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Use of Human Skin to Demonstrate Antinuclear Antibodies in Lepromatous Leprosy Patients[1]

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In leprosy patients, multiple disturbances of humoral and cellular immunity have been described. Apparently they are related to the patient's susceptibility to infection with *Mycobacterium leprae* and are responsible for the clinical varieties of the disease ([3, 5–17]). Among the altered humoral immune responses in lepromatous leprosy, the presence of rheumatoid factor, C-reactive protein, hypergammaglobulinemia, abnormal antibodies, and immune complexes have been demonstrated ([8]). In addition, autoimmune mechanisms manifested by the presence of antibodies against nuclear, thyroid, mitochondrial, gastric parietal cells and smooth muscle antigens have been described by several authors ([1, 2, 9]). This study shows that the use of the patient's skin, a device previously utilized to demonstrate hidden antinuclear antibodies (ANA) in systemic lupus erythematosus ([19]), reveals a higher incidence of ANA in the patient with lepromatous leprosy when compared with the conventional rat liver substrate.

MATERIALS AND METHODS

Thirty-five patients with lepromatous leprosy attending the Instituto Dermatologico de Guadalajara, Mexico—26 males and 9 females from 18 to 77 years of age (average 48.4), who were under treatment with dapsone (diaminodiphenyl sulfone, 100 mg daily from months to years; 15.9 years average)—were studied.

Ten ml of venous blood was drawn from each patient and allowed to clot. The sera were collected and kept frozen at −20°C for one or two weeks until the tests were done. As controls, five sera obtained from healthy volunteers were also used. From each patient a 3 mm punch skin biopsy was obtained, incorporated into tissue gel (Tissue-Tek, Clay Adams), and cut in sections 5 μ thick in a cryostat (American Optical). As a control, healthy human skin obtained from two patients undergoing plastic surgery procedures was also incorporated into tissue gel and cut in the same way. All of these sections were used as substrate for immunofluorescence reactions to detect ANA by the indirect immunofluorescence method according to Coons and Kaplan ([4]). Sections from rat liver were also used as antigenic substrate in order to compare the ANA affinity for nuclei of diverse origins. Sera from patients and controls were diluted 1:4, 1:16, 1:64, and 1:256 in phosphate buffered 0.15 M NaCl (PBS). Human skin and rat liver tissue sections, previously fixed for 10 min in acetone, were incubated for 30 min with the serum dilutions at 37°C in a humidity chamber. After washing for 5 min with gentle agitation in PBS, the slides were covered either with fluorescein-labeled rabbit anti-IgG, IgM, C3, or C1q sera (OKTB-05, OKTC-05, OKTD-05, OKTM-05) (Hoechst, Behring Institute, Germany), diluted 1:15. They were incubated for 30 min at 37°C, washed as described, dried, and covered with 10% glycerin in PBS. Sections were observed in an ultraviolet microscope (Zeiss).

RESULTS

The results are shown in The Table. The immunofluorescence reactions performed with the sera and human skin biopsies (autologous from each patient and those obtained from healthy people) gave 28 out of 35 positive ANA reactions for IgG class at 1:4, 1:16, and 1:64 dilutions with different

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THE TABLE. *Comparative results for the presence of antinuclear antibodies in the sera of 35 patients with nodular lepromatous leprosy, utilizing as antigenic substrate human skin and rat liver sections.*[a]

Serum anti-	Human skin[b]					Rat liver				
	Dilution	Positive ANA (cases)	Reactivity pattern of positive cases			Dilution	Positive ANA (cases)	Reactivity pattern of positive cases		
			Homogeneous	Annular	Granular			Homogeneous	Annular	Granular
IgG	1:4	12	3	7	2	1:4	3	3	—	—
	1:16	13	3	6	4	1:16	3	2	1	—
	1:64	3	—	2	1	1:64	0	—	—	—
IgM	1:4	21	2	10	9	1:4	1	1	—	—
	1:16	8	1	1	6	1:16	1	1	—	—
	1:64	1	—	1	—	1:64	0	—	—	—
C3	1:4	7	—	7	—	1:4	2	—	1	1
	1:16	5	—	2	3	1:16	0	—	—	—
	1:64	2	—	2	—	1:64	0	—	—	—
C1q	1:4	18	—	18	—	1:4	0	—	—	—
	1:16	10	—	10	—	1:16	0	—	—	—
	1:64	2	—	2	—	1:64	0	—	—	—

[a] The sera of five volunteers used as controls gave negative results with the immunoreagents at all of the dilutions tested.

[b] Autologous skin punch biopsy from each patient and leprosy-free human skin obtained from people undergoing plastic surgery procedures.

reactivity patterns (Figs. 1 and 2). ANA reactions of IgM class were positive in 30 out of the 35 patients, using human skin. ANA bound C3 and C1q in 14 and 30 patients, respectively, using fresh serum. When sera samples were decomplemented by heating, most of them gave a gelatinous precipitate due to hypergammaglobulinemia. Those in which no precipitate was present after heating gave negative results when treated with C3 and C1q anti-sera.

When the patients' sera were similarly tested utilizing rat liver as the antigenic substrate, the number of ANA-positive reactions decreased to only 6 cases that were positive for IgG, 2 for IgM, 2 for C3, and 0 for C1q.

The sera of five healthy volunteers used as controls gave negative results with the immunofluorescent reagents utilized at all of the dilutions tested.

DISCUSSION

Humoral immunity in lepromatous leprosy shows an abnormal reactivity pattern. In addition to hypergammaglobulinemia and circulating immune complexes, in some cases autoantibodies against a variety of tissue constituents can be found, as previously mentioned ([1, 2, 9]). The most accepted explanation for the presence of autoantibodies is that the generalized impairment of cell-mediated immunity in lepromatous leprosy also involves the T subpopulation devoted to homeostatic control of the immune response, leading to an increase in autoantibody production ([9]). However, other factors may explain the increased frequency of autoantibodies. These include tissue damage with the appearance of previously hidden antigenic determinants as a consequence of the infection, an adjuvant-like effect of *M. leprae,* a nonspecific stimulation of auto-reactive B lymphocytes by leprosy bacilli, and the induction by mycobacteria of antibodies that crossreact with self antigens ([9]).

In previous reports concerning the presence of ANA in leprosy patients, the frequency of positive cases ranged between 4.2% and 54% ([1, 2, 9, 14, 18, 20, 21]). The differences could be explained by variations in the methods employed or in the diversity of populations studied; in any case, the reactivity of these ANA for autologous nuclei was unknown.

In our study, the average of positive ANA tests to IgG, IgM, C3, and C1q was 85.7%

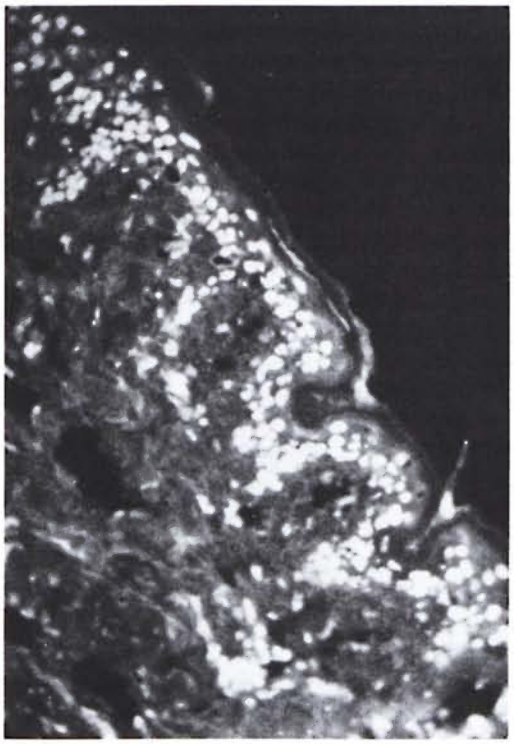

FIG. 1. Indirect immunofluorescence reaction in a skin biopsy section. ANA, IgG type, homogeneous pattern against the nuclei of the epidermis can be observed (original magnification ×100).

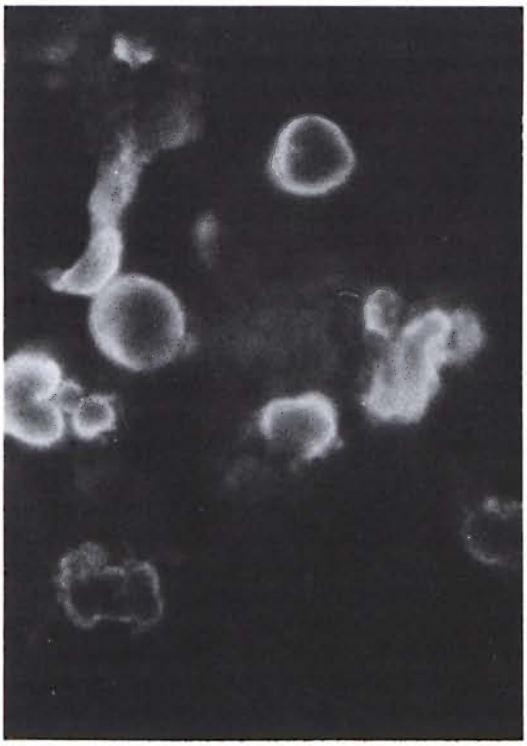

FIG. 2. Indirect immunofluorescence reaction showing ANA, IgG type, annular pattern against epidermal nuclei (original magnification ×1000).

(30 cases) when human skin was used as substrate and 27.1% (9 cases) when rat liver was utilized as substrate. Thus, it seems clear that these autoantibodies had more reactivity for species-specific antigens than against the nuclei of rat cells. Their significance in the pathogenesis of tissue damage in patients with lepromatous leprosy remains to be explained.

SUMMARY

A common finding in the sera of leprosy patients is the presence of antinuclear antibodies (ANA), but their specificity for autologous antigens is unknown. The aim of this work was to investigate the reactivity of these ANA toward the cell nuclei of human skin.

ANA were investigated in the sera of 35 patients with lepromatous leprosy by immunofluorescence reactions performed with sections of human skin biopsies (autologous from each patient and healthy human skin obtained from plastic surgery procedures), and compared with the results obtained when rat liver was used as substrate. ANA titer, immunofluorescence pattern, immunoglobulin classes (IgG and IgM) and complement-binding capability were also investigated. When human skin sections were used as substrates, 30 out of 35 patients (85.7%) gave positive ANA tests; most of them gave a 1:4 to 1:16 titer for IgG with an annular pattern and 1:4 for IgM with an annular or a granular pattern. ANA of 30 patients bound C1q and 14 bound C3. However, when rat liver sections were used as substrates only 9 out of 35 cases (27.1%) gave positive ANA tests.

These results show that human skin sections are a better substrate to demonstrate the ANA present in the sera of patients with lepromatous leprosy. Their significance in the pathogenesis of tissue damage remains to be investigated.

RESUMEN

Un hallazgo común en el suero de los pacientes con lepra es la presencia de anticuerpos anti nucleares (AAN)

de los cuales se desconoce su especificidad por antígenos autólogos. El objeto de este estudio fue investigar la reactividad de estos AAN hacia los núcleos celulares de la piel humana.

Se buscaron AAN en el suero de 35 pacientes con lepra lepromatosa por inmunofluorescencia en secciones de biopsias de piel autóloga (las biopsias de piel sana se obtuvieron cuando se practicaron operaciones de cirugía plástica en personas sanas) y los resultados se compararon con los obtenidos usando hígado de rata como substrato. También se investigaron los títulos de AAN, el patrón de inmunofluorescencia, las clases de inmunoglobulinas (IgG e IgM) y su capacidad para fijar complemento. Cuando se usó piel humana, 30 de 35 pacientes (85.7%) dieron pruebas positivas para AAN; la mayoría de ellos dió un título de 1:4 a 1:6 para IgG, con un patrón anular y de 1:4 para IgM, con un patrón anular o granular. Los AAN de 30 pacientes fijaron C1q y 14 fijaron C3. Sin embargo, cuando se usaron los cortes de hígado de rata, sólo 9 de 35 casos (27.1%) dieron pruebas positivas para AAN.

Los resultados muestran que los cortes de piel humana son mejor substrato que los cortes de hígado de rata para demostrar la presencia de AAN en el suero de pacientes lepromatosos. El significado de estos hallazgos en la patogénesis del daño tisular es un aspecto que queda por estudiarse.

RÉSUMÉ

La présence d'anticorps antinucléaires (ANA) dans le sérum de malades de la lèpre est une observation habituelle. Néanmoins, la spécificité de ces anticorps pour les antigènes autologues n'est pas connue. Le but de ce travail est d'étudier la réactivité de ces anticorps antinucléaires envers les noyaux cellulaires dans la peau humaine. Les anticorps antinucléaires ont été étudiés dans le sérum de 35 malades atteints de lèpre lépromateuse, au moyen de réactions d'immunofluorescence pratiquées sur des coupes de biopsies de peau humaine. Il s'agissait de biopsies autologues provenant de chaque malade et d'échantillons de peau humaine obtenus lors d'interventions de chirurgie plastique. On a comparé les résultats avec ceux obtenus lorsque le foie de rat était utilisé comme substrat. On a également étudié les titres d'anticorps antinucléaires, le profil d'immunofluorescence, les classes d'immunoglobuline (IgC et IgM) et la capacité de fixation du complément. Avec des coupes de tissu cutané humain comme substrats, 30 malades sur 35 (85,7%) ont fourni des épreuves positives pour les anticorps antinucléaires; la plupart ont livré des titres d'IgC de 1:4 à 1:16, avec un profil annulaire, et de 1:4 pour IgM, avec un profil soit annulaire, soit granulaire. Les anticorps antinucléaires de 30 malades liaient C1q et ceux de 14 malades liaient C3. Toutefois, lorsque des coupes de foie de rat étaient utilisées comme substrat, on a relevé des preuves positives pour les anticorps antinucléaires que chez 9 des 35 malades (27,1%).

Ces résultats prouvent que les coupes de tissu cutané humain constituent un meilleur substrat pour mettre en évidence les anticorps antinucléaires présents dans le sérum de malades atteints de lèpre lépromateuse. Leur rôle dans la pathogénèse des lésions tissulaires reste à explorer.

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