EXPERIMENTAL TRANSMISSION OF HUMAN LEPROSY TO LABORATORY RODENTS

A DIGEST OF EXPERIMENTS PERFORMED AND RESULTS OBTAINED FROM JULY 1959 TO DECEMBER 1962[1]

José M. M. Fernández, M.D., Augusto A. Serial, M.D. and Horacio Agüero, M.D.[2]

National University of the Litoral
School of Medical Sciences
Rosario, Argentina

At the Tokyo Congress held in November 1958, and elsewhere, K. R. Chatterjee ([1-3]) reported results of the inoculation of *M. leprae* to a hybrid black mouse obtained by crossing a male Indian house mouse (*Mus musculus*) with a female Swiss albino mouse.

As an inoculum he used a pure suspension of bacilli, particularly freed from tissue elements, obtained by differential centrifugation from fresh lepromas from untreated patients. In animals less than 15 days of age, the material was injected subcutaneously, intraperitoneally, intratesticularly and intracerebrally, in a dosage of approximately 1,000 millions of bacilli.

At the end of 6 months the results were positive in the great majority of the inoculated animals, there being in most of them a true lepromatous septicemia. Passages to hybrids of the same type gave the same positive results in a much shorter period of time.

Lepromin prepared with material from these infected animals behaved immunologically like human lepromin.

Chatterjee believed that the success of the inoculation was due largely to the use of a pure bacillus suspension devoid of all tissue residue.

EXPERIMENT I

MATERIALS AND METHODS

Animals employed.—Thanks to the kindness of Chatterjee, we were enabled to employ in this experiment the same type of hybrid as he used in his experiment. As he had done, we made all inoculations before the animals had reached 15 days of age.

Inoculation material.—Fresh lepromas were obtained from 1 untreated patient, and from 3 patients who had been treated with sulfones but who had abandoned treatment, presenting recurrent active lesions. In all cases the bacteriologic examination showed abundant bacilli, homogenous and well-stained.

The lepromas were cut into small pieces and ground in a porcelain mortar for 90 minutes with the addition of saline. The suspension was filtered through gauze and

[1] Studies carried out in the Center of Dermatologic Investigations, Department of Dermatology, in collaboration with the Department of Microbiology of the School of Biochemistry and Pharmacy, with a subsidy granted by the Division of Communicable Diseases of the World Health Organization.

[2] With the collaboration of Messrs. Osvaldo Garroq and Oscar Furlan.

centrifuged at 1,000 r.p.m. for one-half hour. The sediment was discarded and a pure bacillus suspension, controlled by smears, was obtained. This material was seeded on Lowenstein-Jensen and Petragnani media to determine if there were microbial associations present, and all the cultures were negative.

Technique of inoculation.—The approximate number of bacilli for inoculation—1,000 millions—was ascertained by nephelometry (MacFarland) in 0.25 cc. volume. All the mice were inoculated subcutaneously in the dorsal cervical area. (We now propose to try the intraperitoneal route, alone and in combination with the subcutaneous route.)

The animals were examined periodically, at intervals of 15 to 30 days, but for brevity only the results as seen every 3 to 6 months are recorded.

EVOLUTION OF EXPERIMENT I

First report, December 31, 1959 (3 months after inoculation).—Of the 42 mice used in this experiment, 3 had died. The remaining 39 had been reinoculated at different dates as stated below.

Lot 1. Nine mice (3 males and 6 females) had been inoculated 3 months and 27 days prior to this observation.

Lot 2. Twenty mice (11 males and 9 females) had been inoculated 3 months and 15 days before.

Lots 3 and 4. Twelve animals (sex unidentified) had been inoculated 11 days before.

Results obtained: The periodic examinations of these animals revealed nothing abnormal up to the time of the second report.

Second report, June 30, 1960 (8 months after inoculation).— From December 1959 to date, 7 mice had died, leaving 32 under observation.

Condition of the surviving mice: After intervals varying from 5 to 8 months following the respective inoculation, the animals of this experiment showed no abnormalities of appearance, development, bacteriology, or on histopathologic examination at autopsy. One mouse exhibited partial alopecia of the whiskers.

Third report, December 31, 1960 (from 12 to 15 months after inoculation).—On this date 26 animals survived: 11 males and 15 females. Except for the somewhat decrepit aspect due to age, no abnormal symptoms were observed, although there was a partial or total alopecia of the whiskers in a few of the females. Autopsy of the animals that died showed no signs of infection.

Fourth report, March 28, 1961 (from 15 to 18 months after inoculation).—At this time 18 animals survived: 7 males and 11 females. There was partial alopecia of the whiskers in 4 females.

Fifth report, November 21, 1961 (from 21 to 29 months after inoculation).—Fourteen animals, 5 males and 9 females, survived at this time. Apart from the decrepit condition due to age, no abnormal symptoms were seen except alopecia of the whiskers in 4 females. No abnormality was observed in the autopsies performed.

Sixth report, May 7, 1962 (from 26 to 34 months after inoculation).—Three animals, 1 male and 2 females, still survived at this time, very decrepit but without abnormal symptoms worthy of note. They

were sacrificed on this date and autopsied, thus terminating the experiment.

EXPERIMENT II

This experiment differs from the previous one with respect to the origin of the hybrid animals used, and in the age at which they were inoculated. The purpose was to find out whether by using hybrids of different origin than those of Chatterjee we could obtain results similar to his. With respect to the modification of the age factor, this was due to unavoidable circumstances.

MATERIALS AND METHODS

Animals used.—The hybrid animals used in this experiment were obtained by crossing DBA and C58 male mice with Swiss albino females. This crossing produced a mouse of dark gray color with black eyes, and the animals developed without difficulty.

Contrary to Experiment I, in which we inoculated only mice below 15 days of age, in this instance we inoculated older animals, up to 27 days of age.

The other aspects of the technique of inoculation—inoculation material, route of inoculation, etc.—did not differ from those of Experiment I.

Inoculations.—The inoculated hybrids were divided into two groups according to the origin of the father.

Group A (hybrids from DBA father): Sixty-four such animals (34 males and 30 females) were inoculated, using the dorsal subcutaneous route on dates ranging between May and September 1959. Two uninoculated mice (1 male and 1 female) which were housed in the same cage with the inoculated ones were included in this group.

Group B (hybrids from C58 father): This group consisted of 14 hybrids (5 males and 9 females), also inoculated by the dorsal subcutaneous route in July and September 1959.

As in Experiment I, these animals were examined periodically every 15 or 30 days, but for the sake of brevity only the results observed at the longer intervals are given.

EVOLUTION OF EXPERIMENT II

First report, December 31, 1959 (3 to 7 months after inoculation).—In the periodical examination we found, in the later part of October, in the animals of Group A, about 5 months after the inoculation, an alopecia consisting of falling of the whiskers in the males and falling of the whiskers and plaques of alopecia with regular borders located on the head in the females.

Second report, June 30, 1960 (from 6 to 12 months after inoculation.)—In Group A there were 53 surviving mice, 31 males and 22 females; and in Group B there were 9 survivors, 3 males and 6 females. The condition of these animals was as follows:

Group A: The general condition of these animals was normal with respect to appearance, development and activity. The only symptom of abnormality noted was a peculiar alopecia of the whiskers and the face which affected 26 (i.e., 40.6%) of the 64 inoculated animals. This condition was also observed in the 2 uninoculated mice which were housed in the same cage. The process affected the whiskers of the

males and the whiskers and hairs of the face of the females. The phenomenon appeared at the beginning of the fourth month after the inoculation, affecting at that time 25 per cent of the 64 inoculated mice and both of the uninoculated ones housed with them.

Group B: In 6 animals of this group of 9 there was noted partial or total alopecia of the whiskers.

Control group: Nothing particular was to be seen in the uninoculated mice.

Third report, December 31, 1960 (from 15 to 18 months after inoculation).—Group A: On this date 45 animals survived: 27 males, decrepit but without visible lesions, and 18 females also decrepit, 10 of which showed partial or total alopecia of the whiskers. Autopsies of the animals that had died spontaneously revealed nothing abnormal.

Group B: Out of the 14 mice inoculated at the start of the experiment (15 to 16 months earlier) only 6 survived: 1 male of normal aspect and 5 females, all with total alopecia of the whiskers. Autopsies showed nothing particular.

There was nothing particular about the condition of the controls.

Fourth report, August 20, 1961 (24 to 27 months after inoculation).—At this time the condition of the animals was as follows:

Group A: There were 17 survivors: 8 males and 9 females. Of these, 2 females showed total alopecia of the whiskers, and also plaques on the face, and another 2 showed only total falling of the whiskers. The remainder showed nothing abnormal except decrepitude due to age. From the beginning of the experiment up to date, out of the 64 mice which originally comprised this group, 22 (or 34.4%) showed alopecia.

Autopsies were performed on 18 mice, and in no case were lesions worthy of mention found. In 31 mice which died due to cannibalism, autopsies were not performed.

Group B: Out of the 14 mice of this group only 1 female survived, with total alopecia of the whiskers. Of the other 13, 10 had died due to cannibalism and the other 3 were autopsied. No particular symptoms were found in the autopsy. From the beginning of the experiment up to date, 5 mice (or 35.7%) out of the total number inoculated, showed alopecia.

Fifth report, November 15, 1961 (27 to 30 months after inoculation).—Group A: Five mice survived: 1 male and 4 females. Nothing special with respect to the previous examinations. These animals were sacrificed on this date for autopsy. Group B: The only survivor, a female, recorded in a previous examination, had died.

SYNTHESIS OF EXPERIMENTS I AND II

Experiment I was started with a lot of 39 hybrid mice, obtained by crossing an Indian male mouse with an albino Swiss mother, the

original breeders of which (2 males and 4 females) had been sent to us by Chatterjee of the School of Tropical Medicine of Calcutta. The animals were inoculated between September and December 1959, according to the procedure recommended by Chatterjee, with material from lepromatous patients in full activity.

The periodic examination of these animals, which in the last 3 survivors was performed 34 months after the inoculation, showed no evidence of the occurrence of leprosy infection. In none of them did we find the clinical and bacteriologic symptoms as described by Chatterjee, nor was anything particular found in the autopsies.

Experiment II, begun in May 1959, differed from the first experiment only in that the hybridization was produced by crossing a DBA father (Group A), and a C58 father (Group B), with Swiss albino mothers. Furthermore, the animals were not inoculated within the first 15 days of life but a little later. In other respects the experiment —inoculation material, route of inoculation, etc.—differed in no way from the first experiment. In Group A, 64 animals (34 males and 30 females) were inoculated, all by the subcutaneous dorsal route, on dates ranging between May and September 1959. In Group B, 14 mice (5 males and 9 females) were inoculated by the same route and with the same material in July 1959.

The periodical examination of the animals, which in the last 5 survivors was prolonged up to the 30th month after the inoculation, showed no clinical, bacteriologic or anatomopathologic symptoms such as described by Chatterjee.

The only noteworthy fact to which attention was drawn in both experiments was the appearance of an alopecia of the whiskers and head which affected the females. This condition will be the subject of a special report.

SUMMARY

Our attempts at transmission of human leprosy to hybrid mice, following the method recommended by Chatterjee, have produced only negative results.

Although it is true that in these experiments we endeavored to follow strictly the recommendations of that author, with respect to both the characteristics of the animals used, the inoculum employed, and the technique of inoculation, our failure is no basis to invalidate the results obtained by Chatterjee.

In spite of the fact that the method is not complicated, it may happen that imponderable factors had intervened in our investigations which may explain our failure.

It follows, therefore, that there is need that other investigators should repeat these experiments, in order to gather conclusive evidence to permit a definitive opinion with respect to the validity of the method.

RESUMEN

Nuestras tentativas de transmisión de la lepra humana a ratones híbridos, siguiendo el método preconizado por Chatterjee, han resultado negativas.

Si bien es cierto que en estas experiencias hemos procurado ajustarnos estrictamente a las recomendaciones de este autor, tanto en lo que se refiere a las características de los animales utilizados, como en lo que respecta al inóculo inyectado y a la técnica de inoculación, nuestro fracaso no nos autoriza a invalidar los resultados observados por Chatterjee.

Esto significa que será menester que otros investigadores repitan estas experiencias para poder recoger elementos de juicio que autoricen a emitir una opinión definitiva acerca de su validez.

RESUMÉ

Nos essais de transmission de la lèpre humaine à des souris hybrides, suivant la méthode recommandée par Chatterjee, n' a produit que de résultats négatifs.

Quoiqu'il soit certain qu'au cours de ces essais nous nous sommes efforcés de suivre strictement les recommandations de cet auteur, tant en ce qui concerne les caractéristiques des animaux d'expérience, l'inoculat utilisé et la technique d'inoculation, notre échec n'invalide pas les résultats obtenus par Chatterjee.

En dépit du fait que la méthode n'est pas compliquée, il se peut qu'au cours de nos recherches des inpondérables soient intervenus qui peuvent expliquer notre échec.

Il s'ensuit dès lors que la répétition de ces expériences s'impose menée par d'autres chercheurs, afin de réunir une évidence concluanté qui permettrait de se faire une opinion définitive quant à la validité de la méthode.

REFERENCES

1. Chatterjee, K. R. Experimental transmission of human leprosy in laboratory-bred selected hybrid black mice and Syrian hamsters. Bull. Calcutta Sch. Trop. Med. **6** (1958) 82-85.
2. Chatterjee, K. R. Experimental transmission of human leprosy to an inbred strain of black mice. Trans. VII Internat. Congr. Leprol., Tokyo, 1958; Tokyo (Tofu Kyokai), 1959, pp. 67-75.
3. Chatterjee, K. R. Experimental transmission of human leprosy infection to a selected, laboratory-bred hybrid black mouse. Internat. J. Leprosy **26** (1958) 195-204.