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Human Macrophage Culture

The Leprosy Prognostic Test (LPT)[1]

Tarciso A. Barbieri and Walter M. Correa[2]

The behavior of human macrophages in the presence of *Mycobacterium leprae* has been studied *in vivo* and in tissue culture ([8–13, 16]). Beiguelman and Quagliato ([4]), in a study of biopsies, found that the macrophages of certain persons were able to digest the bacilli, whereas macrophages from others were not. This led them to separate people into two groups according to the capacity of their macrophages to digest *M. leprae*. Beiguelman and Barbieri ([3]) observed that, in tissue culture, the macrophages of Mitsuda-positive but not Mitsuda-negative leprous patients were capable of digesting the bacilli.

Two factors, viz., (1) the possibility that different persons might have lysing or nonlysing macrophages, and (2) the population distribution of Mitsuda-positive and Mitsuda-negative persons, led to the hypothesis that the reactional capacity could be hereditary ([2]). The Mitsuda reaction, however, is prone to errors ([2, 5]). We have tried, therefore, to establish a new and better test to determine human resistance to leprosy, based on observation of the lytic properties of cultured human blood macrophages.

MATERIAL AND METHODS

Tissue cultures with blood of normal and leprous persons. Human blood macrophages were cultured by the method described by Cameron ([6]) in a nutrient medium composed of Hanks' balanced salt solution (BSS) with antibiotics and 10 per cent of human heat-inactivated serum. The cells were cultured in Leighton tubes and the medium was changed every third day.

We studied 125 cultures, 35 of which were from patients with tuberculoid leprosy, 40 from lepromatous patients, and 50 from healthy persons. Both sexes were represented, without age restrictions. All of the persons tested were subjected to the Mitsuda test, the diseased beforé and the healthy after the tissue culture leprosy prognostic test (LPT).

Preparation of *M. leprae*, addition to macrophage cultures and reading of test. After brief centrifugation at 500 rpm, to sediment cellular debris, leprosy bacilli were obtained by washing and precipitating lepromin diluted 1:5 in equal parts of 0.2M sucrose and 1.5M KCl, and then centrifuging the suspension for 20 minutes at 2,000 rpm. The sediment was resuspended in Hanks' BSS, and a drop stained by the Ziehl-Neelsen method. On the basis of the number of organisms observed, the suspension was finally adjusted so as to have six to eight bacilli per microscopic field. The suspension was then placed in 1 ml. ampules, which were autoclaved for 30 minutes at one atmosphere pressure and kept in the refrigerator. For subsequent use the bacillus suspension was diluted by placing one drop in 10 ml. of nutrient medium.

On the sixth day of cell culture the medium of each Leighton tube was changed by that nutrient-infecting medium and the culture was reincubated at 37°C. Thereafter the nutrient medium, without bacilli, was changed every third day. Initially we observed the cells daily. Later we established the reading of the LPT on the tenth day of infection, staining the infected cultures by the Ziehl-Neelsen method. Lysis of bacilli ingested by macrophages was scored for test purposes as LPT-positive and nonlysis as LPT-negative.

RESULTS

Before the inoculation, on the sixth day

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[2]T. A. Barbieri, B.Sc., and W. M. Correa, D.V.M., Faculdade de Ciências Médicas e Biológicas de Botucatu, Caixa Postal 102, Rubião Junior, Estado de São Paulo, Brazil.

TABLE 1. *Correspondence between lepromin reactions and lytic properties of cultured human macrophages for* M. leprae.

Patients and controls	Mitsuda (Lepromin reaction)		Macrophage culture	
	Positive	Negative	LPT +	LPT −
Healthy	35	15	35	15
Tuberculoid	35	0	35	0
Lepromatous	0	40	0	40

of culture, most of the cells had the appearance of macrophages. Some appeared as blastcells, fibroblasts, and epithelioid and multinucleated giant cells. After 30 to 35 days of culture the fibroblast-like forms predominated.

The sequence of events after inoculation of a culture was as follows:

After 24 hours: The macrophages pushed the cytoplasm around the bacilli and ingested them.

After 48 hours: Almost all the bacilli were phagocytized, either as isolated organisms or as globi.

From the 3rd to the 10th day: The macrophages showed lytic or nonlytic capacity. The nonlysers presented a variable number of intact bacilli or globi in the cytoplasm. In the lysers at the 10th day the bacilli appeared thinner than normal, and broken at several points across their bodies, or even completely hyalinized.

On the 16th day: The nonlysers still showed intact bacilli. In the lysers lysis was entirely completed and bacilli could no longer be found. Most of the cells showed epithelioid morphology.

Table 1 summarizes the results of observation. The macrophages cultured from Mitsuda-positive persons, whether healthy or suffering from the tuberculoid (T) form of leprosy, proved to be lysers. Those cultured from Mitsuda-negative persons, whether healthy or afflicted with the lepromatous (L) form of leprosy, proved to be nonlysers.

DISCUSSION

The differentiation and the culture of macrophages from blood lymphocytes has been reported several times ([7, 14, 15, 17, 18]), but the long duration of our cultures was the key to establishment of the LPT. We believe that our macrophages remain without rapid differentiation in fibroblasts, because the nutrient medium is relatively poor and accordingly the cells do not grow in large numbers and the accumulation of catabolites in the nutrient medium is small.

The lepromin or Mitsuda reaction is a good prognostic tool, but it is a reaction of very slow type, which may give some false-positive ([5]), some false-negative ([2]), and some doubtful results ([12]). Also accidents can occur in persons subjected to the lepromin reaction, as noted in textbooks ([1]).

It has been pointed out in the literature ([12]) that in man the macrophages (Virchow cells) and in tissue cultures the fibroblasts ([9, 10]) are lytic or nonlytic for the bacilli, if the persons from whom they came are respectively Mitsuda-positive or negative. We have pointed out that the blood macrophages have the same capacities when cultured ([3]), and now we have established the LPT as a method for determining if a person is resistant or not to *M. leprae*.

Our LPT, we believe, represents an advance over the lepromin reaction, because it is carried out at the cellular level of resistance and not at the level of complex organism reaction, as is the case in the Mitsuda reaction. The LPT does not give false-positive, false-negative, or doubtful reactions. The test requires only 16 days for results and is easy to make.

In our opinion the LPT should be carried out to determine the prognosis and to protect the relatives and contacts of leprosy patients. We believe that physicians, nurses and other persons working in a leprosarium should have lyser macrophages, or, in other words, be LPT-positive.

SUMMARY

With minor modifications of the classic methods white blood cells are cultivated in Leighton tubes and inoculated on the sixth

day with a suspension of killed *M. leprae*. The macrophages phagocyte the bacilli within 48 hours, and after 10 days are observed to behave differently according to the persons from whom they come, being lytic or nonlytic for the bacilli.

The results of 125 observations indicated that tuberculoid leprosy patients and Mitsuda-positive healthy persons have lytic macrophages (LPT+), while lepromatous patients and Mitsuda-negative healthy persons have nonlytic macrophages (LPT—).

The leprosy prognostic test (LPT) based on blood macrophage cultures represents an advance over the lepromin reaction, because it is carried out at the cellular level. It gives no false-positive, false-negative or doubtful reactions, and the results can be read in only 16 days.

The LPT should be useful in the examination of leprosy contacts and persons who plan to work in leprosaria. In our opinion they should have lyser macrophages, as revealed by an LPT-positive test to ensure their resistance against *M. leprae*.

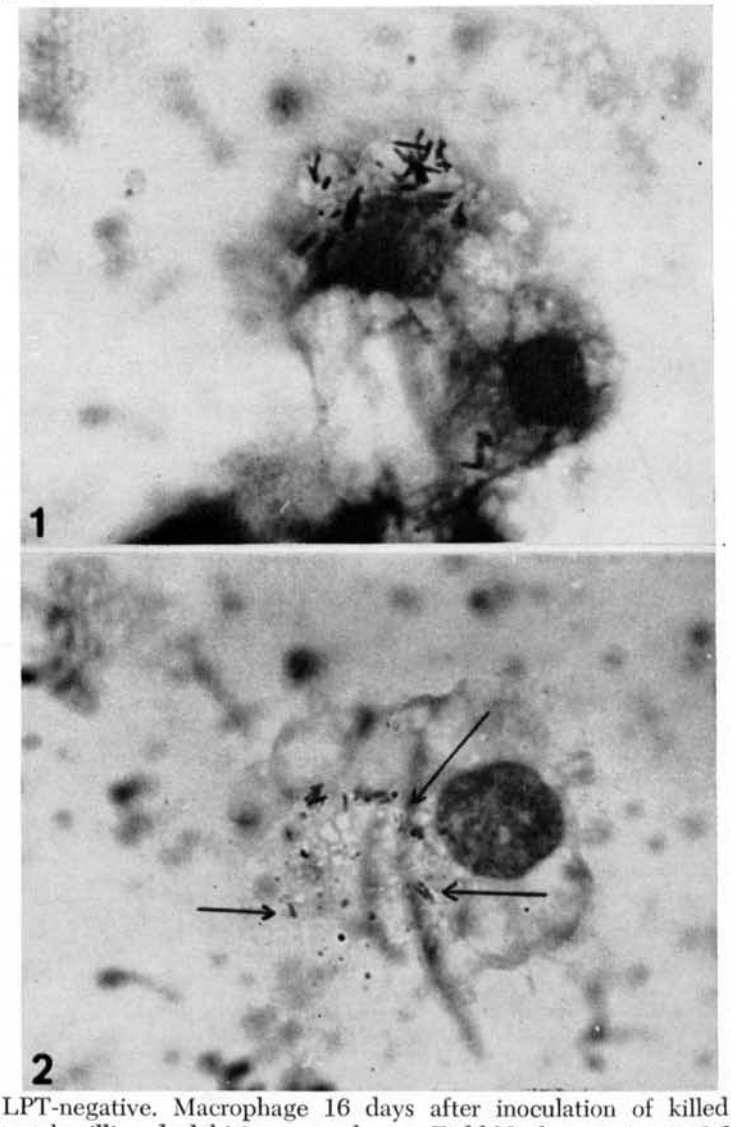

FIG. 1. LPT-negative. Macrophage 16 days after inoculation of killed *M. leprae* culture. Intact bacilli and globi in macrophages. Ziehl-Neelsen stain. × 1,250.

FIG. 2. LPT-positive. Tenth day after inoculation of macrophage culture. Note that the bacilli are thinner, and that some of them are disappearing (arrows) and fragmented (Cf with Fig. 1). Ziehl-Neelsen stain. × 1,250.

RESUMEN

Introduciendo menores modificaciones en el método clásico son cultivadas en tubos de Leighton, las células blancas de la sangre e inoculadas al sexto día con una suspensión de *M. leprae* muerto. Los macróphagos phagocytan los bacilos dentro de las 48 horas y después de 10 días se observa que se conducen diferentemente de acuerdo con las personas donde se originen, teniendo capacidad lytica o no lytica para el bacilo.

Los resultados de 125 observaciones indicaron que enfermos con lepra tuberculoide y personas sanas Mitsuda-positivas tienen macróphagos lyticos (LPT+), mientras que los enfermos lepromatosos y personas sanas Mitsuda-negativas tienen macróphagos con capacidad no lytica (LPT−).

Las pruebas para el pronóstica de la lepra (LPT) que se basan en el cultivo de macróphago de la sangre constituyen un progreso en relación con la reacción de la lepromina, porque se lleva a cabo a un nivel celular. No da reacciones falsa-positivas, falsa negativas o dudosas y los resultados pueden leerse en solo 16 días.

El LPT debería ser útil en el exámen de los contactos de lepra y de las personas que se proponen trabajar en leprosarios. En nuestra opinión ellos deberían tener macróphagos con capacidad lytica, revelado por un test LPT-positivo para asegurar su resistencia contra el *M. leprae*.

RÉSUMÉ

Utilisant les méthodes classiques légèrement modifiées, des globules blancs ont été cultivés dans des tubes de Leighton et inoculés au sixième jour avec une suspension de *M. leprae* tués. Les macrophages phagocytent les bacilles endéans les 48 heures; aprés 10 jours, on observe des différences dans le comportement de ces cellules, qui se révèlent dotées ou non d'un pouvoir lytique pour les bacilles selon les individus dont ils ont été obtenus.

Les résultats de 125 observations montrent que les malades atteints de lèpre tuberculoïde, ainsi que les individus sains avec épreuve de Mitsuda positive, ont des macrophages lytiques (LPT+). Par contre, les macrophages de sujets lépromateux et de personnes saines témoignant d'une épreuve de Mitsuda négative, ne présentent pas de capacités lytiques (LPT−).

L'épreuve pronostique de la lèpre (LPT) basée sur les cultures de macrophages du sang représente un progrès par rapport à la réaction par la lépromine, car il est pratiqué au niveau cellulaire. Elle ne livre pas de faux-positifs, de faux-négatifs, ou de réactions doubteuses. En outre, les résultats peuvent être lus après seulement 16 jours.

Le LPT pourrait être utile pour l'examen des contacts de malades de la lèpre et pour l'examen de personnes qui se destinent à travailler dans des léproseries. D'après l'opinion des auteurs, ces personnes devraient avoir des macrophages dotés de capacités de lyse, révélées par une épreuve LPT positive, afin de pouvoir s'assurer qu'elles présentent une résistance contre *M. leprae*.

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