Comparison of the Antimicrobial Activity of Deactivated Human Macrophages Challenged with *Aspergillus fumigatus* and *Listeria monocytogenes*

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The anticonidial activity of human monocytes deactivated by cytokines interleukin (IL)-4 and IL-10 and the hormone dexamethasone was studied and compared with antilisterial activity. Dexamethasone had the largest effect on the anticonidial activity and suppressed germination-inhibiting activity and elimination of ingested spores by macrophages more than the cytokines did. Maximally active concentrations of IL-10 had a similar but significantly smaller deactivating effect. IL-4, in contrast to IL-10 and dexamethasone, did not reduce anticonidial activity. However, IL-4 and IL-10 were equally potent in deactivating human macrophages against *Listeria monocytogenes*, whereas dexamethasone was significantly less potent in the *Listeria* model. These observations indicate that all three mediators lessen antimicrobial activity but that this effect depends on the test organism studied and is apparently mediated through regulation of different antimicrobial systems operating against a particular microorganism.

Macrophages take a central role in natural resistance to conidia from *Aspergillus* species as well as *Listeria monocytogenes* in experimental and presumably also natural infection [1–3]. Human macrophages derived from blood monocytes are highly efficient in controlling both organisms, even in the absence of activation by cytokines, but they lose their capability to kill these microorganisms and to suppress their growth when exposed to pharmacologic concentrations of glucocorticoids [4]. Previous studies from our laboratory have shown that dexamethasone apparently interferes with nonoxidative killing systems of resting human macrophages, but the nature of these systems is unknown [4, 5].

In the present study, we compared the effects of dexamethasone as well as interleukin (IL)-4 and IL-10, two other mediators of deactivation [6], on the activity of human macrophages against *L. monocytogenes* and *Aspergillus fumigatus*.

Materials and Methods

Organisms. A strain of *A. fumigatus* from previous studies was used for all experiments, and single-spore suspensions were prepared as described [4]. For challenge, conidia were directly diluted in complete medium at a concentration of 2×10^5 spores/mL. *L. monocytogenes* EGD was cultured and prepared as described [5].

Received 29 January 1996; revised 3 May 1996.

Presented in part: 8th annual conference of the European Macrophage Study Group, Lausanne, Switzerland, September 1994 (abstract OV).

Grant support: Swiss National Science Foundation (31-36564).

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The Journal of Infectious Diseases 1996;174:651–4

0022–1899/96/7403–0033$01.00

Cell cultures. Human monocyte cultures were obtained after gradient separation of mononuclear cells from heparinized blood from healthy volunteers as described [5, 7]. Monolayers of monocytes on 12-mm glass coverslips, freed from contaminating lymphocytes by washing away nonadherent cells, were placed in 35-mm tissue culture dishes (Falcon Plastics, Oxnard, CA) containing four coverslips each with 30,000–40,000 monocytes with a purity of >98% in 2 mL of complete medium [7]. Culture conditions were 37°C, 98% humidity, and 5% CO_2. Complete medium was Medium 199 (GIBCO Europe, Basel, Switzerland) supplemented with 25% pooled normal human serum. A single lot of pooled serum stored in aliquots at −70°C was used throughout the experiments.

Cytokine supplements. Human recombinant IL-4 was from Schering-Plough (Kenilworth, NJ) and was used in a final concentration of 1 ng/mL. Human recombinant IL-10 was from PeproTech (Rocky Hill, NJ) and used in a final concentration of 5 ng/mL. Human recombinant tumor necrosis factor-α (TNF-α) was from Knoll (Ludwigshafen, Germany). The three cytokines were dissolved in PBS and then diluted in medium. Dexamethasone (Sigma, St. Louis) was dissolved at a concentration of 2.5×10^{-2} *M* in ethanol before dilution in medium to a final concentration of 2.5×10^{-7} *M*. All cytokines and dexamethasone have been shown in dose-finding studies to be maximally active at these concentrations [7]. Appropriate solvent controls were added to control cultures at the same time as the supplements.

Tests for antimicrobial activity. Anticonidial activity was assessed as described [4]. In brief, germination of phagocytized spores was enumerated 18 h after challenge of monolayers that had been pretreated for 36 h with the indicated cytokines on Giemsa-stained coverslips [4]. Germination rates of control spores incubated in cell-free complete medium was always >99%. The analysis of germination rates was based on 4× 200 spores from quadruplicate wells per condition and experiment.

Disappearance of spores by digestion was quantitated by enumerating the decrease in spores on the basis of phagocytic index immediately after phagocytosis and removal of noningested spores by washing and incubation for 18 h as described [8]. This analysis

was based on 4× 400 macrophages from quadruplicate wells per condition and experiment and time point.

Antilisterial activity was assessed as described [4, 5] by following the percentage of mononuclear phagocytes harboring bacteria during an incubation period of 7 h after phagocytosis and washing off non–cell-associated bacteria. After non–cell-associated bacteria or conidia were washed off, dexamethasone and cytokines were again added to cultures at the indicated concentrations. Of note, IL-1, IL-4, and dexamethasone did not affect the phagocytic index, as shown previously [7].

Measurement of H_2O_2 secretion and NO production. H_2O_2 secretion in response to phorbol myristate acetate stimulation was measured by chemiluminescence as described [5]. NO was measured as nitrite accumulation during 24 h with the Griess reagent as described [9].

Statistical analysis. Mean and SE (combined data from several experiments) or SD (single experiments) are given. Bonferroni's correction for multiple comparisons and Dunnett's test for comparison of multiple samples with one control were done using a computer-based program (Instat; Graphpad, San Diego).

Results

Deactivation of anticonidial activity. Dexamethasone was most potent in deactivating anticonidial activity of human monocytes, followed by IL-10. IL-4 had no effect on anticonidial activity (figure 1). Assessment of the anticonidial activity by quantification of germination rates of spores 18 h after phagocytosis or digestion of spores during phagocytosis for 18 h can be done in parallel. However, anticonidial activity can probably be even more accurately determined by quantification of the percentage of spores germinating in relation to the spores originally ingested by macrophages (i.e., percentage of spores germinating at 18 h of incubation among number of spores remaining at 18 h plus number of spores digested during 18 h of incubation). In any event, all three methods of assessment indicated that dexamethasone was more potent than IL-10 in deactivating macrophages and that IL-4 had no effect on anticonidial activity.

Comparison of deactivation of antilisterial and anticonidial activity and relation to NO production and H_2O_2 secretion. In contrast to results from experiments with *A. fumigatus,* antilisterial activity was most strongly reduced by IL-4 and IL-10; dexamethasone had a much lower activity than the tested cytokines (table 1, $P < .001$). Furthermore TNF-α, which did not restore anticonidial activity of macrophages deactivated with any of the three tested mediators of deactivation, significantly increased antilisterial activity in macrophages deactivated with IL-10 and IL-4 (table 1). During the experiments, no detectable amount of nitrite accumulated in supernatants of phagocytes before challenge with the microorganisms or after. Only deactivation by IL-10 was associated with a significantly decreased secretion of H_2O_2 during the respiratory burst (table 1).

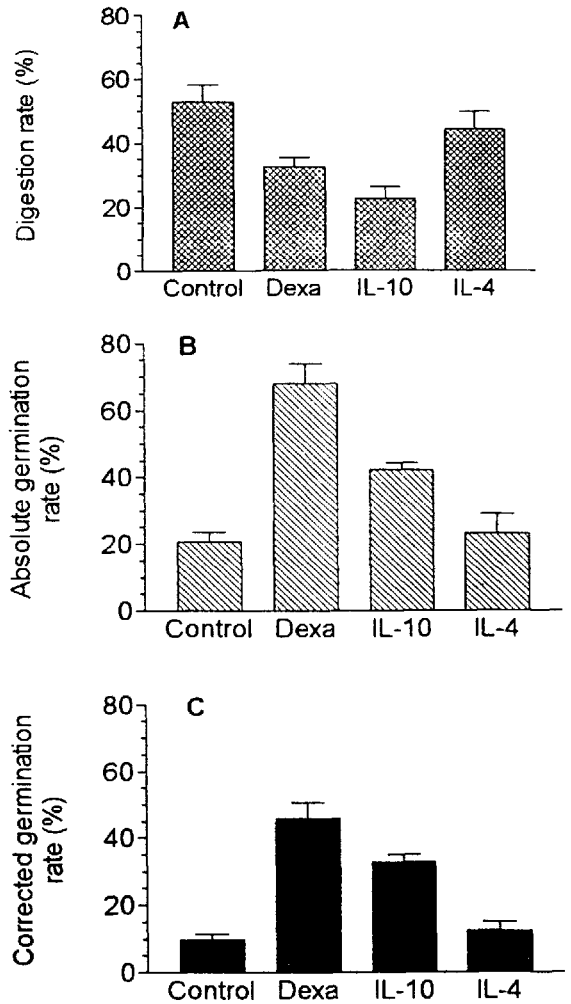

Figure 1. Anticonidial activity of control monocytes and monocytes exposed for 36 h before challenge to dexamethasone (Dexa), interleukin (IL)-10, or IL-4. Concentrations were for dexamethasone, 2.5×10^{-5} *M;* IL-10, 5 ng/mL; and IL-4, 1 ng/mL. **A**, No. of phagocytized conidia completely digested by phagocytes during 18 h of incubation. **B**, Absolute germination rate of phagocytized spores (% of spores germinating among no. of spores enumerated in phagocytes). **C**, Corrected germination rate (% of conidia germinating among no. of spores enumerated in phagocytes + no. of spores digested). Data are mean ± SE from 5 independent experiments with quadruplicate values from quadruplicate wells for controls and each of 3 tested mediators of deactivation per experiment. **A–C**, Control vs. dexamethasone and IL-10, $P < .001$; control vs. IL-4, $P > .05$. **A**, Dexamethasone vs. IL-10, $P > .05$. **B**, Dexamethasone vs. IL-10, $P < .01$. **C**, Dexamethasone vs. IL-10, $P < .05$.

Discussion

In these studies, mediators of macrophage deactivation (IL-10, IL-4, dexamethasone) affected different phagocytic effector systems differently, and deactivation characterized by assessment of antimicrobial activity depended on the test organism studied. The most probable explanation for this finding is that, in human mononuclear phagocytes, disparate effector mecha-

Table 1. Effect of macrophage-deactivating agents on parameters for macrophage activity.

	Deactivating agent			
	None (control)	Dexamethasone	IL-10	IL-4
Germination inhibition of conidia from *Aspergillus fumigatus* (%)	74.7 ± 1.3	24.9 ± 0.7*	54.7 ± 8.2*	75.8 ± 2.0†
Germination inhibition of conidia after TNF-α (%)	67.8 ± 5.9	20.6 ± 5.9	46.6 ± 2.0†	82.1 ± 4.5‡
Change in % of cells infected with *Listeria monocytogenes* (%)	−24.0 ± 2.5	−0.66 ± 16.6‡	25.0 ± 2.8*	31.3 ± 3.5*
Change in % of cells infected with *L. monocytogenes* after TNF-α (%)	−42.0 ± 1.2	−5.3 ± 7.0*	−26.3 ± 5.7†	−0.6 ± 2.1*
H_2O_2 secretion (pmol/10^6 cells)	136 ± 24	132 ± 6†	68 ± 2*	119 ± 8†
Nitrite production (μmol/10^6 cells)	<0.1	<0.1	<0.1	<0.1

NOTE. Data are mean ± SD from typical experiment. IL, interleukin; TNF, tumor necrosis factor. Germination rates of conidia were enumerated 18 h after challenge. Antilisterial activity was assessed by quantifying % of cells eliminating bacteria or acquiring bacteria during incubation for 7 h after phagocytosis.

P *<.001, †>.05, ‡<.01 vs. control in same row.

nisms are responsible for the control of different microorganisms. Accordingly, an effect on a single antimicrobial system by a deactivating mediator cannot explain the observed changes in the antimicrobial activities. No NO-producing activity that could explain antilisterial or anticonidial activity was detected.

The capacity to produce reactive oxygen intermediates (ROI) did not explain all the observed effects, particularly in view of our previous finding that neither killing of conidia nor intracellular control of *L. monocytogenes* by nonactivated human macrophages depends on ROI generation [4, 5]. On the other hand, conidia from *A. fumigatus* are resistant to oxidative killing [1, 4]; yet IL-10 that halved H_2O_2 secretion evidently reduced anticonidial activity by affecting nonoxidative antimicrobial systems as well.

Suppression of TNF-α secretion has been proposed to explain the failure of deactivated macrophages to control intracellular parasites [10]. However, in the present fungal studies, exogenous TNF-α could not restore anticonidial activity.

Therefore, taken together, effects of deactivating mediators on previously undescribed effector functions of human macrophages must be assumed to explain deactivation of anticonidial activity.

In this context, the recent proposition that aspergillosis might be promoted by glucocorticoids through a direct effect on fungal growth is noteworthy [11]. We have previously appropriately controlled for such effects in our experiments that assessed germination rates in macrophages exposed to glucocorticoids [1, 4]. By demonstrating that glucocorticoids affect killing and digestion of conidia as well as germination rates, we further substantiate that the glucocorticoid target is the macrophage rather than the fungal spore in this setting. Such a mechanism is therefore also eliminated as explanation for the success of conidia in resisting glucocorticoid-treated macrophages.

In conclusion, the balancing effects of activating and deactivating signals appear to depend not only on the complexity of a network of simultaneous or sequential activating and deactivating mediators [10, 12] but also on the specific microbial pathogen and the antimicrobial mechanism operative in their control.

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Synthesis of Tumor Necrosis Factor-α mRNA in Bronchoalveolar Lavage Cells from Human Immunodeficiency Virus–Infected Persons with *Pneumocystis carinii* Pneumonia

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Bronchoalveolar lavage fluid cells from a cohort of 34 human immunodeficiency virus–infected persons with established *Pneumocystis carinii* pneumonia were examined for expression of tumor necrosis factor-α (TNF-α) mRNA by fluorescence in situ hybridization with an antisense riboprobe. Video image analysis was used to develop a quantitative assay that evaluates relative single-cell levels of mRNA. The resulting data were analyzed as an antisense-to-sense ratio and examined for correlation between TNF-α mRNA expression and other measures of disease severity. Higher levels of TNF-α mRNA were seen in persons who had higher levels of arterial oxygen.

Pulmonary manifestations are important in determining morbidity and mortality resulting from human immunodeficiency virus (HIV) infection. Increased susceptibility to opportunistic pulmonary infection was recognized when the clinical syndrome associated with HIV was first defined, and many causative organisms have been identified. Perhaps the best-known example is *Pneumocystis carinii*. However, as with other HIV-associated disease, the pathophysiologic mechanism that leads to pulmonary manifestations and the relationship between findings and outcome (e.g., lung function, patient survival) remain poorly understood.

Bronchoalveolar lavage (BAL) specimens have provided much information for diagnosis and research investigation of these conditions. Such studies indicate that identifiable opportunistic disease may be only one form of lung immunopathology in such patients. For example, 25%–50% of all HIV-infected persons show some evidence of alveolitis [1], and a mixed NK and other T cell (chiefly CD8 cells) infiltration has been noted, not only early but also late in the course of HIV disease, when responder cells are depleted [2]. In the lung, the conventional selective target cell for HIV infection, the CD4 T cell, has not been found in appreciable numbers, probably reflecting the progressive dysfunction and eventual depletion of these cells.

The alternative CD4 target cells, mononuclear phagocytes (MP), are present in the lung in the form of alveolar macrophages (AM), and absolute numbers of these cells may be increased in both early stages of infection and late stages of disease [3]. This may be due to relative resistance to cytopathic effects of HIV; therefore, MP may serve not only as a potential reservoir for HIV but also as a primary vector for viral propagation [4]. Both viral propagation in MP and the effector-immunoregulatory function of MP are mediated by cytokines, and studies of cytokine production, either by protein detection or polymerase chain reaction (PCR) [5], have shown that in HIV-infected persons there may be dysregulation of several cytokines, including tumor necrosis factor-α (TNF-α).

TNF-α plays a pivotal role as a mediator in many physiologic and immunologic processes. In the lung, the AM is the first host cell to interact with infectious agents and to synthesize cytokines (such as TNF-α) that may modulate the ensuing inflammatory response, thus serving both as a potential reservoir and as a "latent" source of cytokines. TNF-α released by AM may have

Received 13 February 1996; revised 1 May 1996.
All patients gave informed consent for bronchoalveolar lavage. The project was approved by the University College London Hospitals Ethics Committee.
Grant support: Multiple Sclerosis Society and British Heart Foundation (to D.R.K. and supporting N.R.) and Arthritis and Rheumatism Council (to M.H.B.), all in the United Kingdom.
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The Journal of Infectious Diseases 1996;174:654–9

0022–1899/96/7403–0034$01.00