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Demonstration of an indomethacin-sensitive mechanism regulating immune reactivity in American cutaneous leishmaniasis patients

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SUMMARY

We investigated some aspects of the regulation of the immune response that were sensitive to the effect of indomethacin (INDO), an inhibitor of prostaglandin synthesis, in 84 patients with American cutaneous leishmaniasis (ACL), and in normal controls. The patients were classified on the basis of clinical and histopathological criteria as suffering localized (LCL), mucocutaneous (MCL) or diffuse (DCL) forms of the disease. The responses *in vitro* to mitogens (PHA and Con A) and leishmanial antigens were evaluated in the presence or absence of INDO. It was found that the drug significantly increased *in vitro* the mitogenic stimulation by PHA of peripheral blood mononuclear cells from LCL, MCL and DCL patients, but the effect was less evident in the controls. Considering specific responses to leishmanial antigens, we showed that in the presence of INDO, these were significantly increased in LCL patients, but not in MCL or DCL. Also, only in LCL was an inverse correlation found between the initial response to leishmanial antigen and the increase caused by INDO. Significant correlations were found between the INDO-induced enhancement of PHA and Con A responses in the patient groups, but not in the controls. In LCL patients there was a significant correlation between the increases caused by INDO of the mitogen and antigen responses. It can be suggested that an indomethacin-sensitive (prostaglandin dependent) suppressor mechanism operates in LCL patients, that is possibly responsible for the modulation of the immune response against the parasite. In MCL, where this suppressive mechanism is apparently not functional, the response to the parasite is intense, and a possible consequence of this could be tissue damage. Our results indicate, however, that the anergy observed in DCL patients is not due to an involvement of prostaglandins in the suppression of the specific immune response.

Keywords American cutaneous leishmaniasis indomethacin immune regulation prostaglandins

INTRODUCTION

American cutaneous leishmaniasis (ACL) is a disease caused by infection with a flagellated protozoan that invades and grows within macrophages. It manifests itself in three general clinical forms: localized cutaneous leishmaniasis (LCL), the most benign form of the disease, with limited and spontaneously healing ulcerated skin lesions; muco-cutaneous leishmaniasis (MCL), the intermediate form, with lesions that contain few parasites, but that are progressive and often cause severe histological, anatomical and functional disruption of the oral, nasal, pharyngeal or laryngeal

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mucous membranes; and diffuse cutaneous leishmaniasis (DCL), the malignant form, characterized by the presence of progressive non-ulcerated nodules rich in parasites, and resistant to treatment.

In previous studies (Castes *et al.*, 1983; Castes, Agnelli & Rondon, 1984) have demonstrated that patients with DCL lack responsiveness *in vivo* and *in vitro* to leishmanial antigens and present a marked leishmanial antigen-induced suppression of mitogenic responses *in vitro*. In contrast, patients with MCL have a hyper-responsiveness to the parasite antigen, and do not demonstrate suppressive activity. Patients with LCL, who generally control their infection, have moderate responses to leishmanial antigens, and appear to have established a balance between stimulatory and suppressive activities.

In the present study, we continued the evaluation of the regulation of the immune response of these patients by indirectly examining the role of prostaglandins (PG). A number of basic immunological studies in different experimental and clinical situations have provided evidence that implicates PG E_1 and E_2 as local feedback inhibitors of T cell activation *in vitro* and *in vivo* (Goodwin & Ceuppens, 1983). In many of these studies, the effects of PG were indirectly evaluated by the use of drugs such as indomethacin (INDO), which is an inhibitor of PG synthesis (Vosixa & Thies, 1979; Barsoum *et al.*, 1983). Petersen *et al.* (1982) also employed this system to evaluate patients with a form of diffuse cutaneous leishmaniasis that appears to be particular to the Dominican Republic. We have examined patients with the different forms of ACL, and normal controls, for evidence of the regulation of the immune response by PG. The response to mitogens (PHA and Con A) and leishmanial antigens in the presence or absence of INDO was determined. The results obtained are discussed in relation to the physiopathology of the disease.

MATERIALS AND METHODS

Patients. A total of 84 patients (37 males and 47 females) with ACL were evaluated in the Instituto de Biomedicina, Caracas, Venezuela. They were classified on the basis of clinical and histopathological criteria (Convit & Pinardi, 1974) as suffering LCL, with one or more lesions (65 patients) or MCL (12 patients). None had received treatment during the period immediately before evaluation. Many of the MCL patients had, however, presented with LCL a number of years previously, and the lesions had regressed either spontaneously or after treatment with Glucantime (antimony-N-methyl glucantime, Specia, Paris, France). A small group (seven patients) with DCL was also examined, but only one of these was seen before the institution of any treatment. Despite the treatment protocols applied to the DCL patients, no effective clinical improvement had been noted at the time of the study.

The mean ($\pm$s.d.) period of evolution of the infection in the LCL group was $11{\cdot}40 \pm 11{\cdot}9$ weeks and in the MCL group $20{\cdot}83 \pm 18{\cdot}6$ months. The DCL patients had been followed for periods of 1 to 22 years. The mean ages ($\pm$s.d.) of the patient groups was $32{\cdot}9 \pm 18{\cdot}3$ years for LCL, $30{\cdot}7 \pm 16{\cdot}0$ years for MCL, and for DCL patients the ages ranged from 17 to 55 years.

Normal controls. A total of 39 individuals were studied (18 females, 21 males, mean age $30{\cdot}7 \pm 8{\cdot}3$ years), these being obtained from either the endemic zones of the disease, the Municipal Blood Bank of the Vargas Hospital, Caracas, or the staff of the Instituto de Biomedicina.

Antigen preparations. A *Leishmania* extract, kindly provided by Dr J.L. Avila (I.B., Caracas), was prepared from *L. mexicana pifanoi* 7940 (L.m).

The protozoa were routinely maintained in Minimal Essential Medium containing 2·5% fetal calf serum (GIBCO, Grand Island, NY, USA), but the last passage was in a medium free of extraneous proteins (Steiger & Steiger, 1977). The promastigotes were collected by centrifugation (1800 *g*, 20 min) at 4°C, washed twice with PBS, resuspended to a concentration of 25×10^6/ml, and autoclaved (125°C, 15 min).

Lymphocyte proliferation assay. A standard microtest procedure was used. Briefly, mononuclear cells were separated from heparinized blood by centrifugation over Ficoll-Hypaque, washed three times, and cultured in triplicate in plastic microtitre plates at a concentration of 2×10^5 cells/well.

The medium used was RPMI 1640 (Grand Island Biological Company, NY, USA) containing 100 U/ml penicillin and 100 μg/ml streptomycin, supplemented at 5% with a pool of heat-

inactivated human AB serum. After 3 days of exposure to mitogen, or 6 days to antigen, 1 μCi of [^{3}H]thymidine was added to each well, 6 h before harvesting onto glass fibre strips and β counting by liquid scintillation. Both phytohemagglutinin-M (PHA, B grade Calbiochem, San Diego, CA) and Concanavalin A (Con A, Type IV, Sigma) were used at concentrations of 2·5 and 5·0 μg/ml.

The *L. mexicana* antigen was tested at a concentration equivalent to $12{\cdot}5 \times 10^6$ parasites/well. In the experiments with INDO (Sigma Chemical Company) the drug was used at 0·5, 1·0, 5·0 μg/ml, diluted with RPMI-1640 from a stock solution of 10 mg/ml in ethyl alcohol. Control tests with the diluent alone were also performed.

The results were expressed as the stimulation index (SI), defined as the mean ct/min of antigen or mitogens tests divided by the mean ct/min of the control tests.

The increase of the proliferative response induced by INDO was expressed as the Enhancement Index (EI), which is the SI in the presence of INDO divided by the SI in the absence of the drug. This was calculated for each individual subject, and then the mean $\pm$ s.d. was determined for the group.

Statistical analysis. The effects of INDO were analysed in the following manner: firstly, the significance of the effect for each individual subject was evaluated by the paired Student *t*-test; the basal SI in each case was compared to that in the presence of the drug. Secondly, the group mean SI in the presence or absence of INDO were compared between LCL, MCL, DCL or normal subjects by the conventional Student *t*-test. Similarly, the group mean EI were compared between the different clinical conditions.

In order to determine the significance of an increase caused by INDO, we used the criteria of Bahr, Rook & Stanford (1981), and found that no EI below 1·3 was significant when the mean of unstimulated triplicate cultures without INDO was compared to the mean of those with the drug. The proportions of each study group exceeding this value were compared by the χ^2 test.

The Pearson *r* coefficient was used to determine the significance of the correlation between the basal SI and that in the presence of INDO, as well as that between the EI produced by INDO for the PHA, Con A and antigen responses.

RESULTS

Effect of indomethacin on lymphocyte proliferative responses to mitogens. The SI in the presence or absence of INDO for each subject evaluated are presented in Table 1. For PHA (2·5 μg/ml) the comparison by Student's paired *t*-test of the basal SI with that in the presence of the drug, for each individual subject, revealed significantly altered responses in LCL ($P < 0{\cdot}005$), MCL ($P < 0{\cdot}05$) and DCL ($P < 0{\cdot}05$) patients, but not in controls. The modification of the response by the drug was predominantly an enhancement (Fig. 1). Enhancement indices $\geq 1{\cdot}3$ occurred in 77% of the LCL patients, 60% of MCL, 100% of DCL and 62% of the normal controls. These proportions were not significantly different between the groups, but when the overall EI were considered, the mean value for DCL was significantly higher compared to LCL ($P < 0{\cdot}001$), MCL ($P < 0{\cdot}05$) and controls ($P < 0{\cdot}001$). Also, a significant inverse correlation was found between the basal PHA response and the EI for LCL ($r = -0{\cdot}35$, $P < 0{\cdot}05$), MCL ($r = -0{\cdot}90$, $P < 0{\cdot}01$) and DCL ($r - 0{\cdot}80$, $P < 0{\cdot}05$) patients. This was not the case for the controls ($r - 0{\cdot}17$, NS).

When the group mean SI were considered (Table 1), the responses in the absence of INDO were significantly lower in DCL patients compared to controls ($P < 0{\cdot}01$). However, in the presence of INDO the responses of the DCL patients were not significantly different from those of the controls.

It should be noted that INDO did not significantly affect the thymidine incorporation of unstimulated 3 day cultures (Table 1). Also, while the results presented here were obtained with 2·5 μg/ml of PHA and 5·0 μg/ml of INDO, basically comparable effects were observed with the other doses of mitogen and drug tested (data not shown).

The results obtained for Con A (5·0 μg/ml) were somewhat different to those found with PHA (Table 2), as the alterations of the responses were only statistically significant in the case of controls ($P < 0{\cdot}01$), and not in the patient groups.

Despite this, enhancement indices $\geq 1{\cdot}3$ (Fig. 1) occurred in 34% of LCL patients, 60% of MCL,

Table 1. Effect of indomethacin on the proliferative response (SI) of leishmaniasis patients and controls to PHA (2·5 μg/ml)

Controls ($n=21$)*		LCL ($n=35$)		MCL ($n=5$)		DCL ($n=4$)	
−INDO	+INDO	−INDO	+INDO	−INDO	+INDO	−INDO	+INDO
3·66	11·42	2·08	5·74	3·34	8·87	1·82	6·22
4·17	17·76	2·09	4·63	6·05	16·89	8·82	26·56
9·48	15·30	2·22	16·20	10·33	19·98	12·83	41·11
10·66	12·80	2·59	6·27	11·78	12·50	17·44	43·26
11·34	21·80	3·48	3·70	31·90	33·38		
12·60	35·12	3·93	4·52				
13·86	21·48	3·95	10·96				
14·42	11·50	4·58	11·26				
20·29	27·14	4·65	2·55				
21·97	30·25	4·73	17·12				
23·81	54·43	5·17	15·16				
24·91	28·98	5·39	7·84				
25·11	55·89	5·52	8·36				
27·15	36·87	7·12	36·58				
28·52	5·65	9·63	12.84				
32·20	27·78	9·82	31·39				
32·46	86·74	10·27	19·67				
32·54	16·50	11·50	31·09				
43·72	22·05	12·09	24·90				
46·32	32·26	12·80	17·21				
64·21	130·11	19·04	26·16				
		20·64	13·35				
		22·58	58·63				
		24·07	33·53				
		24·78	14·67				
		30·01	21·16				
		30·58	47·97				
		30·96	19·02				
		32·02	73·50				
		33·46	27·22				
		36·31	79·20				
		41·58	54·74				
		41·81	65·22				
		46·10	104·87				
		47·17	24·56				
23·97†	33·42	17·28	27·18	12·68	18·32	10·23	29·29
±14·91	±28·88	±14·48	± 24·38	±11·26	± 9·42	± 6·62	±17·07
503‡	302	362	338	368	303	377	268
±369	±222	±214	±186	±187	±141	±203	±128

* Number of subjects studied.
† Mean ± s.d. of stimulation indices.
‡ ct/min of unstimulated cultures.

71% of DCL and 33% of normal controls. These proportions were significantly different for DCL patients ($P<0·05$) compared to LCL patients.

In contrast to PHA, the mean EI of the groups in the presence of INDO were significantly different. The mean SI in both the absence and presence of INDO was, however, significantly lower in LCL compared to controls ($P<0·025$–$0·01$). In Table 3 it can be seen that there was a significant

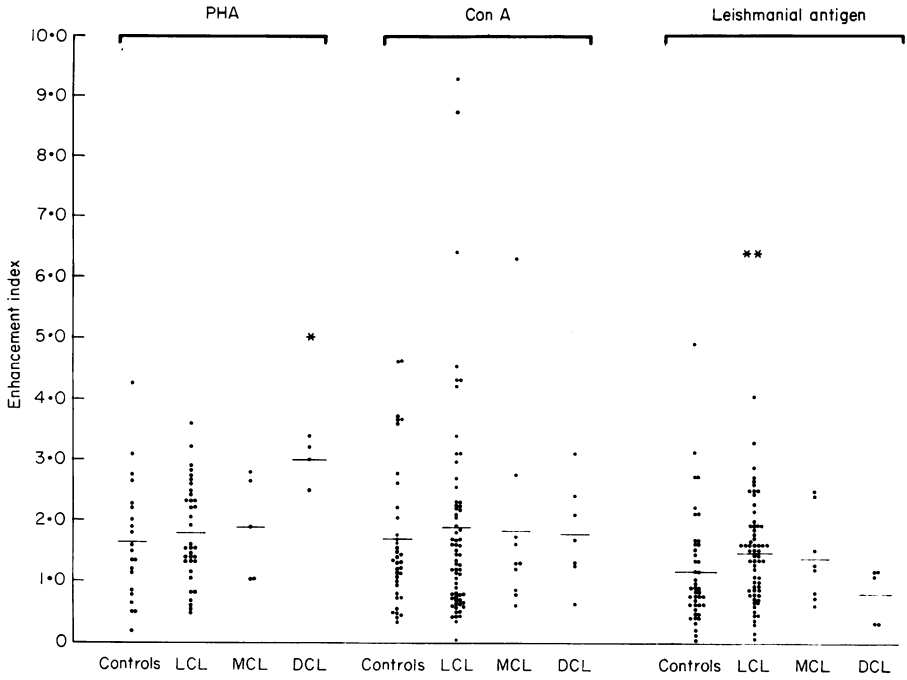

Fig. 1. Enhancement by indomethacin of mitogenic (PHA and Con A) and leishmanial antigen-induced response of Leishmania patients (LCL, MCL and DCL) and control subjects. * $P<0{\cdot}001$–$0{\cdot}05$ for DCL vs LCL, MCL and controls in PHA response, and ** $P<0{\cdot}005$ for LCL vs DCL in leishmanial response.

positive correlation between the INDO-induced EI of PHA and Con A responses, for all the patients taken as a group ($P=0{\cdot}003$), for LCL alone ($P=0{\cdot}02$), and MCL and DCL together ($P=0{\cdot}009$). This was not the case for the controls.

Effect of indomethacin on lymphocyte proliferative responses to leishmanial antigen. The proliferative responses to leishmanial antigen ($12{\cdot}5\times10^6$ parasites/well) were measured in the presence or absence of INDO (Table 4). The paired Student's *t*-test revealed that only in LCL patients did INDO exert a significant effect ($P<0{\cdot}05$). Enhancement indices $\geq1{\cdot}3$ were found in 59% of LCL patients, 37% of MCL, 0% of DCL and 35% of controls, the difference between DCL and LCL patients being statistically significant ($P<0{\cdot}025$).

The mean EI of the LCL group was significantly higher compared to DCL patients ($P<0{\cdot}005$), and in fact, the DCL group presented the lowest EI of all. Also, significant inverse correlations were found between the SI for leishmanial antigen in the absence of INDO and the EI in its presence, in both LCL ($r=-0{\cdot}60$, $P<0{\cdot}01$) and controls ($r=-0{\cdot}39$, $P<0{\cdot}05$).

The group mean SI in the absence of INDO were significantly higher in LCL ($P<0{\cdot}001$) and MCL patients ($P<0{\cdot}005$) compared to DCL patients and controls. This pattern was maintained when the response was measured in the presence of INDO. In the DCL group, which presented the lowest reactivity to leishmanial antigen, a significant increase in SI was not induced by INDO.

Comparison of the EI between the Con A, PHA and leishmanial antigenic response (Table 4) revealed that there was significant correlation when all the patients were taken as a group, and for the LCL patients alone, but not for MCL and DCL groups taken together or for the controls. The correlations between PHA and antigenic responses were generally not statistically significant, probably due to the low number of patients that were studied simultaneously under both conditions.

A final point that should be made here is that the inaccessibility of some of the patients made it impossible to perform multiple evaluations of their responses in the presence of INDO. However, in

Table 2. Effect of indomethacin on the proliferative response (SI) of leishmaniasis patients and controls to Con A (5·0 μg/ml)

Controls ($n=36$)*		LCL ($n=65$)		2MCL ($n=9$)		DCL ($n=7$)	
−INDO	+INDO	−INDO	+INDO	−INDO	+INDO	−INDO	+INDO
2·61	3·96	2·07	18·04	5·91	7·14	3·42	10·58
3·08	5·17	2·09	13·46	6·86	43·25	5·57	9·44
3·10	3·42	2·28	21·10	11·13	9·42	11·70	15·01
3·31	4·31	2·74	11·25	19·76	53·95	17·77	37·60
3·91	6·84	2·89	9·08	27·05	35·13	23·94	15·93
5·61	20·71	3·07	8·25	29·12	24·10	25·24	60·69
6·40	13·22	3·12	6·54	29·85	47·19	36·96	46·79
6·49	4·94	3·49	4·14	36·98	22·84		
7·42	33·99	3·53	14·86	70·53	122·06		
8·13	10·20	3·53	2·35				
8·42	8·49	3·77	2·03				
9·46	26·47	3·83	8·66				
9·82	5·63	3·97	7·45				
10·37	37·60	4·28	18·40				
10·67	10·40	4·46	2·38				
11·45	16·80	4·60	6·06				
11·51	29·72	4·85	4·01				
12·26	14·85	4·98	11·42				
13·26	61·64	5·33	7·02				
19·91	72·76	5·43	11·28				
21·21	47·37	5·60	9·63				
21·24	11·58	5·68	10·31				
21·50	25·53	6·02	13·50				
22·11	30·29	6·04	8·11				
23·75	31·92	6·06	13·51				
24·66	23·33	6·43	27·87				
25·82	21·51	6·53	10·07				
28·98	45·35	6·61	14·58				
29·95	43·15	6·71	17·13				
41·95	49·55	7·09	8·43				
42·33	14·96	7·44	5·71				
43·18	159·25	7·65	4·77				
57·87	44·79	7·92	24·70				
65·01	112·77	8·16	13·20				
69·64	53·58	8·31	5·72				
75·38	40·40	9·50	32·49				
		9·70	8·65				
		9·79	10·55				
		10·57	12·77				
		10·69	31·65				
		10·89	7·78				
		11·39	26·55				
		11·85	7·90				
		12·34	29·90				
		12·64	8·27				
		12·89	8·61				
		13·77	17·76				
		15·64	13·25				
		16·98	6·20				
		17·13	29·15				

Table 2 (*cont*)

		17·47	25·38				
		17·56	28·60				
		18·32	22·40				
		20·64	9·70				
		22·38	16·10				
		22·84	12·26				
		24·54	23·75				
		26·15	44·56				
		28·45	32·95				
		30·62	4·10				
		36·04	69·15				
		37·20	15·97				
		50·80	38·63				
		55·35	45·60				
		75·16	59·99				
21·71 ±19·80	31·85 ±31·83	13·01 ±13·57	16·55 ±13·40	26·35 ±19·87	40·56 ±34·59	17·80 ±11·93	28·01 ±20·32

* Number of subjects studied.
† Mean ± s.d. of stimulation indices.

Table 3. Correlation of indo-induced EI between Con A and PHA or Con A and antigen responses

	Correlation between	
Population	EI for Con A and PHA responses	EI for Con A and Ag responses
LCL+MCL+DCL	0·45*	0·29
	35	53
	0·003	0·01
MCL+DCL	0·84	0·29
	7	10
	0·009	NS
LCL	0·38	0·25
	28	43
	−0·02	0·05
Controls	0·25	0·11
	18	29
	NS	NS

* Correlation coefficient (r)/cases/significance.

some of the subjects this could be done, and the patterns of reactivity were reproduced over a period of at least 6 months.

DISCUSSION

In experimental models, and in a limited number of clinical studies, it has been shown that the response to *Leishmania* infection can be modulated by diverse factors, that include active

Table 4. Effect of indomethacin on the proliferative response (SI) of leishmaniasis patients and controls to leishmanial antigen ($12{\cdot}5 \times 10^5$ parasites/well)

Controls ($n=43$)*		LCL ($n=63$)		MCL ($n=8$)		DCL ($n=5$)	
−INDO	+INDO	−INDO	+INDO	−INDO	+INDO	−INDO	+INDO
0·58	1·28	0·75	0·96	8·13	12·45	1·35	1·40
0·73	0·94	0·99	1·25	9·26	7·35	1·39	0·47
0·81	0·32	1·07	1·91	12·56	15·44	3·09	3·60
1·04	1·45	1·09	2·10	15·65	38·51	4·43	5·09
1·14	1·48	1·26	0·98	23·38	56·60	5·10	1·60
1·14	0·99	1·27	1·92	24·69	30·86		
1·23	1·14	1·31	1·09	27·33	19·51		
1·27	2·07	1·48	1·17	53·20	31·59		
1·48	0·94	1·50	2·30				
1·50	1·24	2·13	3·42				
1·61	2·32	2·51	2·10				
1·93	1·19	2·94	2·24				
1·98	0·80	3·96	4·52				
2·07	5·63	4·10	6·66				
2·19	1·60	4·30	6·81				
2·29	3·78	4·48	11·34				
2·32	1·74	4·54	6·98				
2·39	1·78	4·55	12·35				
2·47	3·77	5·13	13·62				
2·53	5·43	5·53	18·09				
2·69	0·90	5·59	22·42				
2·95	4·19	5·75	7·98				
3·29	1·54	6·00	11·34				
3·50	7·12	7·31	12·16				
3·77	2·06	7·79	14·94				
3·92	3·36	8·03	9·65				
3·94	3·86	8·23	23·42				
3·99	2·41	8·28	18·05				
4·01	5·33	9·12	15·30				
4·30	20·99	9·15	20·75				
4·43	3·54	9·46	13·33				
4·75	12·86	10·73	11·07				
4·87	3·04	11·56	9·04				
5·58	6·43	13·04	25·49				
7·01	14·69	14·05	12·30				
7·30	6·48	14·19	22·34				
8·60	1·68	14·76	7·26				
9·03	6·91	14·79	9·10				
10·15	11·78	15·41	20·39				
10·48	4·25	15·49	16·30				
11·10	7·44	15·65	38·51				
24·75	10·91	15·72	10·83				
		17·07	2·80				
		17·16	7·31				
		17·78	28·75				
		18·18	45·23				
		18·78	24·68				
		18·83	32·43				
		19·73	50·10				
		19·99	17·87				
		20·59	3·70				

Table 4 (*cont*)

		20·81	14·99				
		21·03	19·61				
		25·23	48·84				
		25·32	4·64				
		27·09	12·62				
		29·24	53·50				
		36·12	14·33				
		36·94	12·39				
		42·98	28·28				
		82·83	111·62				
4·43†	4·26	14·15	17·20	21·78	26·54	3·05	2·43
± 4·46	± 4·38	±15·37	± 19·56	±14·61	±16·17	±1·74	±1·87
422‡	495	398	358	659	737	409	392
±355	±384	±239	±232	±539	±498	±145	±156

*, †, ‡ As in Table 1.

suppression of lymphocytes bearing receptors for the antigen (Liew, Hale & Howard, 1982), serum factors (Pearson & Steigbigel, 1980), antigenic load (Preston & Dumonde, 1976) and the genetic constitution (Howard, Hale & Liew, 1981) or nutritional status (Perez, De la Rosa & Malave, 1984) of the host.

Prostaglandins are ubiquitous in the body, and interest has recently developed in the immunoregulatory properties of these hormones (Ellner, 1981; Raff & Stobo, 1981). In particular, prostaglandin E_2, has been associated with suppressor activity in response to mitogens *in vitro* (Goodwin, Bankhurst & Messner, 1977). The role of PG as mediators of suppression in chronic infections has not, however, been fully defined. Considering this, we studied the possible existence of an indomethacin-sensitive mechanism regulating the immune response in the three major clinical forms of ACL.

It was found that mitogenic stimulation *in vitro* by PHA of peripheral mononuclear cells from patients with LCL, MCL, and DCL was significantly increased by INDO. In fact, an inverse correlation existed between the initial response to PHA and the subsequent increase in the presence of INDO, for all the groups, particularly in MCL and DCL patients. This provides indirect evidence that a PG-sensitive regulatory mechanism is operative, and that the net effect of PG is suppression. Considering responses to Con A, when the changes were evaluated by the paired *t*-test, these were only statistically significant in the controls, and not in the patients' groups. The apparent non-identity of PHA- and Con A – responsive T cell subsets (Stobo & Paul, 1977) may explain some of the observed differences between the results obtained with these two mitogens. Even so, a high proportion of the MCL and DCL patients showed marked increases in their Con A responses in the presence of INDO. As cells of the monocyte/macrophage series are the main producers of PG, and these inhibit mitogen responsiveness (Goodwin *et al.*, 1977; Ellner & Spagnoulo, 1979), it may be postulated that such cells are active in all groups of patients as well as in the controls.

Considering specific responses to leishmanial antigens, we showed, as in an earlier report (Castes *et al.*, 1983), that these were significantly higher in LCL and MCL patients compared to the controls, and that DCL patients demonstrated a complete absence of response to specific antigen.

In the presence of INDO, the responses were significantly increased only in the LCL group. An inverse correlation was also found for this group between the initial response to leishmanial antigen and the subsequent increase in the presence of INDO. It would appear, therefore, that a PG-dependent inhibitory mechanism for stimulation by leishmanial antigen is active in LCL patients, but not in MCL or DCL. It is important to note that when we took all the patients together as a group we found a significant correlation between EI produced by INDO in the Con A and PHA responses, and for the Con A and leishmanial antigenic responses. This was not the case for the controls. When the LCL patients were taken as a single group and the DCL and MCL patients as

another, the correlations were consistent with that described earlier. For the LCL group, significant correlations occurred for the EI for both mitogens and the antigen. This contrasts to MCL and DCL patients, where we only observed significant correlations between the EI for the mitogens, but not between Con A and antigen responses. This supports the suggestion that PG are not involved in the modulation of the leishmanial antigen response in these latter patients.

In this respect, it is relevant to note that MCL patients have higher specific proliferative responses *in vitro* and a lower degree of specific suppression than the other study groups. This could be due to deficient antigen-specific suppressor mechanisms in these patients (Castes *et al.*, 1984). It has been reported (Carvalho *et al.*, 1985) that MCL patients have significantly decreased CD4 inducer/helper T cells, and we have also found decreased levels of CD4 cells in response to leishmanail antigen (unpublished results). It is possible that the cells responsive to PG regulation are the CD4 inducer of suppression (Damle *et al.*, 1985) and that these are defective in the MCL patients.

On the other hand, DCL patients, who show a lack of responsiveness to leishmanial antigen *in vivo* and *in vitro*, demonstrated a marked leishmanial antigen-induced suppressive activity (Castes *et al.*, 1984). From the results obtained in the present study, it does not, however, appear that the inability of lymphocytes from DCL patients to respond to leishmanial antigens is due to a mechanism in which PG are involved. This contrasts with the study of Petersen *et al.* (1982), in which four patients with a diffuse type of cutaneous leishmaniasis were found to increase their proliferative response to leishmanial antigens in the presence of INDO. These authors suggested a role for PG in this form of the disease. One explanation for this lack of agreement with our results may be that leishmaniasis in the Dominican Republic, as suggested by the same authors (Petersen *et al.*, 1984), presents differences with that found in other regions. They report (Petersen *et al.*, 1982), for example, that the strains isolated from two of their patients differed from any known members of the *L. braziliensis* or *L. mexicana* complexes.

It is of interest to remark here that although not statistically significant, 35% of the normal controls also showed augmented responses to leishmanial antigen in the presence of INDO. As some of the controls originated from the endemic area, the possibility exists that they had experienced sub-clinical infections by *Leishmania*, or other cross-reactive parasites (Castes *et al.*, 1983). In fact, 33% of the controls demonstrated positive (SI > 4) responses *in vitro* to leishmanial antigen. Also, an inverse correlation was found for this group between the initial response to leishmanial antigen and the subsequent increase in the presence of INDO. A comparable situation has been described by Bahr *et al*, (1981), who demonstrated that there is a prostaglandin-dependent, indomethacin-sensitive regulatory mechanism operating on the response of tuberculosis and tuberculoid leprosy patients to mycobacterial antigens, that was also present in normal individuals. A similar situation was found in the response to *T. cruzi* antigens in a group of normal subjects (Castes, Panagiotopoulos & Mosca, 1986). It would appear from our results that a normal PG-dependent, indomethacin-sensitive suppressor mechanism operates in LCL patients, possibly creating an equilibrium between the presence of the parasite and the protective immune response, without producing significant tissue damage. In MCL, where this suppressive mechanism is apparently not functional, the response to the parasite is intense, and tissue damage might occur as a consequence. The anergy observed in DCL patients does not, however, appear to be due to an involvement of PG in the suppression of the specific immune response.

As a final point, it should be remarked that PG are not the only derivatives of arachidonic acid that can modulate the immune response, as leukotrienes also might be implicated (Rola-Pleszczynski, 1985). Further study of this possibility is required to elucidate the importance of this mechanism in leishmaniasis patients.

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