized models of acute
inflammation. For example, parenterally administered SOD has been shown to
suppress the reversed passive Arthus reaction (13), immune complex-induced
alveolitis (3) and pleural effusions (5) or footpad edema (4) induced by
carrageenan. However, few studies have implicated a role for oxygen radicals
in chronic inflammation.

    In a previous report we demonstrated the capacity of macrophages from
hepatic granulomas generated in murine schistosomiasis mansoni to spontaneously
produce 0_2^- during the vigorous phase of granulomatous hypersensitivity (7). In
the present report we employed a model of synchronous schistosome egg-induced
pulmonary granuloma formation in order to more specifically delineate the role
of oxygen radicals in granulomatous hypersensitivity. Indeed, our results

confirm and extend our original observation providing evidence that oxygen radicals contribute to granulomatous inflammation. It is noteworthy that significant spontaneous O_2^- release by macrophages was not detected at day 4. Since this time precedes the accelerated hypersensitivity phase of granuloma formation, this observation may be related to the lack of macrophage activation by immune T cell-derived products. This is further supported by the observed low levels of O_2^- production by macrophages from Sephadex bead granulomas which lack antigen-specific T-cell involvement. All of the populations produced O_2^- with an exogenous stimulus, PMA or zymosan. Thus, regardless of the inciting nidus granuloma macrophages have a higher activation state than resident peritoneal macrophages which fail to produce O_2^- in response to exogenous stimuli (3).

The ability of vitamin E, an oxygen radical scavenger, and oxygen radical degrading enzymes to ameliorate hypersensitivity granuloma formation provides further evidence for a proinflammatory function of reactive oxygen species. The suppressive effect of SOD and catalase implicate both O_2^- and H_2O_2 as participants in granuloma formation. Similar findings were previously reported in a model employing kaolin-induced granulomas (5). The precise mechanism by which reactive oxygen species promote inflammation is not known, but a variety of mechanisms such as local inactivation of neutral proteinases (14), generation of chemotactic factors (13), and lymphocyte activation (15) have been suggested. Interestingly, the release of reactive oxygen species by macrophages has been linked to arachidonic acid metabolism. Specifically, arachidonic acid or its lipoxygenase derivative, 15-HETE, were shown to induce oxygen radical synthesis and may represent second messengers for oxygen metabolism (16,17). This may have relevance to our recent demonstration that schistosome egg-induced granuloma macrophage are capable of significant spontaneous arachidonic acid release and metabolism by lipoxygenases (18). Moreover, granuloma formation could be significantly suppressed by agents with anti-lipoxygenase activity. Further analyses are currently underway to delineate the specific biochemical determinants of granulomatous hypersensitivity.

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