

In Vitro And In Vivo Effects of Dapsone on Neutrophil and Lymphocyte Functions in Normal Individuals and Patients with Lepromatous Leprosy

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The effects of dapsone on polymorphonuclear leukocyte functions and lymphocyte mitogen-induced transformation were assessed in vitro and in vivo in normal individuals and in newly diagnosed untreated patients with lepromatous leprosy. The effects of dapsone on the cell-free generation of superoxide by the xanthine: xanthine oxidase system and iodination of bovine serum albumin by horseradish peroxidase were also investigated. In normal individuals dapsone mediated stimulation of polymorphonuclear leukocyte migration in vitro and in vivo. Dapsone had no effects on postphagocytic hexose monophosphate shunt activity or superoxide generation in vitro, but caused slight inhibition of peroxidase-mediated protein iodination in vitro and in vivo and hexose monophosphate shunt activity in vivo. Similar effects were found in patients with lepromatous leprosy. Dapsone also decreased the inhibitory activity of serum from patients with lepromatous leprosy on normal polymorphonuclear leukocyte migration in vitro. Progressive loss of serum-mediated inhibition of migration was observed after ingestion of dapsone by the patients. Further experiments showed that stimulation of polymorphonuclear leukocyte motility was related to inhibition of the peroxidase-H₂O₂-halide system in vitro. The drug caused inhibition of lymphocyte transformation at high concentrations in vitro, but had slight stimulatory activity on phytohemagglutinin-induced transformation in controls and patients in vivo.

The precise therapeutic mechanism of dapsone (4,4'-diaminophenyl sulfone) in the treatment of leprosy is unknown (14). In vitro experiments with other bacteria have shown that the chemotherapeutic agent has sulfonamide-like antimicrobial properties and causes inhibition of the enzyme dihydropteroic acid synthetase (13). However, it seems unlikely that this is the only mechanism (if it is indeed a true in vivo mechanism) since dapsone has been reported to have beneficial effects in non-mycobacterial diseases such as dermatitis herpetiformis (9), generalized pustular psoriasis (19), and leukocytoclastic vasculitis (27) and in some cases of rheumatoid arthritis (26) and Crohn's disease (23). The effects of dapsone therapy observed in these conditions may relate to the anti-inflammatory activity of the drug (22).

We have recently reported that commencement of dapsone therapy in newly diagnosed patients with lepromatous leprosy was associated with a progressive recovery of polymorphonuclear leukocyte (PMN) motility and increased

lymphocyte responsiveness to mitogens (2). In view of these findings we undertook a study to investigate the in vitro and in vivo effects of dapsone on PMN functions and lymphocyte transformation in normal individuals and patients with lepromatous leprosy.

MATERIALS AND METHODS

Patients. Sixteen new untreated leprosy patients (4 borderline, 2 subpolar, and 10 with lepromatous leprosy [LL]) were classified by the clinical and histopathological criteria of Ridley and Jopling (16). All patients showed negative skin tests to lepromin. Rifampin therapy was withheld for 1 week to assess the effects of dapsone therapy (100 mg daily) alone. For in vivo studies cellular immune functions were performed before dapsone therapy, 2 h after ingestion of a single oral dose of 100 mg of dapsone, and after 1 week of ingestion of a single oral daily dose of 100 mg of dapsone.

Control subjects. Normal adults were used to assess the duration of dapsone effects on neutrophil and lymphocyte functions after the ingestion of a single oral dose of 100 mg of dapsone. Tests of cellular

function were performed before dapsone intake and at 2, 24, and 48 h after the ingestion of the drug.

Dapsone. Dapsone was partially dissolved in the appropriate suspending medium according to the cell function being assessed, and its effects over a concentration range of 0.01 μ M to 100 mM were investigated.

PMN functions: studies of motility. PMN were obtained from heparinized venous blood (5 U of heparin per ml) and were resuspended to a final concentration of 6×10^6 /ml in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; Sigma Chemical Co., St. Louis, Mo.)-buffered Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 0.05% bovine serum albumin after hypotonic lysis of residual erythrocytes with 0.84% ammonium chloride as previously described (3).

Two leukoattractants were used: (i) EAS, namely, fresh autologous serum activated with 100 μ g of bacterial endotoxin (*Escherichia coli* O127:B8; Difco Laboratories, Detroit, Mich.) per ml, which was diluted eightfold with Hanks balanced salt solution before use; and (ii) the synthetic chemotactic tripeptide, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (f-met-leu-phe; Miles Laboratories Inc., Elkhart, Ind.), which was used at a final concentration of 0.5 μ M (previously found to be the optimal leukotactic concentration).

In random migration systems the leukoattractant was replaced with an equal volume of Hanks balanced salt solution (0.8 ml). The various dapsone concentrations were added to PMN before assessment of motility and remained in the cell compartment throughout the incubation period. The final PMN concentration in all studies of migration was 3×10^6 /ml. The assays of motility were performed in modified Boyden chambers (25) with membrane filters (5- μ m pore size, Sartorius-membranfilter, Göttingen, West Germany) and a 2-h incubation period. The results are expressed as the number of cells which have completely traversed the filter as cells per microscope high-power field as an average of triplicate filters.

Serum inhibition studies. For investigation of the effects of dapsone in vitro and in vivo on the migration inhibitory effects of serum from patients with LL, 10% heat-inactivated normal or LL serum was added to PMN in the cell compartment of the Boyden chamber.

Leukotactic potential of the test agent. For determination of whether dapsone possessed intrinsic leukotactic potential, stimulatory concentrations were placed above and below the filter to give positive and negative gradients and chemokinesis systems for each drug concentration in the absence of a leukoattractant.

Phagocytic studies. In studies of postphagocytic metabolic activity the cell suspending medium used was 0.15 M phosphate-buffered saline (pH 7.2). These activities were stimulated by using *Candida albicans* at concentrations which under our laboratory conditions gives maximal stimulation of postphagocytic metabolic activity. Pure PMN suspensions prepared as previously described (3) and of >90% purity and viability were used in these studies.

Hexose monophosphate shunt activity. Hexose monophosphate shunt activity was measured by the method of Wood et al. (28), with minor modifications (3), by potassium hydroxide absorption of $^{14}\text{CO}_2$ derived from D-glucose radiolabeled in the 1-C position

(New England Nuclear Corp., Boston, Mass.). The reaction mixture contained 2×10^6 PMN (0.1 ml) and 0.1 ml of autologous serum, 10^7 *C. albicans* cells (0.1 ml), 0.1 ml of the drug at an appropriate concentration, and 0.6 ml of radiolabeled glucose (0.06 μ Ci). Incubation was for 1 h after which the reaction was terminated, and CO_2 was released by the addition of 2 ml of 1 N HCl. After 1 h the radioactivity associated with KOH was determined in a liquid scintillation counter. Results are expressed as nanomoles of glucose metabolized per 60 min.

Measurement of H_2O_2 production. The effects of the drug on release of H_2O_2 into the extracellular medium after ingestion of opsonized *C. albicans* were determined. Opsonization was performed at 4°C by using 1 ml of fresh pooled serum per 10^8 organisms per 2 h. The organisms were washed twice and resuspended to a concentration of 5×10^9 /ml. Each experimental tube contained 10^7 PMN (0.2 ml), 0.1 ml of *C. albicans* (to give a PMN/*C. albicans* ratio of 1:50), 0.1 ml of the drug at an appropriate concentration, 0.1 ml of 10 mM sodium azide, and 0.4 ml of phosphate-buffered saline supplemented with 10 mM glucose. Tubes were preincubated for 15 min before the addition of *C. albicans*. After a 30-min incubation period the tubes were centrifuged at $5,000 \times g$ for 10 min, and the supernatant fluid was assayed for H_2O_2 by the method of Root et al. (17) by reduction of scopoletin fluorescence. Each assay tube contained 2.5 ml of distilled water, 50 μ l of horseradish peroxidase (HRP; type VI; Sigma) at a stock concentration of 13.8 U/ml, 20 μ l of 200 μ M scopoletin (Sigma), and 50 μ l of the cell-free supernatant. Standard curves were constructed in the range of 0.1 to 10 nmol of H_2O_2 (Merck & Co., Inc., Rahway, N.J.). The H_2O_2 -dependent reduction in fluorescence was monitored on a Perkin-Elmer model 204 Hitachi fluorescence spectrophotometer at an exciter wavelength of 390 nm and an analyzer wavelength of 460 nm. Results are expressed as nanomoles per 10^7 PMN per minute.

PMN superoxide production. Superoxide production by PMN was assayed as described by Curnutte and Babior (10). The reaction mixtures contained 5×10^6 PMN in 0.5 ml, 0.1 ml of preopsonized *C. albicans* to give a cell/microorganism ratio of 1:50, 0.1 ml of 1 mM horse heart ferricytochrome *c* (cyt *c*; type VI; Sigma) and 0.1 ml of the drug at an appropriate concentration or 0.1 ml of phosphate-buffered saline in controls. The final reaction volume was 1 ml. Tubes were incubated on a turntable at 37°C for 45 s. (reaction linear to 90 s), after which the reaction was terminated with 1 ml of ice-cold phosphate-buffered saline, tubes were centrifuged, and the supernatants were assayed for reduced cyt *c* in a Unicam SP 1700 ultraviolet spectrophotometer at 550 nm. The amount of reduced cyt *c* was calculated by using an absorbance coefficient of 15.5 mM at 550 nm (12). Superoxide-dependent reduction of cyt *c* was expressed as the difference in cyt *c* reduction between reaction mixtures containing no superoxide dismutase (Sigma) and those containing 200 U of superoxide dismutase per ml.

Quantitative MPO assay. Myeloperoxidase (MPO) was assayed by the increase in fluorescence at 470 nm which accompanies the oxidation of guaiacol. In these experiments 10^8 PMN (1 ml) were sonicated

in an MSE ultrasonic disintegrator (three 20-s bursts) at an amplitude of 10 μm peak to peak, and the sonicate was centrifuged at $5,000 \times g$ for 10 min. The supernatant was used as a source of MPO, and 0.1-ml volumes were incubated with 0.1 ml of the drug at an appropriate concentration for 30 min. After incubation, 0.1-ml samples of the mixtures were then assayed for MPO by the method of Paul et al. (15), with minor modifications. The assay system consisted of 1 ml of glycine-NaOH buffer (pH 10), 1 ml of 3×10^{-2} M guaiacol, 1 ml of 10^{-2} M H_2O_2 , and 0.1 ml of the reaction mixture. The rate of oxidation was measured spectrophotometrically at 470 nm. Results are expressed as enzyme units per 10^8 PMN, calculated from a standard curve in the range of 0.83 to 275 U of HRP per ml.

MPO-mediated iodination of ingested protein. MPO-mediated iodination of ingested protein was determined by the method of Root and Stossel (18), with minor modifications. To 0.1 ml of PMN suspension ($10^7/\text{ml}$) was added 0.1 ml of *C. albicans* cells ($10^8/\text{ml}$), 0.1 ml of fresh autologous serum, 0.1 ml of the drug at various concentrations, 0.1 ml of a ^{125}I solution (0.6 $\mu\text{Ci}/\text{ml}$) (New England Nuclear; sodium iodate), and 0.5 ml of phosphate-buffered saline. Incubation was for 60 min at 37°C on a turntable, after which the extent of incorporation of ^{125}I into acid-precipitable protein was determined by solid scintillation counting. Results are expressed as nanomoles of ^{125}I in the protein precipitate.

Effects of dapsone on the HRP-iodide- H_2O_2 system. To investigate the effects of the drug in a cell-free system the effects on the HRP iodination of bovine serum albumin were assessed. Each reaction system contained 0.27 U of HRP, 10 μmM H_2O_2 , 0.06 μCi of ^{125}I , 5 mg of bovine serum albumin, and the drug at an appropriate concentration in a total reaction volume of 1 ml. Tubes were incubated and processed as above for the MPO system.

Exposure of neutrophils to the HRP-iodide- H_2O_2 system. The effects of the HRP-iodide- H_2O_2 system in the presence and absence of dapsone on neutrophil motility were investigated. PMN (3×10^6) in 0.5 ml were preincubated with 0.27 U of HRP, 1 μM hydrogen peroxide, 1 mM sodium iodide, and drug at a concentration which caused maximal stimulation of motility in a final reaction volume of 1 ml of Hanks balanced salt solution without bovine serum albumin. Control systems contained no drug. The reaction systems were incubated at 37°C for 30 min, after which the supernatants containing the reactants were removed after centrifugation, and the cell pellets were resuspended to 3×10^6 cells per ml in Hanks balanced salt solution supplemented with 0.05% bovine serum albumin. The differently processed neutrophils were then tested for reactivity to EAS.

Inhibition of peroxide availability. For determination of whether dapsone bound or inactivated H_2O_2 the drug was incubated with 2 μM peroxide for 30 min, and the mixture was assayed for peroxide by the reduction of scopoletin fluorescence as described above.

Effects on the xanthine:xanthine oxidase system. For assessing whether dapsone had superoxide scavenging properties, the effects on xanthine:xan-

thine oxidase-mediated reduction of cyt *c* were assessed. Each reaction system contained 1.7 mM xanthine, 100 μM cyt *c*, the drug at an appropriate concentration and 0.12 U of xanthine oxidase (Sigma; grade 1, from buttermilk) in a reaction volume of 3 ml of 0.05 M phosphate-buffered saline (pH 7.2). Superoxide-mediated reduction of cyt *c* was assessed after 3 min (still on the linear portion of the reaction curve) at 22°C spectrophotometrically at 550 nm.

Lymphocyte transformation. Blood for lymphocyte transformation studies was defibrinated and fractionated by density gradient centrifugation (Ficoll-sodium metrizoate gradients) at 400 g/25 min. The mononuclear cell layer was removed and washed twice in TC 199 (GIBCO) (pH 7.2) supplemented with 2 g of HEPES per liter and 10% heat-inactivated autologous serum. The cell suspension was adjusted to 4×10^6 mononuclear cells per ml. Samples of 50 μl (2×10^5 cells) were placed in wells of 5-mm Linbro tissue culture plates (Flow Laboratories, Inc., Inglewood, Calif.) together with 100 μl of serum-supplemented TC 199. The mitogens used in this study were phytohemagglutinin (Wellcome Reagents, Ltd., Beckenham, England) and concanavalin A (Sigma), each at concentrations of 25 and 50 $\mu\text{g}/\text{ml}$. Mitogens were added in 20- μl volumes to triplicate wells, and unstimulated controls received 20 μl of TC 199. The different concentrations of the test agent (0.01 μM to 10 mM) were added in 20- μl volumes to triplicate wells. The final volume in each well was brought to 200 μl by the addition of serum-supplemented TC 199. The plates were mixed and incubated for 48 h in a humidified atmosphere of 3% CO_2 in air, 20 μl of [*methyl*- ^3H]thymidine (New England Nuclear) containing 0.2 μCi was added to each well, and the plates were incubated for a further 18 h. Harvesting was performed with a multiple automated sample harvester (MASH-II; Microbiological Associates, Bethesda, Md.). Incorporation of [^3H]thymidine was assessed in a liquid scintillation spectrophotometer.

RESULTS

Calculation and expression of results. Unless otherwise stated the results are expressed as the mean value with standard error for each investigation. Statistical analysis of results was performed by using the Student's *t*-test (*t* statistic for two means). Pre-dapsone values for in vivo studies and control systems without added dapsone for in vitro experiments were compared with the corresponding experimental values.

In vitro studies of PMN motility. Dapsone at concentrations of > 0.1 mM (Fig. 1) caused stimulation of normal PMN migration to EAS which was statistically significant at 1, 2.5, and 5 mM, with *P* values of < 0.05 for each concentration (Student's *t* test for paired means). Inhibition of migration was observed at dapsone concentrations of > 10 mM. Dapsone in vitro caused no stimulation of PMN random migration or migration to *f*-met-leu-phe, and progressive inhibition was observed at drug concentrations of > 0.1 mM (Fig. 1).

Dapsone at concentrations found to stimulate migration of normal PMN caused significant stimulation of PMN from 10 patients with LL to normal and autologous EAS. The mean PMN per high-power field \pm standard errors were as follows: without dapsone, 55.9 ± 17.8 ; with 1 mM dapsone, 128.8 ± 19.4 ; with 2.5 mM dapsone, 148.0 ± 22.8 ; with 5 mM dapsone, 153.4 ± 23.3 ($P < 0.01$ for all values with dapsone). Further experiments showed that incubation of normal PMN with serum from three patients with LL caused inhibition of migration to autologous EAS which was eliminated by migration-stimulatory concentrations of dapsone. The mean PMN per high-power field \pm standard errors of three separate experiments were as follows for PMN incubated with the following: 10% autologous serum, 185.3 ± 7.9 ; 10% LL serum, 75.0 ± 26.0 ; 10% LL serum with 2.5 mM dapsone, 159.7 ± 44.1 ; 10% LL serum with 5 mM dapsone, 159.3 ± 41.0 ; 10% LL serum with 10 mM dapsone, 200.3 ± 23.2 .

In vivo studies of PMN motility. Ingestion of a single 100-mg oral dose of dapsone by eight normal volunteers caused increased migration to EAS with no detectable effects on random migration or migration to f-met-leu-phe (Table 1). Ingestion of dapsone by the leprosy patients was

accompanied by significantly increased PMN migration to normal and autologous EAS (Table 1) and a progressive loss of the migration-inhibitory activity of their sera. The mean PMN per high-power field \pm standard errors of sera from three patients with LL were as follows for PMN incubated with the following: 10% autologous serum, 158.2 ± 17.4 ; 10% LL serum before dapsone therapy, 62.6 ± 19.6 ; 10% LL serum at 2 h after ingestion of 100 mg of dapsone, 104.8 ± 30.0 ; 10% LL serum after 1 week of daily ingestion of 100 mg of dapsone, 125.0 ± 36.2 . Dapsone possessed no intrinsic leukotactic potential (data not shown).

Phagocytosis and postphagocytic metabolic activity. Dapsone at concentrations up to 10 mM had no detectable effects on the phagocytosis of *C. albicans* by normal PMN (data not shown). Slight inhibition of hexose monophosphate shunt activity was observed with dapsone concentrations of 10 mM, with a mean inhibitory value (three experiments) of $10.1 \pm 1.2\%$. No inhibition of PMN H_2O_2 or superoxide generation and superoxide generation by the xanthine: xanthine oxidase system at dapsone concentrations up to 2.5 mM was observed. Investigation of the effects of higher dapsone concentrations on these functions was not possible, as the drug

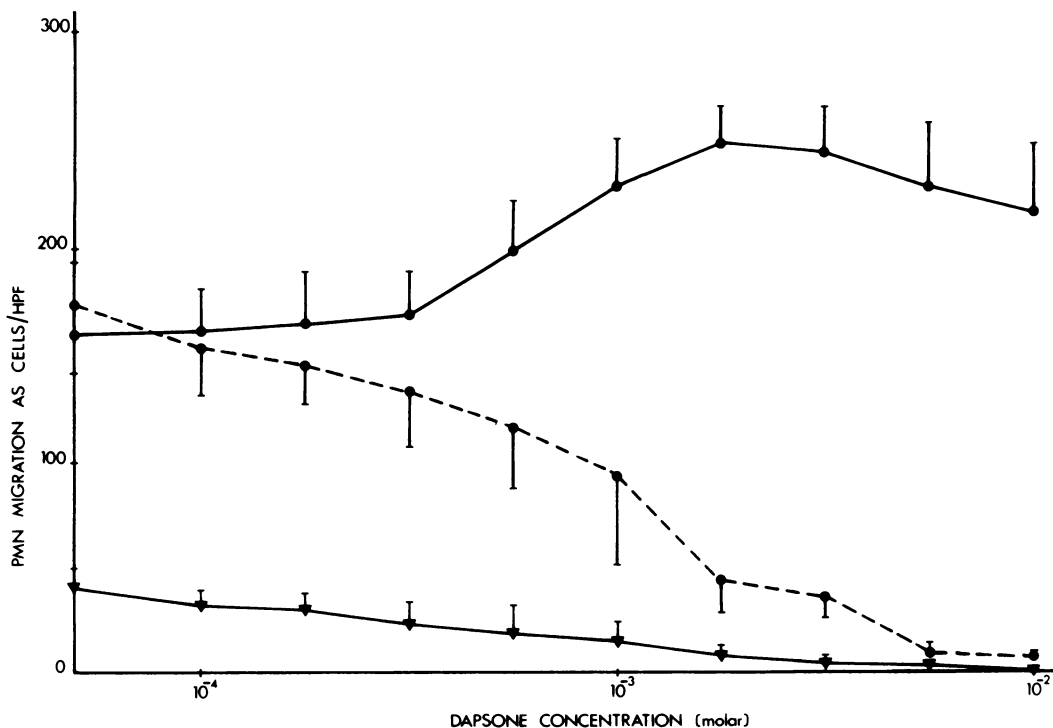


FIG. 1. Effects of dapsone on the migration of blood neutrophils obtained from normal adult volunteers to autologous EAS (●—●) and f-met-leu-phe (●---●) and on random motility (▼—▼).

TABLE 1. Effects of ingestion of a single 100-mg oral dose of dapsone in normal adults and patients with LL^a

Subjects	Migration to autologous EAS (PMN/HPF) ^b		HMS activity (nmol of 1- ¹⁴ C]glucose metabolized/60 min)		MPO-mediated iodination (nmol of ¹²⁵ I precipitated)				
	Before dapsone	At 2 h ^c	At 24 h ^c	Before dapsone	At 2 h	At 24 h	Before dapsone	At 2 h	At 24 h
Normal adults	174 ± 24	286 ± 41 ^d	212 ± 41	14.7 ± 0.5	13.9 ± 0.8	14.8 ± 0.9	0.76 ± 0.07	0.062 ± 0.07	0.08 ± 0.08
Patients with LL	36 ± 10	65 ± 19 ^d	38 ± 5	15.5 ± 1.2	12.9 ± 1.1	14.9 ± 1.2	0.70 ± 0.06	0.58 ± 0.11	0.74 ± 0.08

^a Results are expressed as the mean value with standard error obtained from eight normal individuals and 12 patients with LL. Random motility and migration to f-met-leu-phe were unaffected.

^b HPF, High-powered microscope field.

^c Time after dapsone ingestion.

^d $P < 0.05$, calculated by Student's *t* test.

per se interfered with the assay system. Dapsone at concentrations up to 2.5 mM had no detectable H₂O₂ binding or inactivating effects (data not shown).

MPO- and HRP-mediated protein iodination. MPO- and HRP-mediated protein iodination was progressively inhibited by dapsone at concentrations of >0.01 mM (Fig. 2). The activity of MPO per se as assessed by the halide-independent oxidation of guaiacol was unaffected by dapsone at concentrations up to 2.5 mM with values of 29.1 ± 3.1 and 26.4 ± 4.3 U/10⁶ PMN for control and 2.5 mM dapsone systems, respectively.

Effects of the HRP-H₂O₂-halide system on PMN migration to EAS. For investigation of the possible relationship of dapsone-mediated stimulation of PMN migration and inhibition of the HRP-H₂O₂-halide system, PMN were exposed to this system in the presence and absence of migration-stimulatory concentrations of dapsone. The HRP-H₂O₂-halide system mediated considerable inhibition of PMN migratory responsiveness to EAS. Inclusion of migration-stimulatory concentrations of dapsone protected the PMN from the HRP-H₂O₂-halide system-mediated inhibition of migration (Table 2).

Postphagocytic hexose monophosphate shunt activity and MPO-mediated iodination. Postphagocytic hexose monophosphate shunt activity and MPO-mediated iodination were inhibited in normal individuals and leprosy patients (Table 1) after ingestion of a single 100-mg oral dose of dapsone. These differences did not, however, achieve statistical significance.

Mitogen-induced lymphocyte transformation. Mitogen-induced lymphocyte transformation was unaffected by dapsone concentrations of up to 0.1 mM in vitro. Concentrations of >0.1 mM caused progressive inhibition of responsiveness to both mitogens. The mean inhibition (three experiments) for 1 mM dapsone at 25 µg of phytohemagglutinin per ml was 28.3 ± 5.5%. Ingestion of a single 100-mg oral dose of dapsone was accompanied by a slight but insignificant stimulation of transformation in three patients and six healthy adult volunteers (Table 3).

DISCUSSION

We have previously reported that dapsone therapy was associated with improved PMN chemotaxis and mitogen-induced transformation in a group of patients with LL (2). The relationship between dapsone therapy and improved leukocyte functions was not investigated. We suggested that it may be due to a primary immunopharmacological effect or a secondary effect as a result of decreased activity of serum

inhibitors of leukocyte functions due to the antimicrobial activity of the drug. In the present study we have found that dapsone causes in vitro and in vivo stimulation of motility of PMN from normal individuals and patients with LL. It has previously been reported that PMN migration is decreased in patients with both tuberculoid leprosy and LL due to intrinsic cell

defects and serum inhibitors of leukotaxis (7, 20, 24). Dapsone in vitro and in vivo reduced the migration inhibition activity of sera from patients with LL. The stimulation of motility by dapsone in vitro was observed only when EAS was used as the leucoattractant. Inhibition of random motility and migration to f-met-leu-phe was observed. This difference could be explained

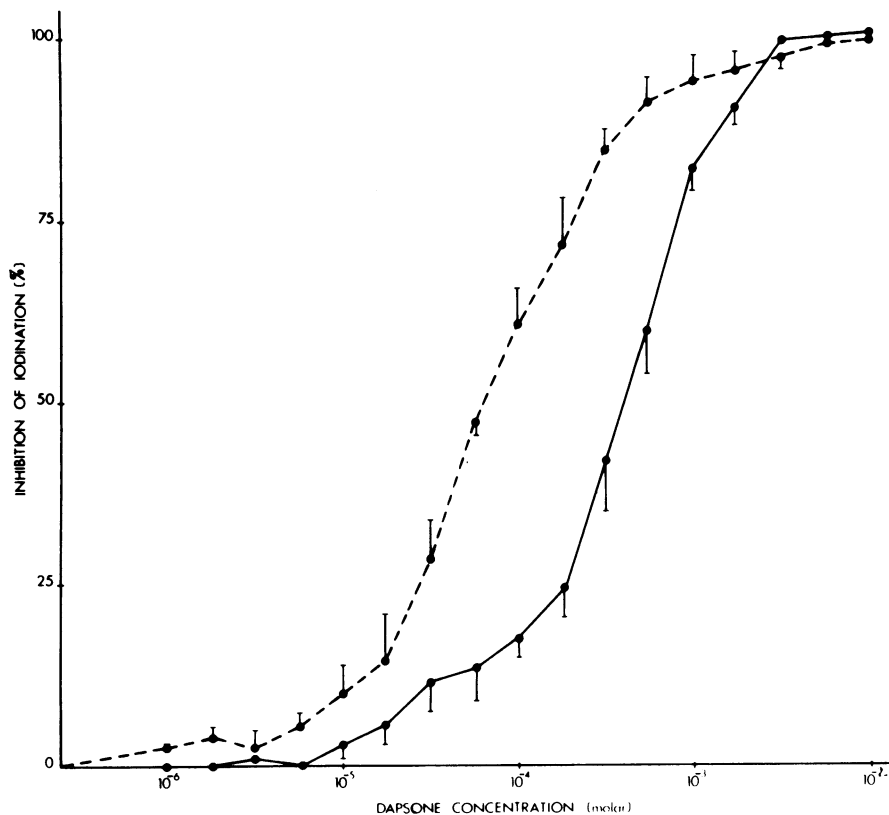


FIG. 2. Effects of dapsone on MPO-H₂O₂-¹²⁵I-mediated iodination of ingested *C. albicans* (●—●) and BSA (●—●).

TABLE 2. Effects of migratory-stimulatory concentrations of dapsone on inhibition of PMN migration to autologous EAS mediated by the HRP-H₂O₂-halide system

Reaction system	PMN migration to autologous EAS ^a
PMN + HBSS	196 ± 17
PMN + HRP	182 ± 18
PMN + H ₂ O ₂ + I ⁻	102 ± 8 ^b (<i>P</i> < 0.01) ^c
PMN + HRP + H ₂ O ₂ + I ⁻	17 ± 8 (<i>P</i> < 0.005) ^c
PMN + HRP + H ₂ O ₂ + I ⁻ + 1 mM dapsone	206 ± 16
PMN + HRP + H ₂ O ₂ + I ⁻ + 2.5 mM dapsone	236 ± 21
PMN + HRP + H ₂ O ₂ + I ⁻ + 5 mM dapsone	212 ± 19

^a Results are expressed as mean cells per high-power field ± standard error of six separate experiments. Cells were preincubated with the various concentrations of agents at 37°C for 30 min followed by washing. The chemotactic responsiveness of the cells was tested with autologous EAS.

^b The low migratory value obtained with the H₂O₂-I⁻ (without HRP) may be due to MPO release from PMN.

^c *P* values were obtained by comparing the experimental values with the control values (Student's *t* test). Experimental systems containing dapsone were not significantly different from the control value.

TABLE 3. Effects of ingestion of a single 100-mg oral dose of dapsone on lymphocyte transformation to phytohemagglutinin (PHA)^a

Subjects	Lymphocyte responsiveness to 25 µg of PHA/ml			Lymphocyte responsiveness to 50 µg of PHA/ml		
	Before dapsone	At 2 h ^b	At 24 h ^b	Before dapsone	At 2 h	At 24 h
Normal adults	58,790 ± 5,028	65,157 ± 6,420	63,810 ± 10,980	52,805 ± 5,052	61,719 ± 7,645	49,766 ± 11,404
Patients with LL	54,116 ± 9,884	58,050 ± 11,527	53,819 ± 9,979	45,941 ± 8,581	52,711 ± 12,458	40,521 ± 8,899

^a Results are expressed as mean counts per minute with standard error of six normal individuals and patients with LL.

^b Time after dapsone ingestion.

by the requirement of a serum factor for dapsone-mediated stimulation of PMN motility as has been previously described for ascorbate (4). Alternatively, f-met-leu-phe may cause lesser stimulation of MPO release and H₂O₂ production than does EAS. In recent experiments (data not included) we have shown that substitution of bovine serum albumin with 5% fresh autologous serum in the presence of stimulatory concentrations of dapsone causes stimulation of neutrophil motility. Although synthetic leukotactic peptides are important in the evaluation of biochemical events associated with leukocyte locomotion, the present results indicate that the use of serum-associated leukoattractants may be necessary to assess drug effects.

There is a considerable difference in the dapsone concentrations required to stimulate neutrophil motility in vitro and in vivo. After ingestion of a single 100-mg oral dose of dapsone, peak serum levels occur at approximately 4 to 6 h, with an average serum value of 2 µg/ml (1). Concentrations required to cause stimulation of neutrophil motility in vitro are approximately 100-fold greater (1 mM). This indicates that enhancement of neutrophil migration in vivo requires considerably less dapsone than is required to achieve the same effect in vitro. Similar findings have been reported for levamisole-mediated stimulation of neutrophil motility in vitro and in vivo (3).

Dapsone in vitro had no effect on phagocytosis or superoxide and H₂O₂ production. However, the drug caused inhibition of postphagocytic MPO-mediated iodination of *C. albicans* and HRP-mediated iodination of bovine serum albumin. This inhibitory activity was not related to an effect on MPO per se. It is possible that dapsone acts as a scavenger of oxidative intermediates generated by the HRP-H₂O₂-halide system. Stendahl et al. have previously reported that dapsone is an inhibitor of MPO-mediated iodination (21). These authors also reported that dapsone in vitro had no effects on PMN migration to zymosan-activated serum, which is not in agreement with this report. These differences are probably due to the different concentration ranges investigated in the two studies and the limited sensitivity of the agarose technique used by Stendahl et al. to measure PMN migration (11). Inhibition of MPO-mediated iodination as well as postphagocytic HMS activity was observed in normal individuals and patients with LL after the ingestion of dapsone. The effects on HMS activity were unexpected since inhibition in vitro was observed only with dapsone concentrations of >10 mM.

For investigation of the relationship between

dapsone-mediated stimulation of PMN motility and inhibition of the HRP-H₂O₂-halide system, normal PMN were exposed to this system in the presence and absence of stimulatory concentrations of dapsone in vitro and tested for migratory responsiveness to EAS. The HRP-H₂O₂-halide system caused considerable inhibition of PMN motility. Inclusion of dapsone, however, protected the PMN from HRP-mediated inhibition of motility. It is possible that dapsone may further contribute to improved leukocyte motility by protecting leukoattractants from inactivation by the HRP-H₂O₂-halide system (8).

On the basis of these results it is possible to suggest a mechanism of dapsone-mediated stimulation of PMN migration in vivo and in vitro. Interaction of leukoattractants with PMN causes generation of superoxide and H₂O₂ and degranulation with release of MPO (5, 6). The presence of MPO and H₂O₂ with a halide in the extracellular milieu could be expected to restrict PMN locomotion. This inhibitory effect could be moderated by dapsone.

The in vivo effects of dapsone on mitogen-induced lymphocyte transformation in three patients with LL and six normal adults and in vitro effects in blood from six normal adults were less striking. Inhibition was observed at dapsone concentrations of > 0.1 mM in vitro. However, slight stimulation of transformation was observed in both groups at 2 h after ingestion of dapsone, but not at 24 h. The relationship between inhibition of peroxidase-mediated iodination and slight stimulation of lymphocyte transformation in vivo was not investigated.

This report has demonstrated that, in addition to antimicrobial activity and anti-inflammatory activity, dapsone also possesses immunostimulatory activity which is related to inhibition of the HRP-H₂O₂-halide system. Dapsone may therefore cause at least three potentially beneficial effects in patients with leprosy.

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LITERATURE CITED

- Alexander, J. O., E. Young, T. McFadyen, N. G. Fraser, W. P. Duguid, and E. M. Meridith. 1970. Absorption and excretion of ³⁵S dapsone in dermatitis herpetiformis. *Br. J. Dermatol.* **83**:620-631.
- Anderson, R., E. M. S. Gatner, F. M. J. H. Imkamp, and S. H. Kok. 1980. *In vivo* effects of propranolol on some cellular and humoral immune functions in a group of patients with lepromatous leprosy. *Lepr. Rev.* **51**: 137-148.
- Anderson, R., R. Oosthuizen, A. Theron, A. J. van Rensburg. 1979. The *in vitro* evaluation of certain neutrophil and lymphocyte functions following the ingestion of 150 mg oral dose of levamisole in normal volunteers. *Clin. Exp. Immunol.* **35**:478-483.
- Anderson, R., and A. Theron. 1979. Effects of ascorbate on leucocytes. Part 1. Effects of ascorbate on neutrophil motility and intracellular cyclic nucleotide levels *in vitro*. *S. Afr. Med. J.* **56**:394-400.
- Becker, E. L., H. J. Showell, P. M. Henson, and L. S. Hus. 1974. The ability of chemotactic factors to induce lysosomal enzyme release. I. The characteristics of the release, the importance of surfaces and the relation of enzyme release to chemotactic responsiveness. *J. Immunol.* **112**:2047-2054.
- Becker, E. L., M. Sigman, and J. M. Oliver. 1979. Superoxide production induced in rabbit leukocytes by synthetic chemotactic peptides and A23187. *Am. J. Pathol.* **95**:81-97.
- Bullock, W. E., M. F. Ho, and M. J. Chen. 1974. Quantitative and qualitative studies of the local cellular exudative response in leprosy. *RES J. Reticuloendothel. Soc.* **16**:259-268.
- Clark, R. A., and S. J. Klebanoff. 1979. Chemotactic factor inactivation by the myeloperoxidase-hydrogen peroxidase-halide system. *J. Clin. Invest.* **64**:913-920.
- Cornbleet, T. 1951. Sulfoxone (diasone) sodium for dermatitis herpetiformis. *Arch. Dermatol.* **64**:684-687.
- Curnutte, J. T., and B. M. Babior. 1974. Biological defence mechanisms. The effect of bacteria and serum on superoxide production by granulocytes. *J. Clin. Invest.* **53**:1662-1672.
- Kreutzer, D. L., J. T. O'Flaherty, W. Orr, H. J. Showell, P. A. Ward, and E. L. Becker. 1978. Quantitative comparisons of various biological responses to different active and inactive chemotactic factors. *Immunopharmacology* **1**:39-47.
- Margoliash, E., and N. Frohwirt. 1979. Spectrum of horse-heart cytochrome c. *Biochem. J.* **71**:570-573.
- McCullough, J. L., and T. H. Maren. 1973. Inhibition of dihydropterolate synthetase from *Escherichia coli* by sulfones and sulfonamides. *Antimicrob. Agents Chemother.* **3**:665-669.
- McDougall, A. C. 1979. Dapsone. *Clin. Exp. Dermatol.* **4**:139-142.
- Paul, B. B., R. J. Selvaraj, and A. J. Sbarra. 1978. A sensitive assay method for peroxidases from various sources. *RES J. Reticuloendothel. Soc.* **25**:407-410.
- Ridley, D. S., and W. H. Jopling. 1966. Classification of leprosy according to immunity: a five-group system. *Int. J. Lepr.* **34**:255-273.
- Root, R. K., J. Metcalf, H. Oshino, and B. Chance. 1975. H₂O₂ release from human granulocytes during phagocytosis. I. Documentation, quantitation, and some regulating factors. *J. Clin. Invest.* **55**:945-955.
- Root, R. K., and T. P. Stossel. 1974. Myeloperoxidase mediated iodination by granulocytes: intracellular site of operation and some regulating factors. *J. Clin. Invest.* **53**:1207-1215.
- Shedden, I. B., and D. S. Wilkinson. 1956. Sub-corneal pustular dermatitis. *Br. J. Dermatol.* **68**:385-394.
- Sher, R., R. Anderson, A. Glover, and A. A. Wadee. 1978. Polymorphonuclear cell function in the various polar types of leprosy and erythema nodosum leprosum. *Infect. Immun.* **21**:959-965.
- Stendahl, O., L. Molin, and C. Dahlgren. 1978. The inhibition of polymorphonuclear leukocyte cytotoxicity by dapsone. *J. Clin. Invest.* **62**:214-220.
- Thompson, D. M., and R. Souhami. 1975. Suppression of the Arthus reaction in the guinea-pig by dapsone. *Proc. R. Soc. Med.* **68**:273.
- Ward, M., and J. P. A. McMannns. 1975. Dapsone in Crohn's disease. *Lancet* **i**:1236-1237.
- Ward, P. A., S. Goralnick, and W. E. Bullock. 1976.

- Defective leukotaxis in patients with lepromatous leprosy. *J. Lab. Clin. Med.* **87**:1025-1032.
25. **Wilkinson, P. C.** 1971. Chemotaxis of phagocytic cells towards proteins. The effect of denaturation, p. 59. *In* N. R. Di Luzio and K. Flemming (ed.), *The reticuloendothelial system and immune phenomena*. Plenum Publishing Corp., New York.
26. **Williams, K., R. B. Capstick, D. A. Lewis, and R. Best.** 1976. Anti-inflammatory actions of dapsone and its related biochemistry. *J. Pharm. Pharmacol.* **28**:555-558.
27. **Winkelmann, R. K., and W. B. Ditto.** 1964. Cutaneous and visceral systems of necrotizing or "allergic" angitis: a study of thirty-eight cases. *Medicine (Baltimore)* **43**: 59-89.
28. **Wood, H. G., J. Katz, and B. R. Landau.** 1963. Estimation of pathways of carbohydrate metabolism. *Biochem. Z.* **338**:809-817.