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Research Article

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Hydrogen Peroxide Release by Alveolar Macrophages from Sarcoid Patients and by Alveolar Macrophages from Normals after Exposure to Recombinant Interferons αA, β, and γ and 1,25-Dihydroxyvitamin D_3

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Abstract

We measured H_2O_2 release by human alveolar macrophages (AM) from normals and sarcoid patients in suspension immediately after bronchoalveolar lavage in the presence and absence of the triggering agent, phorbol myristate acetate (PMA). AM from 11 sarcoid patients produced a mean (±SE) of 21.7±2.3 and 5.9±3.4 nmol $H_2O_2/10^6$ macrophages in the presence and absence of PMA, respectively. By contrast, AM from normals (n = 6) produced 9.8±1.7 and 1.6±0.7 nmol $H_2O_2/10^6$ macrophages with and without PMA, respectively. Macrophage activation, as monitored by H_2O_2 production, did not correlate with the angiotensin-converting enzyme levels, the result of gallium-67 scans, or the percent of lymphocytes in the bronchoalveolar lavage. To determine whether AM from normals could be stimulated to increase their H_2O_2 production to the level seen in patients with sarcoid, we measured H_2O_2 released by adherent AM after incubation in each of four potential activating agents: recombinant interferons αA, β, γ (rIFNαA, rIFNβ, and rIFNγ, respectively), and 1,25-dihydroxyvitamin D_3. H_2O_2 release in the range seen in sarcoid patients could be induced in PMA-triggered AM from normals by rIFNγ in a time- ($t_{½}$ ~ 1 d) and dose-dependent fashion (threefold increase, EC_{50} 5 antiviral U/ml) and by rIFNαA and rIFNβ at higher concentrations, but not by 1,25-dihydroxyvitamin D_3.

Introduction

A dramatic change in oxidative metabolism, known as the respiratory burst, occurs in macrophages triggered by phagocytosis or soluble secretagogues such as phorbol myristate acetate (PMA) (1); prior priming of the cell by immunologic stimuli enhances this response (2). A major feature of the respiratory burst is the generation of superoxide anion and its dismutation product, H_2O_2. A portion of the oxygen metabolites produced by these cells escapes intracellular degradation and is released outside the plasma membrane, where it can damage surrounding cells (3). In lung diseases such as sarcoidosis, which are characterized by an increased and immunologically activated population of macrophages, H_2O_2 production may itself be a mechanism of parenchymal injury. Also, H_2O_2 production can be seen as a marker of macrophage activation. Prior studies have shown that mononuclear cell activation can be accompanied by the release of neutral proteases (4), acid hydrolases (5), growth factors (6), and inflammatory cytokines such as interleukin 1 (IL-1) (7, 8). Although the majority of sarcoid patients recover with minimal or no damage to the lung parenchyma, lung disease is seen 5 yr after initial evaluation in ~ 18% of patients presenting with stage I disease (9). In this subset of patients the chronic release of secretory products by activated macrophages may play a role in causing progressive lung disease.

In the present investigation we have looked at H_2O_2 release by alveolar macrophages (AM)[1] from normals and sarcoid patients in the presence and absence of the triggering agent, PMA, and correlated this with commonly used parameters of disease activity. Also, to determine whether the mature, low H_2O_2-releasing AM found in normals are capable of being activated to the level of H_2O_2 release observed in the sarcoid patients, we have studied the capacity of these resident cells in monolayer cultures to respond to four potential activating agents: recombinant interferons αA, β, γ (rIFNαA, rIFNβ, and rIFNγ, respectively), and 1,25-dihydroxyvitamin D_3. IFNγ and 1,25-dihydroxyvitamin D_3 are of particular interest in sarcoid. IFNγ, a product of antigen-stimulated T cells and natural killer cells, increases oxidative metabolism in other populations of mononuclear phagocytes (2) and has recently been shown to enhance oxidative and antimicrobial activity in AM from acquired immunodeficiency syndrome (AIDS) patients and normals (10). It has also been shown to induce a variety of other markers of cellular activation such as the expression of DR Ag (11) and Fc receptors (12, 13), increased cellular IL-1 production (7), and complement production (14). In sarcoid patients, lung lymphocytes have been shown to release interleukin 2 (IL-2) (15, 16), a factor that stimulates T cell production of IFNγ. IFNγ production by lung lymphocytes from these patients has also been demonstrated (17), and there is evidence that IFNγ may, also, be released by the AM themselves (17, 18). Finally, AM from some sarcoid patients hydroxylate vitamin D_3 to its active form, 1,25-dihydroxyvitamin D_3 (19). This molecule has a wide range of immunologic activity (20) and has been reported to enhance oxidative metabolism in monocytes (21). Thus both IFNγ and 1,25-dihydroxyvitamin D_3 are agents by which AM in sarcoid could potentially be activated.

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1. *Abbreviations used in this paper:* ACE; angiotensin-converting enzyme; AM, alveolar macrophage(s); BAL, bronchoalveolar lavage; HIA, high intensity alveolitis; HPO, horseradish peroxidase; rIFNαA, rIFNβ, and rIFNγ, recombinant interferons αA, β, and γ, respectively.

Methods

Study population. Three men and three women, all nonsmokers with no prior history of pulmonary disease, were designated as normals. These subjects ranged from 24 to 33 yr of age with a mean (±SD) of 27±4 yr. The sarcoid group consisted of 11 patients with untreated, biopsy-proven sarcoid who were referred to the New York Hospital Pulmonary Clinic. There were five female and six male patients. Their ages ranged from 25 to 63 yr with a mean of 34±11 yr. All but two of these patients were nonsmokers. Of these two, one had smoked one-half pack per day for 6 yr and the other, three to four cigarettes per day for 2 yr. All the diagnostic biopsies were performed at New York Hospital. Using the standard roentgenographic classification of sarcoid (22), seven patients had stage I disease and four, stage II. All patients had an angiotensin-converting enzyme (ACE) level determined by radioimmunoassay (SmithKline Corp., Bioscience Laboratories, Lake Success, NY) within 1 wk of their bronchoscopy and had gallium-67 (^{67}Ga) scans performed within 2 wk of bronchoscopy. The ^{67}Ga scans were evaluated by visual scanning. ^{67}Ga scans were rated on a scale of one to four by nuclear medicine radiologists at New York Hospital. Lung scans were rated as one if they had an uptake of radioactive material in the lungs that was less than or equal to that of the background, as defined by non-specific uptake in the abdomen and extremities. ^{67}Ga scans were rated two if the uptake in the lungs was greater than background, but less than that of the liver. A rating of three was given to scans where the uptake in the lungs was equal to that in the liver, and a rating of four, to scans in which the lung uptake exceeded that of the liver. For this study, a negative scan was defined as a scan that received a rating of one and a positive scan, as one that was rated ≥ 2. Of the ^{67}Ga scans performed on patients in this study, none received a rating of 2.

Isolation of human AM. AM were obtained by fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) in patients and normals, who had given informed consent. After 2% lidocaine anesthesia of the nose and upper airways, the bronchoscope (model FB-19D; Pentax Precision Instruments, Norwood, NJ) was passed transnasally and wedged into a subsegmental bronchus of the right middle lobe or lingula. Sterile saline (0.9%), 210 ml in 30 ml aliquots, was instilled into and then withdrawn from the lung. The lavage fluid was centrifuged at 130 *g* for 10 min at 5°C, and the cells were suspended in phosphate-buffered saline (PBS) and centrifuged again. The cells were then either resuspended in PBS at 2×10^6 cells/ml for immediate assay in suspension or at 2×10^5/ml in RPMI 1640 medium (Gibco, Grand Island, NY), which contained 15% AB positive human serum plus 100 U/ml penicillin and 100 μg/ml streptomycin (culture medium).

Characterization of BAL cell populations. In the sarcoid patients 4.6×10^6–40.0×10^6 nucleated cells were obtained per procedure (mean [±SD] $15.7 \times 10^6 \pm 10.2 \times 10^6$ cells). In the normals 4.3×10^6–18.0×10^6 nucleated cells were harvested per procedure (mean[±SD] $10.0 \times 10^6 \pm 5.2 \times 10^6$ cells). Of these, > 90% were viable as determined by trypan blue staining. Differential counts of cytocentrifuged, Wright's stained preparations indicated that ≤ 2% of the cells from each lavage were PMNs. 2% or less of the cells were positive when stained for myeloperoxidase (23). The differential of the normals' BAL cells (mean±SD) was 91.5±3.0% macrophages, 6.8±2.4% lymphocytes, and 1.4±0.4% PMNs. The differential of the sarcoid patients' BAL cells was 64.4±20.3% macrophages, 32.0±19.7% lymphocytes, and 1.2±0.9% PMN. The sarcoid patients' BAL lymphocyte counts ranged from 12 to 64% and those of normals, from 3 to 10% of the total cells.

Cell cultures. Where indicated, cells were cultured in 6-mm diam, flat-bottomed wells in 96-well plates (Costar, Cambridge, MA) at 4×10^4 cells/200 μl culture medium. Separate wells were designated for cell-free controls and for protein standards as previously described (24). After allowing the cells to adhere for 2 h at 37°C in 95% air/5% CO_2, the medium was aspirated through a 21-gauge needle and replaced with 200 μl fresh medium with or without various concentrations of test agents. Each condition was tested in triplicate. The RPMI was tested for endotoxin (chromogenic limulus amebocyte lysate test) and found to have < 10 pg/ml. Pure rIFNγ (specific activity 2×10^7 antiviral U/mg protein) was provided by Genentech Inc. (San Francisco, CA). The rIFNγ was determined by limulus amebocyte lysate test to have < 0.125 endotoxin U/mg protein. Pure rIFNαA and rIFNβ (specific activity 2×10^8 U/mg protein) were provided by Hoffman-La Roche, Inc. (Nutley, NJ), where the recombinant stock solution was found to be nonpyrogenic in rabbits. 1,25-Dihydroxyvitamin D_3 was kindly provided by Dr. Milan Uskokovik (Hoffman-La Rouche, Inc.).

H_2O_2 assay. H_2O_2 was assayed by measuring the loss of fluorescence of scopoletin after its oxidation by H_2O_2 as catalyzed by horseradish peroxidase (HPO). The assay was performed with cells in suspension (25) or with adherent cells (24) as described below.

In the first system the assay mix was as follows: to the first and last of four quartz cuvettes (4 ml vol, 1 cm light path) were added 40 nmol scopoletin, 15 purpurogallin U HPO, 0.3 mM NaN_3, 0.4 ml cells in PBS at 2.5×10^6 cells/ml, and modified Krebs-Ringer phosphate buffer with glucose (145 mM NaCl, 4.86 mM KCl, 0.54 mM $CaCl_2$, 1.22 mM $MgSO_4$, and 5.7 mM sodium phosphate with 5.5 mM glucose) to a final volume of 3 ml. To confirm that decreases in fluorescence were due to H_2O_2, controls consisted of a second cuvette in which HPO was omitted and a third cuvette to which 3,220 U of catalase (Sigma C-100; Sigma Chemical Co., St. Louis, MO) were added. The contents were mixed by inversion and equilibrated to 37°C in a thermostatted fluorometer (Hitachi MPF-4A; Perkin-Elmer Corp., Instrument Div., Norwalk, CT). A baseline reading was made for 2 min; then the triggering agent, 300 ng PMA, was added to cuvettes one to three; the contents were mixed; and fluorescence was recorded at 4-min intervals for each cuvette until no further change was noted (usually 90–120 min). The kinetics of the respiratory burst did not differ between the normals and the sarcoid patients.

In the second assay system (24), at the indicated time points, the cultures were washed three times by submerging the plate in normal saline at 37°C, and inverting and flicking the plates. Next, 100 μl of an assay mix consisting of 24 μM scopoletin (Sigma Chemical Co.), 1 mM NaN_3 (Fisher Scientific Co., Fair Lawn, NJ), and 1 U purpurogallin/ml HPO (Sigma Type II; Sigma Chemical Co.) with or without 100 ng/ml PMA (Sigma Chemical Co.) in Krebs-Ringer phosphate buffer with glucose were added to each well. The plates were placed in a filter fluorometer (Micro Fluor MR 600; Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, VA), and the fluorescence was recorded for each well. The plates were then incubated in water-saturated air at 37°C for 60 min, and the fluorescence of each well was again recorded.

Cell protein assays and cell counts. After the three rinses of the 96-well plates, designated wells, on the same plate as the wells to be assayed for H_2O_2 release, received 100 μl of a solution containing 1% (wt/vol) cetyltrimethylammonium bromide in 0.1 M citric acid with 0.05% (wt/vol) naphthol blue black, pH 2.2. This solution lyses cells and stains the nuclei (26). After a 10-min incubation in water-saturated air at 37°C the solution was pipetted vigorously and aliquots were removed for three hemocytometer counts per well of stained nuclei.

Protein determinations on the 96-well plates were made by a modification of the method of Lowry et al. (27) as previously described (25). Protein standards (0–10 μg bovine serum albumin in 100 μl) were prepared in the H_2O_2 assay mix used for the same plate. The absorbance at 690 nm was measured in each well using a filter-absorbance reader (Bio-Tek EL307, Bio-Tek Instruments, Inc., Burlington, VT).

For adherent cells, nanomoles of H_2O_2 per milligram of cell protein and nanomoles of H_2O_2 per 10^6 macrophages were calculated as described (25). For cells in suspension, H_2O_2 was expressed in nanomoles per 10^6 macrophages.

Results

H_2O_2 release by freshly collected AM in suspension. AM from normals (n = 6) released 9.8±1.7 (mean±SE) nmol $H_2O_2/10^6$ AM when assayed on day 0 in suspension and triggered by PMA (range 3.6–15.4 nmol/10^6 AM). In AM from the sarcoid patients (n = 11), release after addition of PMA was 21.7±2.3 nmol $H_2O_2/10^6$ AM (range 13.3–33.3 nmol/10^6 AM) (Fig. 1 *A*). In

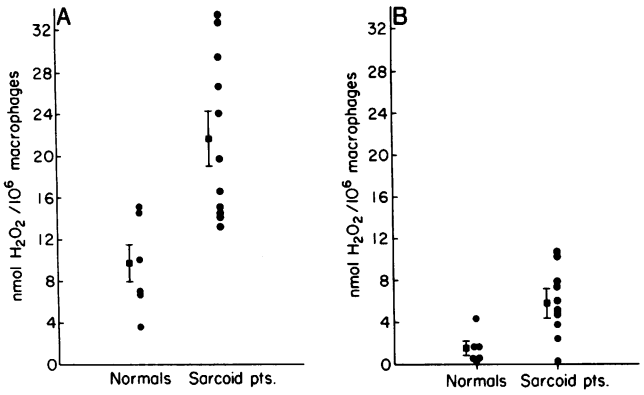

Figure 1. H_2O_2 release by AM assayed in suspension: (*A*) H_2O_2 release by AM from normals (n = 6) and patients (Pts) with sarcoid (n = 11) after stimulation with 100 ng/ml PMA. (*B*) H_2O_2 release by AM from normals (n = 6) and patients with sarcoid (n = 9) in the absence of PMA. Vertical lines represent the standard errors. Closed squares represent mean values.

the absence of PMA, H_2O_2 secretion by AM from normals was 1.6±0.7 whereas that by the AM from sarcoid patients, 5.9±1.1 nmol $H_2O_2/10^6$ AM (Fig. 1 *B*).

In the sarcoid patients no correlation was found between the percent of lymphocytes in the BAL and H_2O_2 release by AM (r = 0.09). Note however, that in this study only five patients had a BAL total lymphocyte count of ≥ 28%. Of these five patients, four had ^{67}Ga scans, three of which were negative. Two patients in this study, therefore, potentially had a high intensity alveolitis (HIA), as defined (28). It is possible therefore that with a larger population of patients with HIA such a correlation might have been seen. Gallium-scan positivity also was not predictive of AM activation in the small group of patients tested. The AM from patients with negative (n = 5) and positive (n = 3) scans showed a wide spectrum of H_2O_2 production. Finally, the level of ACE (a parameter which has been shown to correlate poorly with response to therapy [29] and the presence of the alveolitis [30]) also showed no correlation with H_2O_2 release (n = 9, r = 0.06, $P > 0.19$). Thus, none of the tests commonly used to measure disease activity appeared to predict macrophage activation as manifest by H_2O_2 release.

H_2O_2 release by adherent macrophages after exposure to rIFNαA, rIFNβ, rIFNγ, or 1,25-dihydroxyvitamin D_3. AM from normals (n = 3) that were exposed to rIFNγ on day 0–3 and then triggered with PMA showed a dose-dependent response with maximum values of 93.0±17.0 nmol H_2O_2/mg cell protein and 35.2±9.0 nmol $H_2O_2/10^6$ macrophages (Fig. 2). The concentration of rIFNγ producing 50% of the maximum response was ~ 5 U/ml (4.3×10^{-12} M), which suggests a physiologic role for this mediator. In the absence of PMA, there was no H_2O_2 release on day 3 of culture by control cells. However, with high dose of rIFNγ, a modest but consistent elevation of H_2O_2 release was seen: 4.0±2.1 nmol H_2O_2/mg protein and 1.8±1.0 nmol/10^6 macrophages.

AM (n = 3) that were given rIFNγ on days 3–6 (Fig. 3) again showed a dose-dependent response to this agent and a 50% maximum response with ~ 5 U rIFNγ/ml. The maximum response to rIFNγ after PMA stimulation (53.5±6.9 nmol H_2O_2/mg protein) was less than that on day 3. However, when expressed in terms of cell number, the maximum H_2O_2 release (27±2 nmol $H_2O_2/10^6$ macrophages) was similar to that on day 3. On day

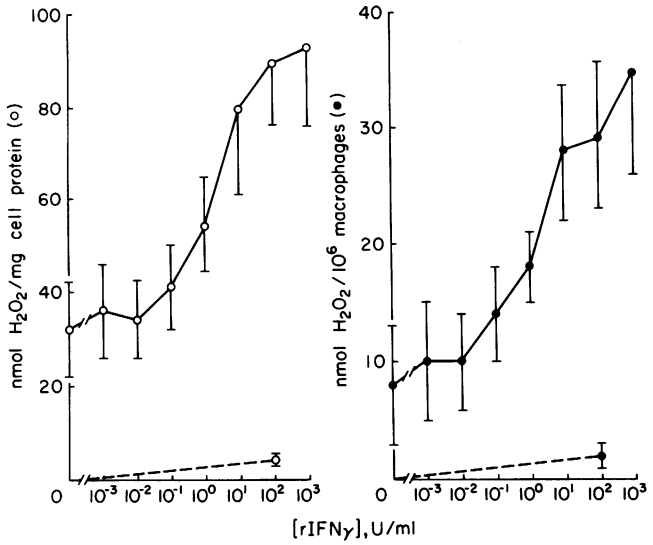

Figure 2. H_2O_2 release by AM harvested from normals (n = 3) and cultured in triplicate for 3 days in the indicated doses of rIFNγ. PMA-triggered H_2O_2 release demonstrates a dose dependence with 50% of maximum response occurring at ~ 5 U/ml rIFNγ. H_2O_2 release in the absence of PMA, after exposure to 100 U/ml rIFNγ, is greater than that of control cells. Open circles represent nanomoles of H_2O_2 per milligram of cell protein; closed circles, nanomoles of H_2O_2 per 10^6 AM; solid lines, cells triggered with PMA; dashed lines, cells not triggered with PMA; and vertical bars, standard errors.

6, small amounts of PMA-independent H_2O_2 release were again observed with high dose of rIFNγ (10^2 U/ml): 10.0±3.6 nmol H_2O_2/mg protein and 5.0±1.6 nmol/10^6 macrophages. In three

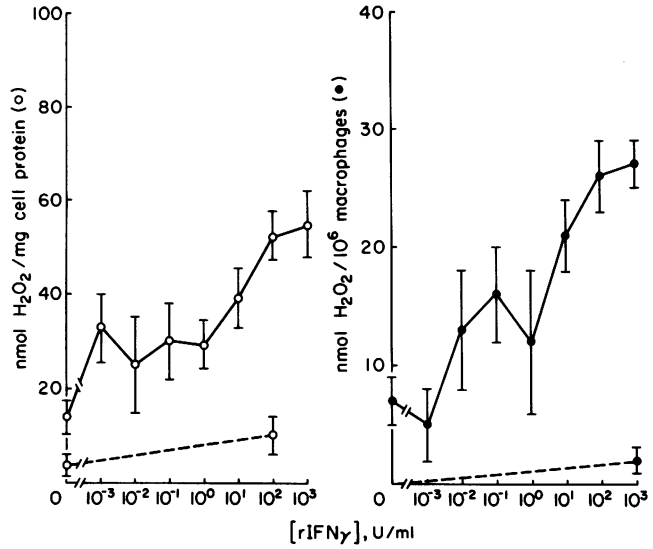

Figure 3. H_2O_2 release by AM harvested from normals (n = 3) and cultured in triplicate for 6 d. rIFNγ in the doses indicated was added to the cells on day 3. A dose-dependent response is demonstrated with 50% of the maximal response occurring at ~ 5 U/ml. Although the secretion of H_2O_2 per milligram cell protein in response to rIFNγ is less than that seen on day 3 (Fig. 2), the H_2O_2 released per 10^6 cells in response to rIFNγ is approximately the same. H_2O_2 secretion in the absence of PMA, after exposure to 100 U/ml of rIFNγ, is greater than that of control cells. Symbols are same as in the legend for Fig. 2.

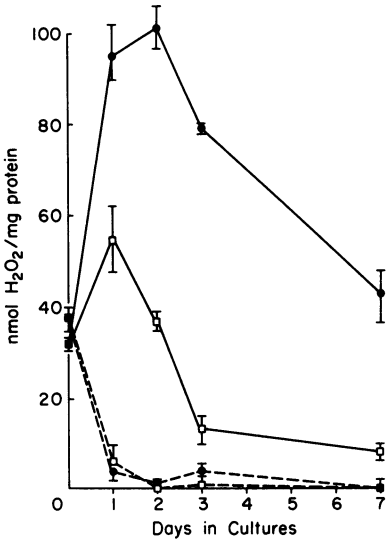

Figure 4. H_2O_2 release by AM from a normal subject cultured in triplicate for 7 d in the presence and absence of rIFNγ. 100 U/ml rIFNγ was added to the cells on day 0. At the time points indicated, H_2O_2 secretion by the cells with and without PMA was assayed. H_2O_2 release in cells receiving rIFNγ and triggered with PMA peaked on day 2. At day 7 these cells' H_2O_2 release was still greater than that of the control cells. Solid circles represent cells given rIFNγ; open squares, control cells; solid lines, cells triggered with PMA; and dashed lines, cells not triggered with PMA.

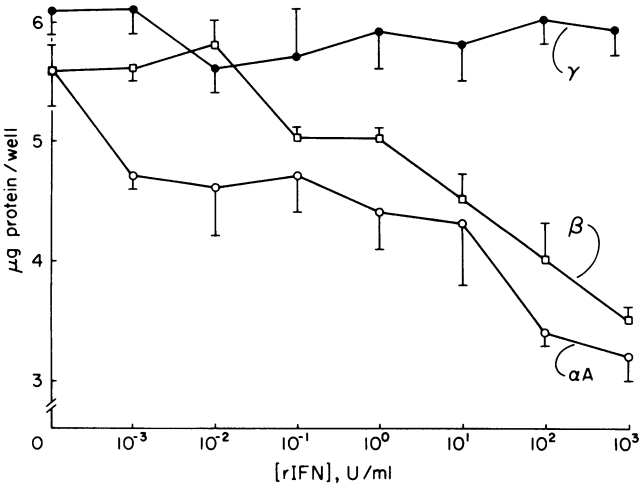

Figure 6. The micrograms of protein per well were determined on day 3 after triplicate cultures of AM from normals (n = 3) in the presence of the indicated concentrations of rIFNαA, rIFNβ, or rIFNγ. rIFNαA and rIFNβ, but not rIFNγ induced a dose-dependent decrease in protein per well suggesting cellular toxicity. Closed circles represent cells exposed to rIFNγ; open squares, cells exposed to rIFNβ; and open circles, cells exposed to rIFNαA.

experiments with rIFNγ, starting on day 0, H_2O_2 release peaked on days 2 to 3 (Fig. 4).

Both rIFNαA and rIFNβ given to AM from days 0 to 3 (n = 3) increased H_2O_2 release in the presence of PMA when given at high doses (10^2–10^3 U/ml) (Fig. 5). The lower doses failed to elicit a significant response. The time course for H_2O_2 release by AM stimulated with rIFNαA and rIFNβ from days 0 to 3 (n = 3) showed maximal stimulatory activity on days 1 to 2. rIFNαA and rIFNβ, but not rIFNγ produced a dose-dependent decrease in cell protein, as demonstrated in Fig. 6, which suggests that these agents caused a loss of adherent cells under the conditions used.

1,25-Dihydroxyvitamin D_3 (n = 3) given in a range of doses from 2.4×10^{-10} to 2.4×10^{-4} M caused no increase of H_2O_2 release on days 1, 2, or 3.

Discussion

Sarcoid is a disease characterized by the activation of mononuclear cells within the alveolus. In this study we have focussed on the activation status of AM, as reflected by their capacity for H_2O_2 release. This is potentially significant both as a measure of the cells' immunologic status and as a possible mechanism of chronic damage to the lung.

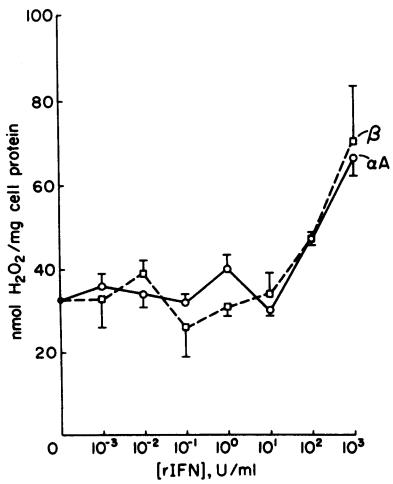

Figure 5. PMA-induced H_2O_2 release by AM from normals (n = 3) cultured in triplicate for 3 d after exposure to the indicated doses of rIFNαA or rIFNβ. High dose rIFNαA or rIFNβ (10^2–10^3 U/ml) caused a significant increase in H_2O_2 secretion. Open circles connected with solid line represent cells exposed to rIFNαA; open squares connected with dashed lines, cells exposed to rIFNβ; and vertical lines, standard errors.

Activated macrophages are believed to play a central role in the chronic granulomatous inflammation characteristic of sarcoid. They secrete IL-1 (8), as well as a host of other biologically active molecules. T cells activated during this process produce IL-2, a lymphokine which induces the clonal expansion of helper, suppressor, and cytolytic T cells and their secretion of IFNγ. IFNγ activates mononuclear phagocytes and potentiates their production of IL-1 (7). Also, AM may themselves be capable of secreting IFNγ (17, 18). AM from sarcoid patients also express the HLA-D antigens believed to be necessary for antigen presentation to lymphocytes. Indeed, enhanced antigen presentation by AM from sarcoid patients has been demonstrated (31). Thus, both signals required for antigen-specific activation of T cells may be provided by AM from patients with sarcoid. In addition to their critical role in regulating the immune response, activated macrophages secrete factors that may contribute to the permanent parenchymal damage seen in a subset of sarcoid patients. Among such factors are reactive oxygen metabolites (2). These species are capable of causing tissue damage through a wide variety of mechanisms including disruption of nucleic acids and peroxidation of lipids (32, 33).

In this paper we have looked at the release of H_2O_2 by AM from sarcoid patients and normals. Prior work in this area has been contradictory, one abstract showing an increase (34), and another study showing no difference in the release of oxygen metabolites by AM from sarcoid patients when compared with those from normals (35). In an animal model of granulomatous disease, however, it has been demonstrated that oxygen metabolite secretion by macrophages is increased and that this increase occurs at the time of maximal granuloma formation (36).

We have shown that AM from patients with sarcoid have an enhanced release of oxygen metabolites both in the presence and in the absence of the triggering agent, PMA. Furthermore,

AM from normals in vitro increased their release of H_2O_2 to the level seen in sarcoid patients after exposure to rIFNγ. rIFNαA and rIFNβ also caused a dose-dependent increase in cellular H_2O_2-releasing capacity; however, a dose-related loss of adherent cells was also observed using these agents. It is therefore unclear whether the increased H_2O_2 release with rIFNαA and rIFNβ was due to the selection of a subset of cells with high H_2O_2 production or to an enhanced production of this metabolite by the entire cell population. 1,25-Dihydroxyvitamin D_3 did not appear to affect H_2O_2 secretion. Our results are in accord with a recent study by Murray et al. (10) who demonstrated enhanced microbicidal capacity as well as increased H_2O_2 secretion in AM stimulated with rIFNγ.

Macrophage activation, as reflected by the cells' H_2O_2-releasing capacity, did not correlate with three currently used tests for disease activity: the ACE level, gallium-scan positivity, or the lymphocyte count in the BAL. The ACE level was previously demonstrated to be an insensitive test for the presence of sarcoid (37), a poor predictor for response to therapy (29), and within the normal range in a large percent of patients with elevated BAL lymphocyte counts (38). It appears therefore to be a relatively insensitive measure of disease activity in the lung. The significance of a positive ^{67}Ga scan is also unclear. Although some investigators have postulated that ^{67}Ga in the lung of sarcoid patients is taken up by activated macrophages (39, 40), others have suggested that it is taken up by activated lymphocytes (41, 42). Similarly it is disputed whether there exists a correlation between the T cell alveolitis of sarcoid and ^{67}Ga scan positivity (40, 42, 43). Since ^{67}Ga scan positivity can be seen in diseases such as interstitial pulmonary fibrosis, where there is neither a T cell alveolitis nor granuloma formation, it is difficult to postulate that this finding reflects a specific immunologic abnormality found in this disease. Rather it appears to be a non-specific marker of acute parenchymal inflammation and may not adequately reflect the chronic inflammatory process seen with progressive sarcoid. The lack of correlation between the lymphocyte count in the BAL and the activation status of the AM is more difficult to explain. This finding may in part reflect the fact that this study contained few patients with roentgenographic evidence of parenchymal involvement and, as noted previously, few patients who could potentially be defined as having HIA. It is possible that with a larger population of stage II patients and/or patients with HIA, such a correlation might have emerged. Alternatively, it is possible that the activation status of the lymphocytes, rather than their absolute numbers, is the critical factor. Consistent with this is the recent finding of a T cell alveolitis in patients with regional enteritis with no clinically apparent pulmonary disease (44). It is possible, therefore, to hypothesize that factors that cause the parenchymal damage, such as AM activation, do not require the intense, local inflammation and large T cell population defined as HIA or may in fact require other factors such as the persistence of local antigen. In this view it would be the chronic, cumulative damage to the lung by macrophage products that would cause morbidity. This effect would be further amplified by the increased macrophage population found in sarcoid patients (28).

The findings of increased H_2O_2 release by sarcoid patients' cells in suspension, in the absence of a triggering agent such as PMA, suggest that constitutive H_2O_2 secretion by activated AM may itself be a mechanism of chronic lung damage. In agreement with this was the observation that after stimulation with 100 U/ml of rIFNγ, the cultured AM had a small but consistent increase in PMA-independent H_2O_2 secretion. The possibility that both the cells in culture and in suspension were triggered by an unidentified factor cannot, however, be ruled out.

We cannot exclude the possibility that the observed increase in H_2O_2 release by AM from sarcoid patients was contributed to by a population of relatively immature mononuclear phagocytes. Recent evidence strongly suggests that such a population exists in BAL cells from sarcoid patients (45). However, it appears unlikely that this was a significant factor in this study. Myeloperoxidase stains showed no increase above normals in the extremely small number of monocytes present in BAL from our patients with mostly low-intensity alveolitis. Also, the capacity of monocytes to release H_2O_2 decreases markedly over several days in vitro as they mature into macrophages (46). Mononuclear cells cultured by the method used for AM in this study and triggered with PMA have been shown to release 34, 10, and 6 nmol $H_2O_2/10^6$ cells on days 0, 1, and 2, respectively (47).

In conclusion, AM harvested from patients with sarcoid appear activated by the criterion of increased H_2O_2 production. AM from normals can be activated to similar levels by exposure to rIFNαA, rIFNβ, and rIFNγ, although only IFNγ caused activation in the absence of cellular toxicity. Further study is required to determine whether macrophage activation monitored in this way will be a useful parameter for predicting the course of the disease.

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