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Similar Alterations of Lymphoblastic Dedifferentiation in Lepromatous Leprosy Patients and Their Healthy Lepromin-Negative Consanguineous Offspring[1]

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Clearly differing immunologic responses between lepromatous and tuberculoid leprosy have been well-established. However, from the humoral point of view ([18, 20, 30]) the facts do not yet warrant clear differentiation.

There is a high level of humoral antibodies against *M. leprae* in lepromatous patients as contrasted with the scarcity of humoral antibodies in tuberculoid patients ([13]). This suggests that in leprosy, as is the case in other infections caused by mycobacteria and viruses, the role of humoral antibodies is not fundamental and that the real immunological effectivity lies in cell-mediated immunity ([3, 19, 21, 24, 29]).

Cultures of lymphocytes *in vitro* in the presence of specific or nonspecific stimulants have revealed the existence of a depressed response in lepromatous leprosy patients, while in tuberculoid patients most authors agree to the existence of an immunological response similar to that observed in normal persons ([10, 14, 22, 27]).

Studies of macrophage lytic activity as related to *M. leprae* are contradictory ([2, 4, 12, 15]). On the other hand, studies of the macrophage migration inhibition test (MIF) ([6, 25]) even involving few cases, show a clear difference between tuberculoid and lepromatous patients. The former gives a response similar to that of normal controls, while lepromatous patients show no inhibition of migration ([11, 16, 17]). Additionally, the cutaneous tests, specially that with dinitroclorobenzene, a substance producing positive reactions in 95% of normal individuals, demonstrates the existence of a high number of negative responses in lepromatous patients, which is the opposite of what is observed in tuberculoid patients and healthy controls ([8, 9, 28, 31]). These facts speak to the existence of a depression in the delayed hypersensitivity and in cell-mediated immunity in lepromatous patients. The causes of this depression are not evident but probably they are not due to a single factor but to the interaction of several factors.

The basis of this immunologic deficiency may be related to the hypothesis, advanced by various authors, relating to a genetically related susceptiblity to leprous infection. This has long been postulated on a clinical and epidemiologic basis, a factor that would condition the evolution of the disease towards one polar form or the other, having been pointed out in 1937 ([23]).

Later studies have strengthened this hypothesis of a constitutional predisposition ([1, 5, 7]); it being accepted actually by some that patients who present a deficiency in cell-mediated immunity may have an immunological tolerance to *M. leprae*, that would preclude an adequate response to this organism when the infection occurs. This deficiency would not be absolute because despite the evidence noted above, it is remarkable that lepromatous leprosy patients give normal response to virus and parasitic infections which in patients with a serious deficiency of cell-mediated immunity (e.g., children with thymic aplasia) are fatal. Additionally, lepromatous patients with a negative cutaneous reaction to dini-

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troclorobenzene may eventually yield a positive response after more or less prolonged applications. This would suggest that the immunologic deficiency is not permanent.

Against this background it was decided to study lymphoblast transformation in untreated lepromatous leprosy patients and in lepromin-negative and positive consanguineous relatives (healthy sons and grandsons) of lepromatous patients and in health, lepromin-positive and lepromin negative controls.

MATERIALS AND METHODS

Integral lepromin with a bacillary concentration of 10×10^6 was utilized and the lepromin reaction was read at 48 hours and 21 days.

Lymphocyte cultures were sustained on T.C. 199 heparinized culture medium (DIFCO), mixing equal parts of culture medium and plasma rich in cells. Integral lepromin (bacillar concentration 10×10^6) and phytohemagglutinin (Burroughs-Wellcome) in a solution of 0.1 mg/ml were used as the stimulating agents. Three different cultures were performed for each lepromatous patient, consanguineous and healthy control, that is to say:

a) control culture with plasma rich in cells and T.C. 199 medium;
b) the same with the addition of 1 ml phytohemagglutinin;
c) with the addition of 0.1 ml of integral lepromin instead of phytohemagglutinin.

One hundred milliliters of blood were obtained aseptically and were placed in the culture chamber in test tubes and allowed to sediment for 45 minutes. The supernatant was placed in 50 ml culture bottles in the proportions of 4 ml plasma rich in cells, and 4 ml of heparinized T.C. 199 culture medium. To this was added 1 ml of phytohemagglutinin or 0.1 ml of integral lepromin as appropriate. The cultures were incubated at 37°C for a period of 72 hours, at the end of which time a sample was removed. This was repeated at 120 hours. One milliliter of each of these samples was mixed with 1 ml of a solution consisting of two parts distilled water and one part isotonic saline solution and the mixture was allowed to rest for 30 minutes.

These culture samples were then centrifuged for five minutes at 800 rpm and the supernatant discarded. One milliliter of Carnoy's solution (5 parts methyl alcohol, 1 part glacial acetic acid) was added and the culture left undisturbed for 30 minutes. It was then again centrifuged for five minutes at 800 rpm and the supernatant discarded. Again, Carnoy's solution was added and centrifugation was repeated, discarding the supernatant.

A new suspension with Carnoy's solution was made and a smear made on cold glass. The preparation was allowed to dry and then stained with May Grünwald-Giemsa stain for microscopic observation.

The culture medium used was prepared in the Immunohematology Section, Instituto de Investigaciones Hematologicas, Academia Nacional de Medicina.

RESULTS

In addition to the subjects mentioned, a culture of lymphocytes from a healthy consanguineous offspring (lepromatous grandson) having a Fernandez negative reaction but with a Mitsuda positive reaction, was studied. Since there was only one case it was not included in the statistical evaluation.

The evaluation of the results is based on the property that human lymphocytes have of differentiating, when placed in contact with antigens to which the subject has already been exposed. This transformation to lymphoblasts can be reproduced *in vitro* by a technic recently described.

The blastic transformation occurs without special stimulation in about 10% of normal lymphocytes.

By adding to culture a specific antigen (here total lepromin) to which the lymphocytes are already sensitized, the percentage of transformation rises in normal instances.

There are also nonspecific or mitogenic stimulating agents which are able to stimulate transformation to blasts in a high percentage of lymphocytes (80% to 90%) in normal individuals. These are phytohemagglutinin, American phytolaca (a concanav-

TABLE 1. *Lymphocyte culture in untreated lepromatous patients.*

			Percentage of lymphocyte transformation					
			Control		PHA		Lepromin	
No.	Age	Sex	3rd day	5th day	3rd day	5th day	3rd day	5th day
1	45	M	11.3	10.8	17	15.7	12	11.3
2	39	F	9.1	8.5	16.2	14	11	10
3	18	M	11	9.2	13	12	11.6	10
4	36	M	9	8.5	14	12.7	12.3	11.6
5	50	M	18	14	68	57	—[a]	—[a]
6	52	F	12	10.2	44	41	—[a]	—[a]
7	31	M	11.8	9.7	30	28.2	—[a]	—[a]
8	48	M	14	6.8	66	55	—	—
9	24	M	25	21.8	46	45	29	26
10	45	M	11.8	10.2	19	15.4	18.3	16
	Averages		13.3	10.97	33.4	29.6	16.31	13.8

[a] These cultures had to be discarded because of contamination. They could not be repeated because the basic conditions for the experiment had changed.

TABLE 2. *Lymphocyte culture in healthy lepromin-negative consanguineous relatives of lepromatous patients.*

			Percentage of lymphoblast transformation					
			Control		PHA		Lepromin	
No.[a]	Age	Sex	3rd day	5th day	3rd day	5th day	3rd day	5th day
1	16	F	11	8.5	31	28	22	17
2	15	F	10.1	9.6	34.8	30.5	14	13
3	26	M	7.1	6	21	16.2	—[b]	—[b]
4	29	M	11	10.2	19	16.3	—[b]	—[b]
5	13	M	9	8.2	28	19	—[b]	—[b]
6	9	M	13	12	41	35	18	15.3
7	12	M	12	10	31	29.3	23	17.2
8	36	F	10	8.5	23.8	20.2	18	16
9	31	M	9.3	8.8	38	35.4	26	24.3
10	19	F	9.5	9	36	35.3	22	20.4

[a] Numbers 5 and 11 were grandsons of a lepromatous patient. The rest were sons of lepromatous patients.
[b] Culture discarded because of contamination.

ine), as well as others. The reacting lymphocytes are the thymus dependent lymphocytes in neonatal life. Lower responses are seen in patients who have alterations in cellular immunity; for example, in thymic displasia, primary biliary cirrhosis, sarcoidosis, Hodgkin's disease, chronic lymphatic leukemia, agammaglobulinemia, Sjögren's disease, etc.

Similar lowering of response has also been observed *in vitro* with the addition to the cultures of substances such as prednisone, cloroquine, 6 mercaptopurine, thalidomide and several other agents. In Tables 1, 2, 3, and 4 the obtained results can be observed.

Statistical evaluation. Statistical evaluation was carried out by means of the Student's test for small samples. On the basis of this evaluation it was evident that there is no meaningful difference between the values for the controls (without the stimulants addition), for the lepromatous patients, and for the normal lepromin-positive and negative controls ($p>0.05$). The cultures stimulated with integral lepromin present meaningful differences between lepromatous patients and healthy controls

TABLE 3. *Lymphocyte culture in lepromin-positive healthy consanguineous relatives of lepromatous patients.*

			Average percent of lymphocyte transformation					
			Control		PHA		Lepromin	
No.	Age	Sex	3rd day	5th day	3rd day	5th day	3rd day	5th day
1	24	M	9.3	8	72	70	25	24
2	13	M	12	13	68	72	28	29
3	10	M	10.6	9.5	80	78	28	26.7
4	11	M	8.5	8.1	84	80.7	29	28.2
5	18	F	10.1	9.6	76	75.1	27.3	26.3
6	23	M	9	8.5	78	77	26	24
7	14	F	10.2	9.6	84	83.1	28	25.6
8	20	M	9.4	8.7	79	82.1	28.4	30.7
9	16	F	8.6	8	76	74	26	25.3
10	19	M	10.1	9.6	78	76	27	24
	Average		9.78	9.26	77.5	76.8	27.3	26.18

TABLE 4. *Lymphocyte culture in healthy lepromin-positive controls.*

			Percent of lymphocyte transformation					
			Control		PHA		Lepromin	
No.	Age	Sex	3rd day	5th day	3rd day	5th day	3rd day	5th day
1	43	M	11	9.8	84.6	76	30	26
2	27	M	13.9	8.1	85	82	31.3	25
3	32	M	10	9	75	68	—[a]	—[a]
4	24	F	11.3	8.1	80	73	—[a]	—[a]
5	36	M	9.7	8	76	70	24	20
6	41	M	10.8	9.3	82.3	75.4	29	25.2
7	26	M	15	11.3	89	85	30	26
8	30	M	10.3	9	83	80	26	24
9	22	M	10.1	9.2	75	72	21	19
10	23	F	16.4	11	85	80	—[a]	—[a]
	Averages		11.65	9.28	81.49	76.24	27.3	23.6

[a] Cultures discarded because of contamination.

($p<0.01$). The cultures stimulated with phytohemagglutinin show highly meaningful differences between lepromatous patients and healthy controls ($p<0.001$).

There was no meaningful difference in the values of the control cultures ($p>0.05$. The cultures stimulated with integral lepromin presented meaningful differences between the lepromin-negative consanguineous persons and the control subjects ($p<0.01$. The cultures stimulated with phytohemagglutinin, present highly meaningful differences between consanguineous subjects and healthy controls ($p<0.001$).

There was no meaningful difference between the values of the control cultures ($p>0.05$) of lepromatous patients and lepromin negative consanguineous subjects. There was no meaningful difference between the values of the cultures stimulated with lepromin ($p>0.3$). There was no meaningful difference between the values of the cultures stimulated with phytohemagglutinin ($p>0.3$). There was no meaningful difference of the control cultures ($p>0.1$). The cultures stimulated with integral lepromin presented meaningful differences between the lepromin negative and the lepromin positive consanguineous persons ($p>0.01$). The cultures stimulated

TABLE 5. *Lymphocyte culture in healthy lepromin-negative control subjects.*

			Average percent of lymphocyte transformation					
			Control		PHA		Lepromin	
No.	Age	Sex	3rd day	5th day	3rd day	5th day	3rd day	5th day
1	45	M	8.1	7.5	68	65.2	18	16.3
2	34	M	10	9.3	76	75.2	22	20
3	56	M	11	10.4	83	80.1	13	12.5
4	32	F	9	8.4	71	69	16	14.3
5	46	M	9.5	8.7	77	75.1	17	15
6	57	M	9	8.1	68.6	65	15	13.2
7	45	M	8.4	8.2	80	78.9	19	16.4
8	36	F	10	8.3	78	75.6	26	25.3
9	23	M	9.2	8	68.4	63	25.4	23
10	26	F	9.1	8	82	79	23	22
	Average		9.33	8.49	75.2	72.61	19.44	17.8

TABLE. 6. *Average percent of lymphocyte transforamtion.*

	Control		PHA		Lepromin	
	3rd day	5th day	3rd day	5th day	3rd day	5th day
Lepromatous	13.3	10.97	33.4	29.6	16.31	13.18
Healthy lepromin—postive controls	11.65	9.28	81.49	76.24	27.13	23.6
Healthy lepromin—negative controls	9.33	8.49	75.2	72.61	19.44	17.8
Healthy lepromin—negative consanguineous	10.11	9.08	30.8	27.12	19.3	18.3
Healthy lepromin—positive consanquineous	9.78	9.26	77.5	76.8	27.23	24.18

with phytohemagglutinin present highly meaningful differences between lepromin positive and lepromin negative consanguineous persons ($p<0.001$).

DISCUSSION

From these studies it may be concluded that there is an altered immunological response in lepromatous patients. It is also possible that such alterations may not be uniform in all cases because not all responded in the same way even when all the patients were untreated. There is a variation in the immunological response from marked deficiency to those near normal values, always with a low capacity of lymphoblastic transformation.

The study of healthy lepromin-negative consanguineous subjects yielded results similar to those observed in lepromatous patients, and statistical analysis showed no meaningful differences between the values of these two groups. This strengthens the hypothesis of the existence of a predisposing genetic factor in lepromatous leprosy patients. This we regard not as being provoked by the presence of the leprosy bacillus but existent prior to parasite contact and therefore probably of genetic origin ([26]).

Finally it is worth pointing out that lymphocyte cultures without the addition of stimulants do not present significant differences between those from lepromatous patients, normal controls and consanguineous subjects. The immunologic deficit that we have pointed out seems to lie in the inabili-

ty of lymphocytes to react adequately in the presence of antigenic substances or nonspecific stimulants.

Further research in this field may provide answers to the questions of whether the immunologic defect is actually of a genetic origin and if so, where it lies. With this aim we have begun the culture of lymphocytes in a control group of healthy lepromin-negative subjects as an extension of this study.

SUMMARY

We have cultured lymphocytes from ten lepromatous leprosy patients, ten lepromin-negative consanguineous, ten lepromin positive consanguineous, ten healthy lepromin positive control subjects and ten healthy lepromin-negative control subjects and have found a percentage of lymphoblast transformation to phytohemaglutinin and to lepromin, which is the same in lepromatous and in lepromin-negative consanguineous persons with a significant statistic difference from that of control subjects. This variation in the immunological response of lepromatous and lepromin-negative consanguineous persons seems to lie in the inability of lymphocytes to react adequately with lymphoblast transformation in the presence of antigenic substances or nonspecific stimulants.

RESUMEN

Hemos cultivado linfocitos de diez pacientes con lepra lepromatosa, diez consanguíneos lepromino-negativos y diez consanguíneos sanos, habiendo encontrado que el porcentaje de transformación a linfoblastos frente a fitohemaglutinina y lepromina es el mismo en lepromatosos y en individuos consanguíneos lepromino-negativos, pero que existe una diferencia estadísticamente significativa con el porcentaje encontrado en individuos controles. Esta variación de la respuesta inmunológica en individuos lepromatosos e individuos consanguíneos lepromino-negativos parece basarse en una incapacidad de los linfocitos para reaccionar con una adecuada transformación linfoblástica en presencia de sustancias antigénicas o estimulantes no específicos.

RÉSUMÉ

On a procédé à la culture de lymphocytes provenant de dix malades lépromateux, de dix sujets consanguins négatifs à la lepromine et de dix sujets consanguins sains. Le pourcentage de transformation lympoblastique à la suite de l'exposition à la phytohémaglutinine et à la lépromine s'est révélé être le même chez les individus lépromateux et chez les personnes consanguines négatives à la lépromine. Par ailleurs, une différence statistique significative a été observée pour les sujets témoins. Cette variation dans la réponse immunologique des personnes lépromateuses et des sujets consanguins négatifs à la lépromine semble résulter d'une incapacité des lymphocytes à réagir de façon appropriée, par une transformation lymphoblastique, en présence de substances antigéniques ou de stimulants nonspécifiques.

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