ENHANCED SEROLOGIC RESPONSE OF LEPROMATOUS PATIENTS TO ANTITYPHOID VACCINATION [1, 2]

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A prominent feature of lepromatous leprosy is the presence of great numbers of Hansen bacilli in large mononuclear cells. These cells are in intimate contact with antigens from the leprosy bacilli, under conditions similar to those brought about by the inoculation of Freund adjuvant ([7]), whose potentiating effect on all kinds of antigens is well known.

The adjuvant action of mycobacteria and smallpox virus has been demonstrated for other antigens that may be present simultaneously. Lewis and Loomis ([11]) were the first to discover that the increased production of antibody against sheep erythrocytes in tuberculous guinea-pigs should be attributed to the tuberculous infection. Denney and Hopkins ([3]) described the activation of specific leprosy lesions following smallpox vaccination and noted later a regression of leprosy in a number of the patients. It is now known that the adjuvant action of mycobacteria results in improvements of antibody production, enhancements of tuberculin-type sensitizations, and increases in resistance. Hanks ([8]) has analyzed, in terms of challenge dosage, the remarkable enhancement of resistance against murine leprosy in rats following immunization with killed *M. lepraemurium* combined with BCG for its adjuvant effect. In another publication Hanks ([9]) has summarized pertinent literature and concepts on the use of adjuvants to improve immunologic response in human leprosy.

It is known that leprosy patients sometimes react violently after smallpox vaccination ([3]), with recurrence of specific lesions and severe symptoms of general involvement. After antityphoid inoculation, however, Johansen and Munday ([10]) did not observe activation of lesions.

Lepromatous lesions, with leprosy bacilli and their adjuvants, could have a potentiating effect on antigens not related to acid-fast bacilli. If this holds true, the serologic response to vaccination would be quantitatively greater than that observed in normal persons. In the case of antityphoid vaccination agglutinin titration before and

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after vaccination should provide the desired information. The investigation here reported demonstrates that lepromatous patients do have a higher capacity for agglutinin formation than controls when submitted to the same schedule of antityphoid vaccination.

MATERIALS AND METHODS

1. *Persons tested.*—Lepromatous patients and soldiers were first tested for the presence of antityphoid agglutinins. No titer higher than 1:20 was found in any sera tested before inoculation.

Sixty lepromatous patients, 44 with active infection and 16 with regressive lesions, comprised the experimental group. Their mean age was 45 years (from 17 to 63); the average duration of the infection was 17 years (from 2 to 37). Sixty military recruits comprised the control group. They had not received any vaccination for enteric fevers. Their mean age was 19 years (from 17 to 26). Their health was good.

2. *Typhoid vaccine.*—Formalin-killed typhoid vaccine, as described by Siler ([13]), was used. It was prepared by growing the strain Panama 58 (Watson type of *Salmonella typhi* obtained from the Walter Reed Army Institute of Research, Army Medical Center, Washington, D.C.) on Roux flasks with bacto-beef infusion agar. After incubation for 24 hours at 37°C, the growth was harvested with buffered saline. The bacterial content was determined by plate counting, with the use of MacConkey medium, and the suspension was diluted to a standard strength of 10^9 organisms per ml. The organisms were killed by addition of 0.1 per cent formalin to the diluted vaccine.

The vaccine was dispensed in 10 ml. flasks and released for inoculation after the controls had shown it was sterilized properly by formalin and free from toxic effects for 250 gm. guinea-pigs inoculated with 1.5 ml. of the vaccine subcutaneously. Two doses of the vaccine, 0.5 and 1.0 ml., were given subcutaneously, with an interval of 8 days.

3. *Human sera.*—Bleeding was carried out before vaccination and 21 days after the second dose of vaccine. Blood was collected in sterile tubes and kept at low temperature on its way to the laboratory, where sera were lyophilized in volumes of 2 ml. to permit paired comparisons under the same conditions. The lyophilized sera were reconstituted with distilled water and left overnight in the icebox.

4. *Agglutinin titration.*—H antigen ([6]) was prepared from 24-hour cultures of the actively motile strain H-901 of *Salmonella typhi* on 2 per cent sugar-free beef infusion agar, contained in Roux flasks. The growth was suspended in buffered salt solution at pH 7.6 containing 0.2 per cent of formalin. The suspension was adjusted to a turbidity of 5 on the MacFarland nephelometer scale. It was stored at room temperature for 48 hours, tested for sterility, and kept in the icebox.

The H agglutination tests were set up in rows of 12 round-bottomed tubes of 12 × 75 mm. size. To two-fold dilutions of sera in saline, 0.5 ml. per tube, 0.5 ml. of the H antigen was added. The tubes were shaken, incubated in the water-bath at 52°C for 4 hours, and then stored in the icebox overnight for reading next morning.

Each set of reactions comprised paired sera from the same individual, collected before and after vaccination. Agglutinin endpoints were assigned to the dilution showing 50 per cent agglutination, in accordance with the recommendations of Felix and Bensted ([5]).

RESULTS

The frequency of agglutinin titers for H antigen of *Salmonella typhi* for both groups is presented in Table 1.

The distribution of titers was different in the two groups: two-thirds of the lepromatous group had log titers of $\bar{3}.1$ or higher, the

TABLE 1.—*Frequency of H agglutinin titers among lepromatous patients and normal controls after inoculation with typhoid vaccine.*

H agglutinin titer (serum dilutions)	Lepromatous patients	Normals (control group)
−log		
1.6	3	2
1.9	2	7
2.2	4	4
2.5	6	7
2.8	6	17
3.1	11	9
3.4	14	10
3.7	7	2
4.0	5	2
4.3	2	0
Total	60	60
Log geometric mean titer	$\bar{3}.09$[a]	$\bar{2}.79$*

[a]The difference between the mean titers is significant at the level of $P < 0.01$ by t-test.

log of the modal class being $\bar{3}.4$, while in the control group approximately two-thirds had log titers of $\bar{2}.8$ or lower, the log of the modal class being $\bar{2}.8$.

The computed values of the geometric mean titers expressed were $\bar{2}.79$ for the controls and $\bar{3}.09$ for the lepromatous group. The difference is statistically significant by t-test at a level of <0.01.

DISCUSSION

The possible enhancing effect of *M. leprae* on antibody response to nonrelated antigens was pointed out by Hanks ([8, 9]) in a review of literature and of concepts on the adjuvant action of mycobacteria.

As noted above the work of Lewis and Loomis ([11]) showed an enhancement of response to sheep-erythrocytes in guinea-pigs, induced by tuberculous infection, and Denney and Hopkins ([3]) described an adjuvant effect on *M. leprae* antigen caused by smallpox vaccination.

In a recent study of the influence of leprosy on cutaneous and serologic reactions to *Candida albicans,* Buck and Hasenclever ([2]) submitted evidence of serologic hyperreactivity in cases of lepromatous leprosy, but not in patients with the tuberculoid type of the disease. They defined serologic hyperreactivity in terms of quantitative differences in agglutination titers observed among lepromatous patients as compared with normal controls. This hyperreactivity was demonstrated also in relation to isoagglutinin titers to A and B blood group antigens, a fact suggesting that the results obtained with *Candida albicans* represented a partial picture of the behavior of lepromatous patients inoculated with any antigen.

This was the case in lepromatous patients inoculated with typhoid vaccine in our studies. Their response, as judged by H agglutinin titrations, was significantly higher ($P < 0.01$) than responses observed in the uninfected control group.

The higher capacity for antibody formation in lepromatous patients may be related to the presence of *M. leprae* in the lesions, producing a permanent adjuvant condition for antigens, not only from mycobacteria, but from sources not related antigenically.

As a whole our data and those found in the literature point in one direction only, viz., a status of hyperergy in lepromatous patients. The serologic findings give an indication of the magnitude of the antigen-antibody reactions occurring *in vivo*.

Nearly all discussions of immunologic states in lepromatous leprosy emphasize the cutaneous anergy and overlook entirely the remarkable serologic responses to *M. leprae* itself. For the sake of perspective, attention is directed to the fact that in lepromatous patients there may be found: circulating antigen ([12]), high levels of antigen-antibody complexes ([1]), and high levels of antibody ([1]). If it is true that continuous interactions between antigen and antibody can create physiologic disturbances, lepromatous leprosy and its reactional states deserve more adequate investigation from this point of view.

SUMMARY

By inoculation of antityphoid vaccine and titrations for H agglutinins, the capacity for antibody formation against antigens not related to the acid-fast bacteria was investigated in a group of 60 lepromatous patients and 60 soldiers. In both groups no titer higher than $10^{-1.3}$ was found previous to vaccination.

The values of the geometric mean titers expressed in logs were $\bar{2}.79$ for the controls and $\bar{3}.09$ for the lepromatous group. The difference is statistically significant by t-test at a level of <0.01.

The higher capacity for antibody formation found in lepromatous leprosy favors the formation of circulating antigen-antibody complexes. The biologic role of such complexes in leprosy, as a cause of tissue lesions, should be further investigated.

RESUMEN

En un grupo de 60 pacientes lepromatosos y 60 soldados, fué investigada la capacidad para la formación de anticuerpos contra antígenos no relacionados a las bacterias acido-alcohol-resistentes, por inoculación de vacuna antitífica y titraciones para las aglutininas H. En ambos grupos no fué encontrada previa a la vacunación, un título mas alto que el $10^{-1.3}$.

Para el grupo lepromatoso los valores de los títulos medios geométricos expresados en logaritmos fueron $\bar{2}.79$ para los controles y $\bar{3}.09$ para el grupo lepromatoso. La diferencia fué estadísticamente significativa por el t-test al nivel de < 0.01.

La mayor capacidad para la formación de anticuerpos encontrada en la lepra lepromatosa, favorece la formación de complejos circulantes antigenoanticuerpos. El

papel biológico de tales complejos en la lepra, como una causa de lesiones tisulares, debe ser ulteriormente investigada.

RÉSUMÉ

Le pouvoir de former des anticorps contre des antigènes sans relation avec les bactéries acido-résistantes a été étudié chez 60 malades lépromateux et chez 60 militaires au moyen de l'inoculation de vaccin anti-typhique et de titrations des agglutinines H. Aucun titre supérieur à $10^{-1.3}$ n'a été décelé chez l'un ou l'autre groupe préalablement à la vaccination.

Exprimés en logarithmes, la moyenne géométrique des titres était $\bar{2}.79$ chez les contrôles et $\bar{3}.09$ chez les lépromateux. La différence est statistiquement significative au t-test pour un seuil de probabilité inférieur à 0.01.

La capacité de former des anticorps, qui s'est revélée plus développée dans la lèpre lépromateuse, favorise la formation de complexes antigènes-anticorps circulants. Le rôle biologique de tels complexes dans la lèpre, en tant que cause de lésions tissulaires, devrait faire l'objet d'études ultérieure.

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