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Serum Factors Affecting the Cell Migration Inhibition Response to Lepromin [1]

E. L. Fliess, B. Ruibal-Ares and M. Braun [2]

The importance of cell-mediated, T cell dependent, immune reactions in leprosy has been stressed ([16]). This is particularly remarkable in the two polar, lepromatous and tuberculoid types of leprosy.

Lepromatous patients whose prognosis, evolution and response to treatment are poor, have very active B type immune reactions. These are characterized by high serum levels of IgG, IgM and IgA with high titers of specific anti-*M. leprae* antibodies ([4]), while their T cell-mediated functions are poor: delayed skin responses to lepromin are negative ([3,7,9,18]), blastomitogenic responses of peripheral lymphocytes to PHA ([5,7,9]) and/or *M. leprae* are depressed ([7,9,10]); they fail to produce migration inhibitory factor (MIF) as a response to lepromin ([10-12]); and the cortical, thymus dependent, areas of their lymph nodes are depleted ([19]). Also, in recent studies a decrease of the T cell subpopulation in their peripheral blood, as tested by the spontaneous rosetting technic, has been reported ([6,7,9,14]).

In tuberculoid patients, whose prognosis and response to treatment are notably better, all T cell-mediated responses are active, including positive skin tests ([7]), good blastomitogenic response to PHA and lepromin ([5]), while their serum Ig levels are normal and specific antibodies for *M. leprae* are very low or undetectable ([4]).

In the present study we have used the leukocyte migration inhibition (LMI) test, carried out on buffy coat cells, to study cell-mediated reactions and serum factors in both polar types of leprosy. Data confirming the presence of CMI reactions to lepromin in tuberculoid patients and its absence in lepromatous patients is presented, and it is also shown that while serum from tuberculoid subjects has a stimulatory effect on LMI, serum from lepromatous patients has the opposite effect.

MATERIALS AND METHODS

Twenty-six subjects were studied: ten with untreated tuberculoid leprosy, six with untreated lepromatous leprosy, and ten normal controls neither related to nor in contact with Hansen's disease patients. Four of the latter were lepromin negative and six were lepromin positive.

Antigen. Total protein lepromin (TPL) was prepared following WHO standards from a concentration of 80×10^6 bacilli. No preservatives were added to this extract that could alter cell migration *in vitro*. This TPL was added to the culture media at a concentration of 1%, v/v.

Sera. Serum from untreated lepromatous and tuberculoid patients as well as from normal blood donors was obtained. Sera were pooled and each pool was divided in 1 ml aliquots and frozen at -20°C. Sera were added to culture medium at a concentration of 20%.

Cell migration inhibition test. The Søborg and Bendixen technic ([13]), as modified by Braun *et al* ([2]), was used. One hundred milliliters of peripheral blood were obtained from each subject and sedimented 45 minutes at room temperature. Buffy coat cells were harvested and washed three times using Hanks balanced salt solution. All centrifugations were done at 800 rpm for ten minutes. The resulting pellet of white cells was resuspended in TC 199 (Difco Lab) and packed into capillary tubes that were placed in Lucite chambers. The chambers were filled with TC 199 medium containing antibiotics (penicillin, 100 U/ml and streptomycin, 100 μg/ml) and the different sera, with or without TPL, and sealed with glass slides.

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[2] E. L. Fliess, M.D., Head, Clinical Laboratory, "Colonia B. Sommer" Hospital and "Bungey Born" Fellow of the Academia Nacional de Medicina; and B. Ruibal-Ares, M.D., Fellow, Consejo Nacional de Investigaciones Cientificas y Técnicas, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires, Argentina; M. Braun, M.D., Member, Research Career of the Consejo Nacional de Investigaciones Cientificas y Técnicas, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires.

For each subject the following sera were used: a) no serum; b) normal serum; c) lepromatous serum; and d) tuberculoid serum. For each of these combinations half of the chambers were left as control chambers without antigen and TPL was added to the other half. All the tests were done in triplicate at least.

Index of migration (IM). All chambers were incubated for 24 hours at 37°C. Migration areas were measured using a microscope with a reticule in the ocular piece. IM were calculated using the following equation:

$$IM = \frac{\text{mean area of migration with antigen} \times 100}{\text{mean area of migration without antigen}}$$

An IM below 75% was considered a positive reaction.

Statistical analysis. One-way analysis of variance was carried out using the computerized Statistical Analysis System of the Northeast Regional Data Center of State University System of Florida. Both horizontal differences, analyzing the effect of sera, and vertical differences comparing groups of subjects were studied taking each group of data as independent samples. The F values were obtained and comparison between groups was done following Scheffe's procedure.

In order to study the effect of sera in the different groups of subjects, the data for each sera in each individual group of patients and controls were analyzed as "paired samples" and an analysis of variance was repeated for each group.

RESULTS

Results are graphically displayed in Figure 1. Using no serum in the test (Table 1), lepromatous patients gave negative results, their IM ranging from 92% to 101%. Tuberculoid patients, in contrast, all gave positive reactions with indexes ranging from 55% to 75%. Normal controls that had shown positive skin reactions also gave positive LMI tests ranging from 68% to 75%, while negative controls showing a slight inhibition of migration in the presence of TPL, were all over the 75% limit.

The addition of normal serum (Table 2) did not alter these results although in lepromatous patients there was a slight reduction in their mean IM.

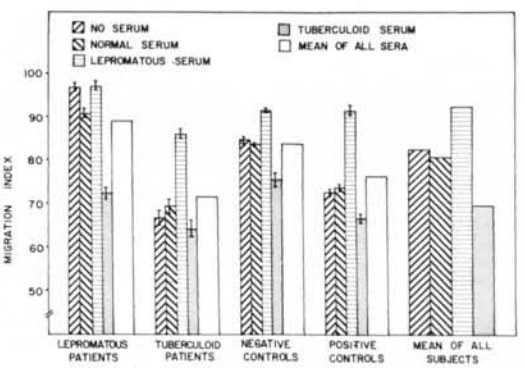

FIG. 1. Effect of sera on all migration inhibition in leprosy and controls. Mean IM ± SE.

TABLE 1. *Migration indexes in patients and controls using no serum.*

Lepromatous patients		Tuberculoid patients		Negative controls[a]		Positive controls[a]	
Subject no.	IM	Subject no.	IM	Subject no.	IM	Subject no.	IM
1	92	11	55	17	86	21	77
2	98	12	72	18	78	22	68
3	94	13	75	19	87	23	75
4	98	14	61	20	88	24	72
5	96	15	69			25	69
6	101	16	68			26	74
7	97						
8	100						
9	98						
10	97						
Mean ± SD	97.1 ± 2.6		66.67 ± 7.4		84.75 ± 4.6		72.5 ± 3.5

[a] Positivity and negativity with reference to lepromin response.

TABLE 2. *Migration indexes in patients and controls using pooled normal serum.*

Lepromatous patients		Tuberculoid patients		Negative controls[a]		Positive controls[a]	
Subject no.	IM	Subject no.	IM	Subject no.	IM	Subject no.	IM
1	86	11	58	17	86	21	77
2	94	12	72	18	78	22	60
3	91	13	78	19	86	23	75
4	95	14	66	20	85	24	70
5	76	15	74			25	73
6	95	16	67			26	78
7	94						
8	103						
9	86						
10	88						
Mean ± SD	90.8 ± 7.3		69.17 ± 7.1		83.75 ± 3.9		73.67 ± 3.7

[a] Positivity and negativity with reference to lepromin response.

TABLE 3. *Migration indexes in patients and controls using lepromatous serum.*

Lepromatous patients		Tuberculoid patients		Negative controls[a]		Positive controls[a]	
Subject no.	IM	Subject no.	IM	Subject no.	IM	Subject no.	IM
1	91	11	83	17	94	21	96
2	103	12	91	18	86	22	80
3	93	13	89	19	95	23	91
4	95	14	74	20	92	24	98
5	94	15	89			25	94
6	105	16	89			26	91
7	97						
8	105						
9	97						
10	92						
Mean ± SD	97.20 ± 5.3		85.83 ± 6.4		91.75 ± 4.0		91.67 ± 6.3

[a] Positivity and negativity with reference to lepromin response.

The addition of lepromatous serum to the culture medium (Table 3) did not alter the IM in lepromatous patients but turned to negative the responses of all but one of the tuberculoid patients and of all positive controls. On the contrary, the addition of tuberculoid serum made positive the responses (Table 4) of all but two of the lepromatous patients and two of four negative controls, and in general enhanced all positive reactions.

Statistical analysis. One-way analysis of variance between groups of patients (Table 5) gave an F value of 21.598, pointing to the presence of differences in their mean IM at the $p < 0.001$ level. Lepromatous patients differed from tuberculoid patients and from positive normal controls at $p < 0.01$ level, and tuberculoid patients differed from normal negative controls at the same level. The difference between normal positive and negative controls was not significant probably because of the small number of cases.

Horizontal comparisons between different sera showed an F value of 151.588, also pointing to a greatly significant difference. All sera differed from each other at the $p < 0.001$ level. Taking into account the data from all the subjects, the absence of sera made no significant difference as compared with normal serum. But when each group of subjects was studied as independent groups

TABLE 4. *Migration indexes in patients and controls using tuberculoid serum.*

Lepromatous patients		Tuberculoid patients		Negative controls[a]		Positive controls[a]	
Subject no.	IM	Subject no.	IM	Subject no.	IM	Subject no.	IM
1	76	11	52	17	81	21	75
2	69	12	72	18	67	22	64
3	72	13	73	19	80	23	66
4	73	14	57	20	74	24	66
5	67	15	66			25	59
6	66	16	64			26	68
7	73						
8	80						
9	72						
10	74						
Mean ± SD	72.2		64.0 ± 8.3		75.5 ± 6.5		66.33 ± 5.2

[a] Positivity and negativity with reference to lepromin response.

TABLE 5. *Mean migration indexes in patients and controls using different sera.*

Group \ Sera	None	Normal	Lepromatous	Tuberculoid	Mean
Lepromatous patients	97.1[b]	90.8[b]	97.2	72.2	89.325[b]
Tuberculoid patients	66.67	69.17	85.83	64.0	71.42[b]
Negative controls[a]	84.75	83.75	91.75	75.5	83.94[b]
Positive controls[a]	72.5	73.67	91.67	66.33	76.04[b]
Mean	82.5[c]	80.77[d]	92.46[d]	69.46[d]	81.30

[a] Positivity and negativity with reference to lepromin skin response.
[b] $p < 0.01$.
[c] Not significant.
[d] $p < 0.001$.

of paired samples, the mean IM was significantly lower using the normal serum than when using no serum at all only in the lepromatous group.

In all other groups, the addition of lepromatous sera significantly raised the IM, and the addition of lepromatous sera significantly lowered the IM while normal serum gave no significant differences.

DISCUSSION

The buffy coat LMI test may not measure directly the production of MIF ([14]), but it has good correlation both for the presence of delayed hypersensitivity ([17]) and the production of MIF ([2]).

Although the method used was slightly different, the data presented here confirm previous papers showing the presence of cell-mediated immunity and/or the production of LMI by lymphocytes from tuberculoid patients and positive controls, and the absence of this reaction in lepromatous patients ([10-12]).

Since lepromatous patients have *M. leprae* and should be sensitized against its anti-

gens, the absence of cell-mediated reactions must be explained. Our approach was the study of the effect of their sera in order to detect circulating factors able to alter the normal reaction. In effect, lepromatous serum added to tuberculoid patient or control lymphocytes produced a marked elevation of IM in the presence of TPL, showing the presence of a "blocking" effect inhibiting the positive reaction in these subjects. This inhibition could be due to a variety of factors.

Blocking antibodies. The anomalous quantities of Ig's with a high content of specific antibodies in lepromatous patients led Bullock and Fasal to postulate that blocking antibodies were present in lepromatous patients, inhibiting normal cellular immune reactions ([4]). These "blocking" antibodies, or perhaps better, Ag-Ab complexes, could be acting *in vitro,* inhibiting LMI reactions.

Unspecific inhibition. CMI can be inhibited by nonspecific factors like antilymphocyte serum. Lepromatous patients have circulating factor(s) that lead to a depressed reaction to PHA ([11]) and perhaps also to the low percentage of rosetting T lymphocytes in their peripheral blood ([6, 7, 9-14]). This same factor(s) could be affecting the LMI response to lepromin. Moreover, this defect could be inherited since one of us has shown abnormal blastogenic responses in healthy descendants of lepromatous patients ([1]).

Some specific unknown inhibiting factor, that could be related to the function of suppressor lymphocytes ([8]). But whatever the inhibiting serum factors are, the lymphocytes of lepromatous patients are able to respond normally. In the presence of tuberculoid serum their response is positive in practically all cases. Even in the presence of normal serum there is a slight but significant difference in IM, as compared with no serum or lepromatous serum while in all other groups of subjects no serum and normal serum gave identical IM's. This could perhaps point to the lack of some normal serum factor in lepromatous patients.

The data presented here show that tuberculoid serum has a factor stimulating LMI, both in patients and normal controls. This factor could very well be circulating transfer factor ([13]). Since all patients in this study were untreated, cell-mediated immune reactions against *M. leprae* were very active when sera were obtained and the destruction of lymphocytes with release of transfer factor to the blood may have happened. On the other hand, it could also be an unspecific stimulatory factor.

The specificity or nonspecificity of both inhibiting and activating activities and the physico-chemical characteristics of these factors require elucidation.

SUMMARY

Cell migration inhibition of white blood cells in the presence of total protein lepromin (TPL) was studied in ten lepromatous patients, six tuberculoid patients, and ten normal controls; adding normal, tuberculoid, lepromatous, or no serum to the culture medium. Using normal or no serum, lepromatous patients and skin negative controls gave negative reactions, while tuberculoid patients and skin positive controls gave positive cell migration inhibitions. The addition of lepromatous serum gave a very significant overall increase of migration indices in all groups of subjects, turning to negative the positive reactions of lepromatous patients and positive controls. On the contrary, the addition of tuberculoid serum gave a decrease of migration index in all groups of subjects, turning to positive the reactions in lepromatous patients. The significance of these circulating factors, able to enhance or inhibit cell migration inhibition responses in patients and controls, is discussed.

RESUMEN

Se estudió la inhibición de la migración de las células sanguíneas blancas en presencia de lepromina con proteína total (LPT) en diez pacientes lepromatosos y en diez controles normales; añadiendo suero normal, lepromatoso, tuberculoide, o sin suero, al media de cultivo. Sin suero o con suero normal, los pacientes lepromatosos y los controles negativos a la prueba intradérmica, dieron reacciones negativas, mientras que los pacientes tuberculoides y los controles con pruebas intradérmicas positivas, dieron inhibición de la migración celular positiva. Cuando se añadió suero lepromatoso se obtuvo un aumento signiticativo de índice de migración en todos los grupos de personas, haciendo negativas las reacciones positivas de los pacientes lepromatosos y controles positivos. Por el contrario, cuando se añadió suero tuberculoide, se observó una disminución de índice de migración en todos los grupos, haciendo positivas las reacciones en los pacientes lepromatosos. Se discute el significado de estos

factores circulantes, que son capaces de aumentar o inhibir las respuestas de inhibición de la migración celular en pacientes y controles.

RÉSUMÉ

Chez dix malades lépromateux, et chez dix témoins normaux, et chez six malades tuberculoïdes on a étudié l'inhibition de la migration cellulaire de globules blancs du sang en présence de lépromine protéinique totale (TPL). Cette étude a été menée en ajoutant au milieu de culture du sérum de témoins, du sérum de malades tuberculoïdes, et du sérum de malades lépromateux, certaines épreuves étant menées en l'absence de sérum. Sans sérum, ou en présence de sérum normal, les malades lépromateux et les témoins avec épreuves cutanées négatives ont fourni des réactions négatives; par contre, les malades tuberculoïdes, et les individus témoins présentant une réaction cutanée positive, ont montré une inhibition de la migration cellulaire. L'addition de sérum lépromateux a entraîné dans l'ensemble une augmentation très significative des indices de migration dans tous les groupes de malades, rendant négatives les réactions initialement positives des malades lépromateux et des témoins positifs. Au contraire, l'addition de sérum de malades tuberculoïdes a entraîné une diminution de l'indice de migration dans tous les groupes de sujets, rendant positives les réactions observées chez las malades lépromateux. On discute la signification de ces facteurs circulants, qui sont capables de stimuler ou d'inhiber les réponses d'inhibition de la migration cellulaire.

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