

Effect of Glucocorticoids and Interferon- γ on the Oxidative Responses of Monocytes from Leprosy Patients and Normal Donors¹

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A characteristic feature of leprosy is its spectrum of clinical symptoms correlating with the cellular immune responsiveness of the patient to *Mycobacterium leprae*. Tuberculoid leprosy patients, in contrast to lepromatous patients, exhibit normal cell-mediated immune responses to *M. leprae* antigens. Mononuclear phagocytes in tuberculoid leprosy are able to ingest and destroy most microorganisms; however, in lepromatous leprosy, phagocytes fail to control the intracellular multiplication of *M. leprae*⁽⁹⁾. Studies have shown that *M. leprae* are susceptible to oxygen radical killing⁽¹¹⁾. Macrophages from leprosy patients reportedly fail to present *M. leprae* antigens in an immunogenic form⁽⁶⁾, are unable to pro-

vide accessory function for lymphocyte proliferation, and have reduced protein synthesis in the presence of *M. leprae*⁽²⁶⁾. It has been reported that T cells from lepromatous leprosy patients fail to produce interleukin-2 (IL-2) after exposure to *M. leprae* but respond by proliferation to *M. leprae* in the presence of exogenous IL-2^(4, 5). Other studies point out that T cells fail to express IL-2 receptors upon challenge with *M. leprae*⁽¹⁵⁾. More recently, it has been shown that T cells from lepromatous patients can respond to *M. leprae* if cultured in medium alone, which suggests that the failure of those T cells to respond to *M. leprae in vivo* may be due to the persistence of antigen⁽¹⁴⁾. Interferon-gamma (IFN- γ), an activator of human monocyte oxidative metabolism and antimicrobial activity^(17, 19), is produced in lower amounts by T lymphocytes from lepromatous patients than by normal T cells or T cells from tuberculoid patients⁽²¹⁾. Patients with leprosy can spontaneously develop erythema nodosum leprosum (ENL) which is characterized by tender skin nodules, fever, malaise, nerve pain, bone pain, and pain in large joints^(1, 23). Current therapy for ENL includes the use of immunomodulators, such as thalidomide or prednisone.

This study was undertaken to determine the effect of IFN- γ on superoxide anion (O_2^-) production by monocytes from leprosy patients. In addition, in view of a recent report that corticosteroids can enhance the binding of IFN- γ to human monocytes in culture due to receptor biogenesis⁽³⁰⁾, and the use of corticosteroids in the treatment of ENL, we have examined the effects of both agents on monocyte O_2^- release in response to various stimuli, including *M. leprae*.

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TABLE 1. Characteristics of patients studied.

Patient	Sex/ age	Lep- rosy type	Bac- terial index	Therapy (duration)
1	M/49	BT	1+	Dapsone (14 mo.); rifampin (14 mo.)
2	M/34	BT	0	Dapsone (6 yr); prednisone (18 mo.)
3	M/29	BT	0	Dapsone (6 yr)
4	M/26	LL	0	Dapsone (5 yr); thalidomide (4 yr)
5	M/24	LL	5+	Dapsone (3 yr); rifampin (3 yr); thalidomide (3 yr); prednisone (1 yr); Dapsone (1 yr); rifampin (9 mo.); thalidomide (3 yr); prednisone (1 yr); clofazimine (16 mo.)
6	M/30	LL	3+	Dapsone (6 yr); rifampin (6 yr); clofazimine (6 yr)
7	M/36	LL	0	Dapsone (3 yr); rifampin (3 yr)
8	M/26	LL	4+	Dapsone (4 yr); rifampin (2 yr); thalidomide (4 yr)
9	M/40	LL	3+	Dapsone (5 mo.); thalidomide (1 mo.); prednisone (5 mo.); clofazimine (5 mo.)
10	M/33	LL	6+	

MATERIALS AND METHODS

Subjects. Informed consent for a blood donation was given by normal adult laboratory personnel (control subjects) and by patients with a confirmed diagnosis of leprosy in the Hansen's Disease Clinic at the University of Illinois Medical Center. Disease classification was according to the criteria of Ridley and Jopling⁽²⁴⁾. Table 1 summarizes the characteristics of the patients used in this study. All were males with ages ranging from 24 to 49, and a median age of 33. Bacterial indices from biopsies performed 0–9 months prior to this study ranged from 0 to 6+, with a median of 2+. All the patients were on dapsone therapy, with some on multiple therapies as indicated in Table 1.

Cells. Peripheral blood was collected from donors as previously described⁽⁷⁾ into 10 U/ml sterile heparin (ICN, Cleveland, Ohio, U.S.A.) and diluted with an equal volume of Hank's Balanced Salt Solution without phenol red, calcium or magnesium (HBSS; Gibco Laboratories, Grand Island, New York, U.S.A.). The blood was layered over Ficoll-Hypaque (Sigma Chemical Company, St. Louis, Missouri, U.S.A., and Winthrop-Breon Laboratories, New York, New York, U.S.A.), and centrifuged at $400 \times g \times 25$ min. The mononuclear cell layer (MNC) was collected, and the cells washed with HBSS. The contaminating erythrocytes were hypotonically lysed. The percentage of monocytes in the MNC suspension was determined by counting the number

of cells ingesting $0.8 \mu\text{m}$ latex particles (Difco Laboratories, Detroit, Michigan, U.S.A.). The monocytes were consistently $> 98\%$ viable, as determined by trypan blue dye exclusion. The cells were washed by centrifugation and resuspended at 2×10^6 monocytes/ml in medium which consisted of RPMI 1640 (Gibco) containing 10% heat-inactivated fetal calf serum (Hyclone Laboratories, Logan, Utah, U.S.A.) and $50 \mu\text{g}/\text{ml}$ gentamicin (Sigma).

Culture conditions. The cells were plated in 96-well, flat-bottom, microtiter plates (Corning, Corning, New York, U.S.A.) at 2×10^5 monocytes/well, and allowed to adhere for 1 hr at 37°C in 5% CO_2 in air. The cells were washed with warm fresh medium to remove nonadherent cells, and then cultured overnight in medium alone or in medium containing 2 nM, 200 nM, or 20 μM dexamethasone (Rugby Laboratories, Rockville Centre, New York, U.S.A.). The cells were then washed and cultured in medium alone or in medium containing up to 500 U/ml IFN- γ (Sigma) overnight. The source of the IFN- γ was supernatants from A23187 and mizerein-induced human buffy-coat cell preparations. In some experiments, the monocytes were cultured simultaneously with the dexamethasone and IFN- γ overnight in the concentrations mentioned above.

Superoxide anion assay. Superoxide dismutase-inhibitable production of O_2^- by monocytes was determined by a modification of the microassay technique of Pick and

Mizel (²²), measuring ferricytochrome c (Type III; Sigma) reduction as described (⁷). The absorbance was determined at 550 nm (EIA Reader, Burlington, Vermont, U.S.A.) immediately after the addition of reaction mixtures (0 time) and 150 min later. We chose to read the plates after 150 min instead of the conventional 90 min because we had previously shown (⁷) that *M. leprae* was a weak and slow stimulus of the metabolic burst of monocytes. The stimuli used in the assays included phorbol myristate acetate (PMA, 100 ng/ml; Sigma), *M. leprae*, and *M. bovis* BCG (50:1 bacteria-to-monocyte ratio). Following the assay, the wells were washed, and protein quantitation on the adherent cells was performed by the Lowry method (¹²) with bovine serum albumin used for the standard curve.

Bacteria. Armadillo-derived *M. leprae* were kindly provided by Dr. Patrick Brennan (Colorado State University, Fort Collins, Colorado, U.S.A.) through National Institutes of Health Contract No. AI-52582. The *M. leprae* were purified from irradiated (2.5 Mrad) armadillo spleens and livers and stored in phosphate buffered saline with 0.1% Tween 80 (PBS-Tween) at -70°C . The *M. leprae* were washed by centrifugation before use in the O_2^- assay. *M. bovis* BCG were obtained from the University of Illinois at Chicago Institute for Tuberculosis Research as lyophilized vaccine, and was grown in Middlebrook broth with 10% OADC enrichment (oleic acid, albumin, dextrose, catalase) and 0.1% Tween 80 (Difco) at 37°C for 4 weeks. The BCG were then heat killed by autoclaving at 121°C for 15 min, and counted using fluorescence microscopy after staining with Auramine-O (Difco) as previously described (⁸). The BCG were stored in PBS-Tween at 4°C , and washed by centrifugation prior to use in the O_2^- assay.

Endotoxin contamination. A quantitative chromogenic limulus amoebocyte lysate assay (Whittaker, Walkersville, Maryland, U.S.A.) with a sensitivity of 10 pg/ml was performed periodically on the reagents used in the preparation and culture of the monocytes. Less than 10 pg/ml of endotoxin was detected in the buffers, media, or reagents.

Statistical analysis. The differences between mean values were evaluated for statistical significance by Student's *t* test.

TABLE 2. Effect of IFN- γ on normal monocyte O_2^- release.^a

IFN- γ (U/ml)	Stimuli		
	<i>M. leprae</i>	BCG	PMA
0	88 \pm 6	111 \pm 51	251 \pm 38
1	95 \pm 21	133 \pm 17	284 \pm 37
10	94 \pm 19	134 \pm 21	269 \pm 13
50	129 \pm 27	164 \pm 36 ^b	279 \pm 42
100	137 \pm 27 ^b	143 \pm 17	313 \pm 12
500	154 \pm 28 ^b	162 \pm 16 ^b	325 \pm 13 ^b

^a Values are mean nmol O_2^- /mg cell protein/150 min \pm S.D. of 3 experiments. Bacteria were used at a particle-to-cell ratio of 50:1; PMA was used at 500 ng/ml.

^b $p < 0.05$ vs 0 IFN- γ group.

RESULTS

Response of monocytes to IFN- γ . To determine the optimal concentration of IFN- γ necessary to enhance the oxidative response of the monocytes, cells from control subjects were cultured with the indicated concentrations of IFN- γ for 24 hr. The enhancement of O_2^- release by IFN- γ is shown in Table 2. IFN- γ at 500 U/ml significantly increased the monocyte O_2^- generation in response to all stimuli (PMA, BCG, and *M. leprae*) with 100 U/ml and 50 U/ml sufficient to augment O_2^- release in response to *M. leprae* and BCG, respectively. It was observed that when monocytes from leprosy patients were pretreated with 50 U/ml IFN- γ , those from patients on prednisone therapy responded with significantly enhanced O_2^- release when challenged with *M. leprae* or with BCG (Table 3). Monocytes from patients not on prednisone responded as did the monocytes from healthy subjects, in that pretreatment with 50 U/ml IFN- γ did not enhance their O_2^- generation upon challenge with *M. leprae*.

Effect of dexamethasone pretreatment on IFN- γ enhancement of monocyte O_2^- release. Monocytes from healthy control subjects were cultured overnight in media containing 0, 2 nM, 200 nM, or 20 μM dexamethasone. They were then washed and cultured for an additional 24 hr in the presence or absence of 500 U/ml IFN- γ . The cells were then challenged with PMA or *M. leprae*. Monocytes pretreated with all concentrations of dexamethasone tested released less

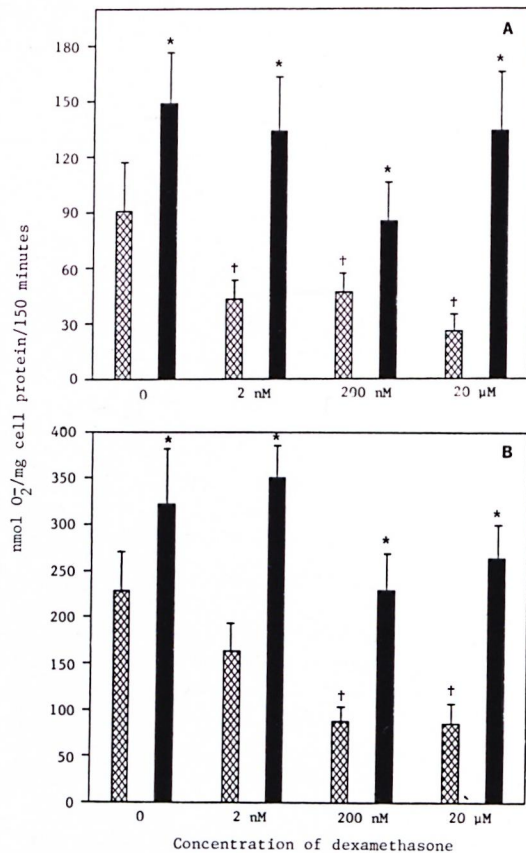


FIG. 1. Effect of dexamethasone treatment prior to interferon- γ treatment on monocyte O_2^- release in response to *M. leprae* (A) or PMA (B). Cells were treated on the day of isolation with indicated concentrations of dexamethasone for 24 hr. Cells were then cultured in the presence ■ or absence ▨ of IFN- γ (500 U/ml) for 24 hr. Results are means \pm S.E. of 8 experiments. † = $p < 0.05$ when compared with untreated control monocytes; * = $p < 0.05$ when compared with no IFN- γ treatment.

O_2^- in response to *M. leprae* than did untreated control monocytes (Fig. 1A). Cells pretreated with 200 nM or 20 μ M dexamethasone but not with 2 nM dexamethasone showed a decrease in O_2^- release when stimulated with PMA (Fig. 1B). IFN- γ enhanced the O_2^- generation of monocytes in response to *M. leprae* and to PMA whether or not the cells had been pretreated with dexamethasone (Fig. 1). IFN- γ restored O_2^- generation to normal levels. In addition, it appears that IFN- γ reversed the suppressive effects of dexamethasone.

Degree of metabolic burst enhancement by IFN- γ . The percent enhancement of monocyte O_2^- release by IFN- γ shown in Table 4 was calculated from data obtained when the monocytes were simultaneously treated with dexamethasone and IFN- γ , and is from the data in Figure 1. Synergism is suggested by the data when dexamethasone treatment preceded IFN- γ treatment. When PMA was the stimulus, all concentrations of dexamethasone appeared to enhance the augmentation of monocyte O_2^- release. When *M. leprae* was used as a stimulus for monocyte O_2^- release, 20 μ M dexamethasone increased the enhancement by IFN- γ . Under culture conditions where dexamethasone and IFN- γ treatment was simultaneous, only the highest concentration of dexamethasone enhanced the IFN- γ activation of monocytes in response to PMA (Table 4).

DISCUSSION

Lymphokines are known to enhance, and hydrocortisone to inhibit, the ability of monocytes to secrete reactive oxygen intermediates (18). IFN- γ is the lymphokine responsible for the activation of macrophages that enhances oxidative killing of intracellular parasites (17, 19, 20, 25). Lymphocytes from patients with the lepromatous form of Hansen's disease are unable to produce normal amounts of IFN- γ upon challenge with *M. leprae* (21). Kaplan, *et al.* (10) have reported that monocyte-derived macrophages from 10 to 14 lepromatous patients studied responded to recombinant IFN- γ with enhanced ability to generate H_2O_2 . More recently, it has been shown that macrophages from leprosy patients have an enhanced ability to kill *M. leprae* in the presence of IFN- γ (2) or delipidified cell components of *M. leprae* (13). Our study shows that monocytes of patients receiving prednisone therapy at the time peripheral blood was drawn responded to lower concentrations of IFN- γ with an enhanced O_2^- production when stimulated with *M. leprae* or BCG, than did monocytes from patients not on prednisone therapy or monocytes from healthy subjects. Even though the number of patients in the study was small and the patient population was heterogeneous, the data suggested a prednisone effect

TABLE 3. Effect of IFN- γ on O_2^- generation by monocytes from leprosy patients.^a

Subjects	IFN- γ (U/ml)	Stimuli ^b		
		<i>M. leprae</i>	BCG	PMA
Patients receiving prednisone (N = 4)	0	102 \pm 10	105 \pm 13	409 \pm 13
	50	169 \pm 29 ^c	187 \pm 28 ^c	371 \pm 18
Patients not on prednisone (N = 6)	0	150 \pm 52	119 \pm 26	360 \pm 71
	50	149 \pm 26	154 \pm 38	350 \pm 89
Healthy subjects (N = 8)	0	133 \pm 38	92 \pm 21	328 \pm 68
	50	119 \pm 32	168 \pm 31 ^c	327 \pm 63

^a Values are mean nmol O_2^- /mg cell protein/150 min \pm S.D.

^b Bacteria were used at a particle-to-monocyte ratio of 50:1; PMA was used at 500 ng/ml.

^c $p < 0.05$ vs 0 IFN- γ group.

and led us to examine the effect of dexamethasone on IFN- γ activation of normal monocytes *in vitro*.

Monocytes pretreated with dexamethasone only released less O_2^- than did control cells. IFN- γ enhanced the O_2^- generation of monocytes whether or not the cells had been pretreated with dexamethasone. In fact, synergism is suggested by these results since when dexamethasone treatment preceded IFN- γ treatment, the IFN- γ enhancement of O_2^- release was further augmented. When PMA was the stimulus, all concentrations of dexamethasone appeared to enhance the IFN- γ augmentation of monocyte O_2^- release. When *M. leprae* was used as the challenge stimulus, the highest concentration of dexamethasone exerted its potentiating effect.

A dexamethasone-induced increase in receptors for IFN- γ on monocytes has been reported (30). We found that IFN- γ augmentation of monocyte O_2^- release was greater in dexamethasone-pretreated cells

than in untreated cells. This would be consistent with an increase in IFN- γ receptors on the monocytes. It appears that pretreatment of monocytes with dexamethasone was more effective in increasing the augmentation of O_2^- by IFN- γ than simultaneous treatment with both agents. This is in agreement with observations that 24 hours or longer was needed for dexamethasone to exert its effects (27). It has been shown that the IFN- γ enhancement of the macrophage respiratory burst could not be prevented by dexamethasone (16), even at the concentrations of dexamethasone used that suppressed the antimicrobial activity of human monocytes (27). Others (31) have reported that IFN- γ can block the O_2^- inhibitory activity of various glucocorticoids. Moreover, dexamethasone has been shown to augment IFN- γ effects on human monocyte effector function as measured by antibody-dependent cellular cytotoxicity (29) and to enhance IFN- γ augmentation of monocyte IgG Fc receptor expression (3, 32). Although it was

TABLE 4. Effect of dexamethasone on IFN- γ augmentation of monocyte O_2^- release.^a

Dexamethasone	Dexamethasone treatment			
	Preceding IFN- γ ^b treatment		Simultaneously with IFN- γ ^b	
	Stimuli ^c		Stimuli ^c	
	<i>M. leprae</i>	PMA	<i>M. leprae</i>	PMA
0	45 \pm 10 (10) ^d	34 \pm 9 (10)	43 \pm 12 (9)	38 \pm 8 (9)
2 nM	61 \pm 10 (9)	45 \pm 8 (9) ^e	42 \pm 9 (7)	52 \pm 5 (7)
200 nM	36 \pm 13 (8)	62 \pm 7 (9) ^e	69 \pm 8 (7)	48 \pm 8 (8)
20 μ M	75 \pm 6 (8) ^e	68 \pm 9 (8) ^e	56 \pm 13 (6)	65 \pm 6 (6) ^e

^a Values are percent enhancement of O_2^- release by IFN- γ \pm S.D.

^b IFN- γ dose was 500 U/ml.

^c *M. leprae* were used at a bacteria-to-monocyte ratio of 50:1; PMA used at 500 ng/ml.

^d Figures in parentheses are number of experiments.

^e $p < 0.05$ vs 0 dexamethasone group.

recently reported that dexamethasone did not suppress H_2O_2 release by monocyte-derived macrophages (²⁸), our studies, using 24- to 48-hour monocyte cultures, show that dexamethasone can suppress O_2^- release as measured by cytochrome c reduction in a sensitive microassay.

IFN- γ and dexamethasone are both important immunomodulators. In our studies, IFN- γ reversed the immunosuppressive effect of dexamethasone on monocytes. In fact, our findings suggest that enhanced effects of IFN- γ may perhaps be achieved by glucocorticoid pretreatment.

SUMMARY

Leprosy patients suffering from erythema nodosum leprosum are frequently treated with glucocorticosteroids. The role glucocorticosteroids and interferon-gamma (IFN- γ) play in regulating the interaction of phagocytic cells with *Mycobacterium leprae* was examined. Monocytes from leprosy patients receiving prednisone therapy responded to lower concentrations of IFN- γ *in vitro* with enhanced superoxide anion release when challenged with *M. leprae* or *M. bovis* BCG than did monocytes from healthy subjects and other leprosy patients. Although the number of patients was small and the population heterogeneous, the data suggested that prednisone could alter IFN- γ efficacy and led to the examination of the effect of glucocorticosteroids on IFN- γ activation of monocytes. IFN- γ treatment following *in vitro* dexamethasone pretreatment of monocytes from healthy subjects resulted in a greater enhancement of superoxide anion generation than that observed with IFN- γ treatment alone. These findings are important considerations in evaluating patient immune function because IFN- γ is being used in a number of clinical trials with leprosy patients.

RESUMEN

Los pacientes con eritema nudoso leproso frecuentemente son tratados con glucocorticoesteroides. Aquí se examina el papel que juegan los glucocorticoesteroides y el interferón gamma (IFN- γ) en la respuesta de las células fagocíticas al interaccionar con *Mycobacterium leprae*. Los monocitos de pacientes con lepra bajo tratamiento con prednisona, respondieron *in vitro* con la producción y liberación de anión superóxido (estimulada con *M. leprae* o con *M. bovis*, BCG) a

concentraciones más bajas del IFN- γ que los monocitos de personas sanas o de otros pacientes con lepra. Aunque el número de pacientes fue pequeño y la población heterogénea, los datos sugirieron que la prednisona podría alterar la eficiencia del IFN- γ . Esto condujo al examen del efecto de los glucocorticoesteroides sobre la activación de los monocitos por el IFN- γ . El tratamiento con IFN- γ posterior al pretratamiento *in vitro* de los monocitos de individuos sanos con dexametasona, dió como resultado un mayor incremento en la generación de anión superóxido que el incremento observado cuando el tratamiento fue con IFN- γ solo. Estos hallazgos pueden ser importantes porque el IFN- γ actualmente se está utilizando en un buen número de ensayos clínicos con pacientes con lepra.

RÉSUMÉ

Les malades de la lèpre présentant un erythème noueux lépreux sont fréquemment traités avec des glyco-corticostéroïdes. Le rôle que jouent les glyco-corticostéroïdes et l'interferon-gamma (IFN- γ) dans la régulation de l'interaction des cellules phagocytaires avec *Mycobacterium leprae* a été examiné. Des monocytes de malades de la lèpre recevant un traitement à la prednisone répondaient *in vitro* à des concentrations plus faibles d'IFN- γ avec une augmentation de la libération d'anion superoxide en présence de *M. leprae* ou de BCG de *M. bovis* que des monocytes en provenance de sujets sains ou d'autres malades de la lèpre. Bien que le nombre de patients était limité et la population hétérogène, les données suggèrent que la prednisone pourrait modifier l'efficacité de l'IFN- γ et ont conduit à examiner l'effet des glyco-corticostéroïdes sur l'activation des monocytes par l'IFN- γ . Le traitement par l'IFN- γ suivant un pré-traitement *in vitro* à la dexaméthasone des monocytes de sujets sains a résulté dans une augmentation de la production d'anion superoxide par rapport à celle observée suite à un traitement par l'IFN- γ seul. Ces observations sont importantes pour l'évaluation de la fonction immunitaire des malades parce que l'IFN- γ est utilisé dans un certain nombre d'essais cliniques avec des malades de la lèpre.

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