

## Abnormalities of Chemotactic Lymphokine Synthesis and Mononuclear Leukocyte Chemotaxis in Wiskott-Aldrich Syndrome

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### Research Article

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The chemotactic responsiveness of MNL from Wiskott-Aldrich syndrome patients was impaired, particularly in those patients with the highest rates of unstimulated LDCF production. Furthermore, normal MNL chemotactic responsiveness could be impaired by preincubation of these cells in either LDCF or plasma from Wiskott-Aldrich syndrome patients. These observations suggest that the regulation of LDCF synthesis is abnormal in Wiskott-Aldrich syndrome, and that a humoral chemotactic inhibitor, perhaps LDCF, "deactivates" the circulating MNL of patients with this syndrome.

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# Abnormalities of Chemotactic Lymphokine Synthesis and Mononuclear Leukocyte Chemotaxis in Wiskott-Aldrich Syndrome

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**ABSTRACT** Wiskott-Aldrich syndrome is characterized by numerous humoral and cellular immune abnormalities including anergy, defective antibody production, and increased immunoglobulin synthesis. To define better the mechanisms of defective cellular immunity in this disorder, lymphoproliferative responses, lymphokine production, and the chemotactic responsiveness of mononuclear leukocytes (MNL) from patients with Wiskott-Aldrich syndrome were quantitated. Peripheral blood lymphocytes from these patients produced normal amounts of a lymphocyte-derived chemotactic factor (LDCF); however, their lymphoproliferative responses were frequently depressed, particularly to antigenic stimuli. In the absence of exogenous antigens or mitogens, lymphocytes from patients with Wiskott-Aldrich syndrome produced significantly more LDCF than unstimulated normal lymphocytes. In fact, this unstimulated LDCF production frequently approached the level produced by normal cells only after antigen or mitogen stimulation.

The chemotactic responsiveness of MNL from Wiskott-Aldrich syndrome patients was impaired, particularly in those patients with the highest rates of unstimulated LDCF production. Furthermore, normal MNL chemotactic responsiveness could be impaired by preincubation of these cells in either LDCF or plasma from Wiskott-Aldrich syndrome patients. These observations suggest that the regulation of LDCF synthesis is abnormal in Wiskott-Aldrich syndrome, and that a humoral chemotactic inhibitor, perhaps LDCF, "deactivates" the circulating MNL of patients with this syndrome.

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## INTRODUCTION

Wiskott-Aldrich syndrome (WAS)<sup>1</sup> is a sex-linked recessive disorder which is characterized by severe thrombocytopenia, eczema, and recurrent infections (1-3). This disease generally culminates in death during infancy or early childhood (4). Patients with this syndrome also manifest a perplexing spectrum of humoral and cellular immune abnormalities (5). Natural antibodies are absent or low in titer, and antibody production after specific immunization, particularly with polysaccharide antigens, is strikingly defective in this disease (6). Patients with WAS, however, have normal numbers of circulating bone marrow-derived (B) lymphocytes (7), elevated serum levels of IgA, IgD, and IgE, and markedly accelerated synthesis and catabolism of immunoglobulins G and A (8).

Evaluation of cellular immune function in WAS has revealed additional abnormalities. Clinically, children with this syndrome are anergic to common microbial antigens, fail to manifest contact sensitivity to dinitrochlorobenzene, and show markedly delayed skin allograft rejection (6). In contrast, lymphocyte-proliferative responses to optimal concentrations of nonspecific mitogens are usually normal (6); lymphocyte-mediated cytotoxicity responses are intact (9, 10); and these patients have a normal proportion of thymus-derived (T) lymphocytes in their circulation (11). However, the proliferative response of WAS lymphocytes to specific

<sup>1</sup> Abbreviations used in this paper: B cells, bone marrow-derived lymphocytes; Con A, Concanavalin A; LDCF, lymphocyte-derived chemotactic factor; MIF, migration inhibitory factor; MNL, mononuclear leukocytes; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SLO, streptolysin O; T cells, thymic-derived lymphocytes; WAS, Wiskott-Aldrich syndrome; [<sup>3</sup>H]TdR, tritiated thymidine.

antigens and allogeneic cells is profoundly impaired (12).

In the present study we investigated the production by WAS lymphocytes of a lymphokine that is chemotactic for mononuclear leukocytes (MNL) and the response of MNL from these patients to standard chemotactic stimuli. These studies were designed to investigate further the basic immunologic defect(s) and possible mechanism(s) of anergy in this disease. Our findings suggest that abnormal regulation and control of lymphocyte-derived chemotactic factor (LDCF) synthesis exists in WAS and that this abnormality may contribute to the impaired delayed hypersensitivity characteristic of this disease.

## METHODS

**Patients.** Seven boys with well-documented WAS were studied at the Clinical Center, National Institutes of Health. Whenever possible, the patients were studied as outpatients during periods of relative well-being. The patients ranged in age from 13 mo to 10 yr. 32 healthy individuals of varying ages served as controls.

**Chemotactic factors.** Human C5a, the biologically active cleavage product of the fifth component of human complement, was isolated from normal human serum after endotoxin activation (13).

Human LDCF was prepared as previously described (14). Briefly, blood from patients and normal donors was drawn into heparinized (20–30 U/ml) syringes. The cells were sedimented by gravity or with dextran, triply washed, and the lymphocyte-rich cell population was cultured in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) at either  $3 \times 10^6$  cells/ml in 1-ml volumes in 1-dram flat-bottomed glass vials, or  $2 \times 10^6$  cells/ml in 2-ml volumes in  $13 \times 125$  mm plastic tubes (Falcon Plastics, Oxnard, Calif.). Media were supplemented with 0.5% vol/vol heated ( $56^\circ\text{C}$  for 30 min) homologous Ab Rh+ plasma, 50 U penicillin, 50  $\mu\text{g}$  streptomycin, and 2 mM glutamine/ml. Leukocytes were incubated for 24 h at  $37^\circ\text{C}$  in a humidified atmosphere of 95% and 5%  $\text{CO}_2$ . After incubation, supernates were cleared of cells by centrifugation (1,500 g for 15 min) and tested for chemotactic activity. Supernates from cultures stimulated with a mitogen or antigen at the beginning of an experiment are referred to as "stimulated", while supernates from cultures reconstituted with the appropriate stimulant after incubation are referred to as "unstimulated".

**Chemotactic assay and expression of results.** A modification (14) of Boyden's original technique (15), using 5  $\mu\text{m}$  Nuclepore (Wallabs, Inc., San Rafael, Calif.) membranes, was employed. MNL from patients and normal subjects, for use in the chemotactic assay, were obtained from peripheral blood by Ficoll-Hypaque equilibrium centrifugation as previously described (16). All experiments were standardized so that each Boyden chamber contained  $4 \times 10^6$  Ficoll-Hypaque-purified MNL. Chemotactic determinations were routinely performed in triplicate and 20 oil immersion fields quantitated per replicate.

The production of LDCF by stimulated WAS or normal lymphocytes is expressed as a function of the number of normal MNL (mean  $\pm$  one SE) which migrated in response to 0.5 ml of WAS or normal culture supernate, respectively. In preliminary studies, volumes less than 0.5

ml were tested, confirming that at this volume saturation of the assay was not obscuring differences between normal and patient samples.

LDCF production by unstimulated lymphocytes is expressed as the

$$\frac{\text{mean LDCF production by unstimulated WAS lymphocytes}}{\text{mean LDCF production by unstimulated normal lymphocytes}} \pm 1 \text{ SE}$$

All experiments that measured LDCF production used normal "second-party" MNL as responder cells.

The chemotactic responsiveness of MNL from WAS patients is expressed as the

$$\frac{(\text{mean migration of WAS MNL})}{(\text{mean migration of normal MNL})} \times 100.$$

The data for WAS MNL migration are presented as a percentage of the simultaneously studied normal cells. This normalized data and the original chemotactic data were statistically analyzed by paired comparison (17) with essentially identical results.

In all experiments WAS and normal MNL were tested simultaneously at identical concentrations. To further standardize these experiments, the number of monocytes in Ficoll-Hypaque preparations of WAS and normal blood was quantitated by phagocytosis of latex beads, with the presence of the receptor for IgG-Fc (18) and the presence of nonspecific esterase (19) as markers for monocytes. In 12 such studies, WAS MNL contained  $17.8 \pm 2.7\%$  (mean  $\pm 1$  SE) monocytes, normal cells  $10.8 \pm 1.7\%$ . These data are statistically significant ( $P < 0.05$ ), indicating that WAS MNL contained slightly more monocytes than normal cell preparations. In addition, in pilot experiments using histochemical methods, we determined that over 90% of cells migrating in the chemotactic assay were monocytes. These data indicate that our experiments are probably a valid measure of monocyte function; however, since histochemical methods were not employed in these particular studies, we have defined the migrating cell population as MNL.

**Lymphocyte transformation.** Lymphocyte transformation was performed as previously described (20), with several modifications. Leukocyte-rich plasma was obtained from heparinized peripheral blood by gravity sedimentation. Duplicate 1-ml cultures containing  $5 \times 10^5$  leukocytes were established in medium RPMI 1640 supplemented with 2 mM glutamine, 100 U penicillin, 100  $\mu\text{g}$  streptomycin/ml, and 10% autologous or homologous plasma. Cultures were incubated at  $37^\circ\text{C}$  in loosely capped flat-bottomed glass vials in a humidified atmosphere of 95% air and 5%  $\text{CO}_2$  for 5 days. Cell suspensions were pulse-labeled with tritiated thymidine ( $[^3\text{H}]\text{TdR}$ ) for the final  $4\frac{1}{2}$  h of the culture period and the acid-precipitable radioactivity was determined. Leukocytes from patients and normal volunteers were always cultured simultaneously.

**Lymphocyte stimulants.** The following stimulants were used in a total of 1 ml of culture medium: phytohemagglutinin (PHA, Burroughs Wellcome & Co., Inc., Research Triangle Park, N. C.), 1  $\mu\text{g}$ ; Concanavalin A (Con A, Calbiochem, San Diego, Calif.), 10  $\mu\text{g}$ ; pokeweed mitogen (PWM, Grand Island Biological Co.), 0.1  $\text{cm}^3$  of a 1:10 dilution of reconstituted powder; streptolysin O (SLO, Difco Laboratories, Detroit, Mich.), 0.1 ml of a 1:3 dilution of the rehydrated reagent; *Candida albicans* extract (candida, Hollister-Stier Laboratories, Spokane, Wash.), 0.1 ml of a 1:20 dilution of the commercial

TABLE I  
*Production of LDCF by Mitogen-Stimulated Normal and WAS Lymphocytes\**

Date	Subject	WAS patients			Normals		
		CON A	PHA	PWM	CON A	PHA	PWM
5/26/72	B. M.		194.0±4.0			157.0±12.0	
6/15/72	M. M.		72.0±3.0			101.0±9.0	
6/15/72	B. M.		99.0±12.0				
6/15/72	A. D.		88.0±8.0				
6/16/72	C. B.		167.0±25.0				
6/16/72						116.0±18.0	
6/30/72	C. B.		85.0±3.0			185.0±31.0	
7/06/72	C. B.		50.0±6.0			145.0±14.0	
8/18/72	A. D.		135.0±14.0	96.0±4.0		107.0±12.0	
9/07/72	A. D.		62.0±10.0			211.0±4.0	
9/19/72	M. M.		155.0±11.0				
10/31/72	A. D.		96.0±9.0			85.0±4.0	
10/31/72						175.0±12.0	
12/11/72	M. M.		123.0±4.0	58.0±4.0	128.0±9.0	78.0±9.0	
12/15/72	M. M.		170.0±7.0	94.0±6.0		41.0±3.0	63.0±8.0
1/08/73	M. M.		117.0±11.0			146.0±18.0	109.0±14.0
1/08/73	B. M.		132.0±10.0			29.0±3.0	
1/24/73	B. M.	150.0±4.0			140.0±10.0	88.0±10.0	
1/24/73	M. M.	153.0±7.0					
2/05/73	A. D.	83.0±3.0					
6/28/73	C. B.		55.0±2.0			157.0±18.0	
6/28/73	B. M.		41.0±4.0			36.0±6.0	
11/07/73	A. D.		145.0±4.0				
12/08/73	M. M.		127.0±7.0			98.0±7.0	
12/08/73	C. B.		50.0±1.0	56.0±5.0		119.0±4.0	
12/10/73	A. D.	109.0±18.0			103.0±4.0	38.0±2.0	48.0±4.0
12/10/73					108.0±8.0		
Mean		137.3±14.2‡	107.0±9.7§	76.0±11.0	119.8±8.6	111.2±12.3	73.3±18.4

\* Expressed as the mean number of migrating monocytes per oil immersion field ±1 SE.

‡ Not significantly different from Con A normal ( $P > 0.20$ ).

§ Not significantly different from PHA normal ( $P > 0.50$ ).

|| Not significantly different from PWM normal ( $P > 0.50$ ).

antigen solution. In preliminary experiments these concentrations had been determined to be optimal for stimulating both lymphocyte transformation and LDCF production.

## RESULTS

*Production of LDCF by stimulated lymphocytes from patients with WAS.* The in vitro production of LDCF by lymphocytes from 4 patients with WAS and 19 controls was compared. Lymphocytes from both groups, when stimulated with the mitogens Con A, PHA, or PWM produced essentially equal amounts of LDCF (Table I). Similarly, lymphocytes from WAS patients and normals, when stimulated with the antigens SLO or candida, produced equivalent quantities of this lymphokine (Table II). These data indicate that stimulated WAS lymphocytes can produce as much LDCF as stimulated normal lymphocytes.

*The chemotactic responsiveness of MNL leukocytes from patients with WAS.* The chemotactic responsiveness of MNL to both C5a, a complement-derived chemotactic factor, and LDCF was examined in 6 children with WAS and 14 normals. MNL from these WAS patients migrated 76.5% as well as normal MNL to C5a and 88.9% as well as normal MNL to LDCF (Table III). Close inspection of these data indicate that there may be a heterogeneity in the chemotactic responsiveness of MNL from WAS patients. MNL from M. M. and B. M., two patients whose cells were studied on multiple occasions, consistently migrated subnormally to both C5a and LDCF (Table III). The average responses of MNL from B. M. and M. M. to C5a were 64.8% and 62.5% of normal, respectively. Similarly, MNL from B. M. and M. M. responded to LDCF 75.4% and 73.4% as well as normal MNL,

TABLE II  
Production of LDCF by Antigen-Stimulated Normal and WAS Lymphocytes\*

Date	Subject	WAS patients		Normals	
		SLO	Candida	SLO	Candida
5/26/72	B. M.	101.0±11.0		163.0±6.0	
6/15/72	M. M.	67.0±5.0		74.0±5.0	
6/16/72	C. B.	133.0±12.0	133.0±20.0	146.0±7.0	
6/30/72	C. B.		43.0±5.0	102.0±14.0	23.0±2.0
7/06/72	C. B.		72.0±10.0		29.0±3.0
8/18/72	A. D.	65.0±2.0			
9/12/72	M. M.	103.0±7.0	125.0±4.0	32.0±3.0	61.0±4.0
9/19/72	A. D.	110.0±12.0		36.0±5.0	
12/11/72	M. M.	48.0±6.0	146.0±6.0	47.0±5.0	100.0±4.0
12/15/72	M. M.	108.0±9.0	163.0±9.0	100.0±5.0	157.0±25.0
1/08/73	B. M.	60.0±7.0		21.0±3.0	31.0±2.0
1/08/73	M. M.	73.0±18.0	112.0±19.0	63.0±6.0	105.0±4.0
1/24/73	M. M.	82.0±6.0			
2/05/73	A. D.	52.0±4.0	88.0±7.0	70.0±7.0	147.0±8.0
Mean		83.5±7.8†	110.3±14.2§	79.1±15.3	81.6±18.9

\* Expressed as the mean number of migrating monocytes per oil immersion field ±1 SE.

† Not significantly different from SLO normal ( $P > 0.50$ ).

§ Not significantly different from Candida normal ( $P > 0.20$ ).

respectively. The response of MNL from patients B. M. and M. M. to both chemotactic agents was significantly less than normal ( $P < 0.05$ ), as was the response of MNL of the entire group to C5a. The response of MNL from all patients to LDCF, although numerically less than normal, did not attain statistical significance. In interpreting these data, it should be noted that the MNL suspensions from WAS patients contained more monocytes than normal MNL preparations. These studies indicate that abnormal MNL chemotaxis occurs in WAS. However, from these data it is unclear if this defect is present in a subpopulation of WAS patients only or if this abnormality occurs in all children with WAS at some time during the course of their disease.

*Production of LDCF by unstimulated lymphocytes from patients with WAS.* The production of LDCF by unstimulated lymphocytes from four patients with WAS is shown in Table IV. These data are expressed as a ratio of the LDCF production by unstimulated WAS lymphocytes divided by the LDCF production by simultaneously and identically cultured unstimulated normal lymphocytes. It is evident that unstimulated WAS lymphocytes produced significantly more LDCF than unstimulated normal cells. The four patients studied define a spectrum of LDCF hyperproductivity, with unstimulated lymphocytes from these patients producing from 159% to 430% of the LDCF produced by unstimulated normal lymphocytes.

*The relationship of LDCF hyperproductivity to impaired MNL chemotaxis in WAS.* The possibility

that "spontaneously" elevated LDCF production might be related to impaired MNL chemotaxis in WAS was investigated in the following series of experiments:

TABLE III  
The Chemotactic Responsiveness of Monocytes in WAS

Date	Patient	Response to	
		C5a*	LDCF*
7/26/71	B. M.	25.4	73.2
7/26/71	M. M.	48.9	86.6
8/16/71	J. D. G.	133.7	124.5
9/14/71	T. R.	—†	97.4
9/29/71	A. D.	92.0	128.3
10/22/71	M. M.	84.1	93.4
10/27/71	B. M.	95.7	103.3
4/24/73	M. M.	44.7	44.1
5/09/73	B. M.	73.4	49.8
5/09/73	M. M.	72.4	69.6
5/24/73	C. B.	69.4	93.7
8/03/73	J. D. G.	101.8	103.6
Group mean§		76.5	88.9
B. M. mean ( $n = 3$ )		64.8	75.4
M. M. mean ( $n = 4$ )		62.5	73.4

\* Expressed as a percentage of normal:

(Response of WAS MNL/response of normal MNL) × 100.

† Not done.

§ Group mean response to C5a is significantly less than normal ( $P < 0.05$ ), as are responses of B. M. and M. M. MNL to both C5a ( $< 0.05$ ) and LDCF ( $< 0.02$ ).

TABLE IV  
Production of LDCF by Unstimulated Lymphocytes from WAS Patients\*

Date	Patient			
	B. M.	M. M.	A. D.	C. B.
5/26/72	1.72±0.08			
6/15/72	1.86±0.13	1.81±0.22	1.29±0.15	
6/16/72				1.71±0.38
6/30/72				1.14±0.59
7/06/72				2.21±0.67
8/18/72			0.89±0.10	
9/12/72		7.12±0.03		
9/19/72		7.11±0.95		
10/31/72			1.43±0.27	
12/11/72		1.79±0.26		
12/15/72		1.30±0.13		
1/08/73	1.68±0.43	2.39±0.50		
1/24/73	4.20±0.69	4.23±0.14		
2/05/73			0.49±0.07	
6/28/73	1.02±0.16			0.82±0.12
11/07/73		10.00±0.99	3.54±1.16	
12/08/73				2.05±0.33
12/10/73		2.98±0.33	2.70±0.35	
Mean	2.10±0.54	4.30±1.02	1.72±0.47	1.59±0.27

\* Expressed as the

(LDCF production by unstimulated WAS lymphocytes/  
LDCF production by unstimulated normal lymphocytes) ±1 SE.

Normal MNL were incubated with LDCF or media alone and washed, and the chemotactic response of these cells to LDCF was tested (Table V). It can be

TABLE V  
Inhibition of Monocyte Chemotaxis by Preincubation with LDCF

Cells preincubated with:*	Chemotactic response	Inhibition§
		%
5% LDCF	82.0±6.0	32.6
10% LDCF	75.0±2.0	38.4
20% LDCF	55.0±2.0	55.0
30% LDCF	51.0±8.0	55.6
50% LDCF	44.0±3.0	64.1
Media alone	121.0±5.0	—
No preincubation†	131.0±8.0	—

\* Normal human MNL were incubated at 37°C for 30 min with RPMI 1640 media alone or with media with the indicated percent volume of LDCF. The cells were then washed twice with media alone, resuspended in RPMI 1640, and tested for chemotactic responsiveness to a 30% solution of LDCF in RPMI 1640.

† Normal human MNL were suspended in RPMI 1640 and then immediately tested for chemotactic responsiveness to a 30% solution of LDCF in RPMI 1640.

§ Expressed as the mean chemotactic response of MNL preincubated with the indicated amount of LDCF, compared to the mean response of MNL preincubated with RPMI 1640 alone.

TABLE VI  
The Effect of Normal or WAS Plasma on the Chemotactic Responsiveness of Normal Monocytes\*

Source of plasma	Chemotactic factor	
	C5a	LDCF
WAS	57.8±7.7	67.8±11.1
Normal	93.0±12.3‡	106.4±13.8§

\* Triplicate samples containing  $4 \times 10^6$  normal MNL were incubated in either normal or WAS plasma for 30 min at 37°C, then washed twice in Gey's salt solution (pH 7.0) and the chemotactic responsiveness of these cells to 0.5 ml of C5a or LDCF was tested. These data represent the mean of five experiments in which plasma samples from four patients and five normals were studied. Results are expressed as cells per oil immersion field (mean±1 SE).

‡ Normal versus WAS  $P < 0.025$ .

§ Normal versus WAS  $P < 0.050$ .

seen that the cells incubated with LDCF migrated substantially less than the cells incubated with media alone.

In a series of subsequent experiments, MNL from normal donors were incubated either in WAS or in homologous normal plasma and washed, and the chemotactic responsiveness of these cells to both C5a and LDCF was tested (Table VI). It is evident that MNL incubated in WAS plasma failed to respond as well as cells incubated in normal plasma. Specifically, the chemotactic response to C5a of MNL incubated in normal plasma was  $93.0 \pm 12.3$ , compared with  $57.8 \pm 7.7$  ( $P < 0.025$ ) for MNL incubated in WAS plasma. Similarly, the response to LDCF was  $106.4 \pm 13.8$  for cells incubated in normal plasma, versus  $67.8 \pm 11.1$  for cells incubated in WAS plasma ( $P < 0.050$ ). These data suggest that prior exposure of MNL to a chemotactic agent can induce a state of refractoriness (desensitization) to a subsequent chemotactic stimulus, and that the plasma of WAS patients also inhibits the chemotaxis of MNL.

Characterization of the inhibitory factor on these WAS plasma samples showed it to be: (a) nondialyzable; (b) stable to heating at 56°C for 30 min; (c) resistant to multiple freezing and thawings; (d) not cytotoxic to MNL as determined by exclusion of trypan blue; (e) not present in the immunoglobulin fraction obtained by precipitation with 50% saturated ammonium sulfate; and (f) soluble in 50% saturated ammonium sulfate, since activity could be recovered from this fraction after dialysis. Parallel studies with LDCF show identical characteristics.

## DISCUSSION

The WAS is a confusing disorder in which a wide variety of abnormalities of both cellular and humoral

immunity have been identified. In the present study, we have examined lymphocyte-proliferative responses, LDCF production, and MNL chemotactic responsiveness in WAS patients. One of the most striking findings of the study was the demonstration that lymphocytes from WAS patients, when cultured in the absence of exogenous stimuli, produced significantly more LDCF than unstimulated normal lymphocytes. In fact, the amount of unstimulated LDCF produced by WAS cells frequently equaled that produced by normal lymphocytes only after antigen or mitogen stimulation.

The cause for this spontaneously elevated rate of LDCF synthesis by WAS lymphocytes is unclear. The possibility that it simply reflects a response to infection appears unlikely. Firstly, these patients were specifically studied during periods when they were not obviously infected. Secondly, we have studied numerous patients with a variety of diseases, including other primary and secondary immunodeficiency disorders, without observing other than sporadic instances of increased spontaneous LDCF production. Included in these other groups were patients with congenital and acquired hypogammaglobulinemia, ataxia telangiectasia, isolated IgA deficiency with chronic sinopulmonary infection, Hodgkin's and non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Sézary's syndrome, and chronic mucocutaneous candidiasis. In fact, many of the patients in these groups were experiencing acute or chronic infections, such as bronchiectasis, acute and chronic moniliasis, and acute herpes labialis.

Elevated LDCF production by unstimulated WAS lymphocytes may, in fact, reflect a more basic defect in this disease. We have previously shown that WAS patients are synthesizing immunoglobulins G and A at up to 10 times the normal rate, in spite of defective specific antibody synthesis (8). In addition, we have identified IgG monoclonal paraproteins in the sera of a significant proportion of patients with this syndrome (21). Thus, this disease is clearly associated with hyperactivity of the B lymphoid cell system. It has generally been assumed that lymphokines such as macrophage inhibitory factor (MIF) and LDCF are products of activated T lymphocytes, and in fact we have previously demonstrated that spleen cells from agammaglobulinemic chickens totally devoid of B cells (22) and lymphocytes from agammaglobulinemic humans<sup>2</sup> produce LDCF. However, Yoshida, Sonozaki, and Cohen (23) have recently shown that in the guinea pig, MIF can be made by B cells and we (24) have data to demonstrate that human B lymphocytes produce LDCF. These observations raise the unique possibility that B lymphocytes may be responsible for the hyper-

synthesis of both immunoglobulins and LDCF in patients with WAS.

Another defect identified in some of the patients in this study was an abnormal response of their MNL to the chemotactic stimulants LDCF and C5a. Defective MNL chemotaxis has previously been observed in a patient with chronic mucocutaneous candidiasis (25) and in some patients with malignant diseases<sup>3</sup> (26). The defective MNL chemotaxis in WAS patients observed in this study was associated with a humoral factor capable of decreasing the chemotactic response of normal MNL. Van Epps and Williams (27) have recently presented evidence of an immunoglobulin that inhibits chemotaxis in anergic patients with a variety of conditions. However, characterization of the inhibitor found in WAS plasma indicates that it is heat-stable, non-dialyzable, and not immunoglobulin in nature. The possibility that this humoral factor develops as a consequence of the repeated infections experienced by WAS patients is unlikely, since we have been unable to identify any inhibitory activity in the plasmas of patients with chronic infections associated with either cystic fibrosis or chronic granulomatous disease.<sup>4</sup> Despite the fact that WAS plasma is inhibitory to MNL chemotaxis, partial characterization showed that the inhibitory factor in these plasma samples shared many properties with LDCF. In this regard, it was found that normal MNL were deactivated by incubation in LDCF, thus becoming less responsive to subsequent chemotactic stimuli. In view of this ability of LDCF to chemotactically deactivate MNL, it is extremely interesting that those WAS patients whose unstimulated lymphocytes produced the highest levels of LDCF also had the most pronounced defect in MNL chemotactic responsiveness. Thus, the two defects described in this report, elevated unstimulated LDCF synthesis and defective MNL chemotactic responsiveness, may be interrelated and may contribute to anergy in WAS by the mechanism of chemotactic deactivation. The increased production of LDCF by unstimulated WAS lymphocytes might also contribute to the anergy characteristic of this disease by a different mechanism. MNL are the predominant cells found in delayed hypersensitivity reactions and are thought to accumulate at these sites in response to chemotactic factors elaborated by specifically stimulated lymphocytes. If LDCF production by unstimulated lymphocytes is increased, then any potential chemotactic gradient produced as a result of a specific lymphocyte response would be diminished. This in turn might result in less active cell migration and

<sup>2</sup>L. C. Altman, and R. M. Blaese. Unpublished observations.

<sup>3</sup>L. C. Altman, and R. M. Blaese Unpublished observations.

<sup>4</sup>R. Snyderman, and R. H. Buckley. Unpublished observations.

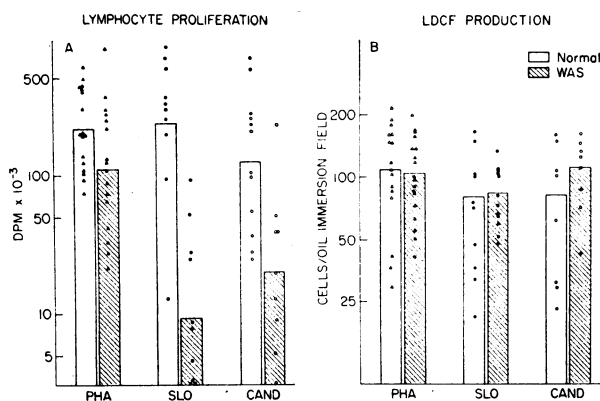


FIGURE 1 Comparison of [ $^3\text{H}$ ]TdR incorporation (A) and LDCF production (B) by cultured lymphocytes from WAS patients. Lymphocytes were stimulated with optimal doses of either PHA, SLO, or *Candida albicans* extract.

failure to manifest an appropriate cellular immune response.

One of the perplexing findings observed repeatedly in WAS is the dissociation between various in vitro parameters of lymphocyte function and in vivo cellular immunity (5). These patients are severely anergic. Yet lymphocyte blastogenic transformation in vitro to nonspecific mitogens such as PHA and Con A is remarkably intact (6). In a previous series of studies (12) we found normal responses to these stimulants in vitro, while the patients represented in the present study (Fig. 1) had mildly reduced responses (6 of 16 equaled or exceeded the normal mean). However, as in our previous observations, in this study lymphocytes from WAS patients showed a markedly defective proliferative response to specific antigens, in this case to SLO and candida. In yet another dissociation, despite the diminished proliferative responses, WAS lymphocytes produced as much LDCF as did normal lymphocytes when stimulated with either specific antigens and nonspecific mitogens. Undoubtedly, some of this dissociation can be explained on the grounds that the cells that synthesize LDCF are distinct from those that proliferate, as has been previously shown for proliferation and MIF production (28). These multiple examples of dissociation of the immune response provide additional support for the concept that one of the major defects in WAS lies in the initiation and control of immune responsiveness (6, 29).

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