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Cold Reactive Lymphocytotoxic Antibodies in Patients with Tuberculoid and Lepromatous Leprosy[1]

Sita Naik, Bhushan Kumar, Surrinder Kaur, and Shoba Sehgal[2]

Cytotoxic antibodies against human lymphocytes that are more reactive in the cold than at 37°C were first described in 1970 by Mottironi and Terasaki ([11]). Their presence has been subsequently reported in autoimmune diseases ([14]), following a number of viral infections ([3, 4, 8]), and even in some healthy persons ([15]). Cold-reactive lymphocytotoxic antibodies (LCAs) have also been found in nonviral infectious diseases such as tuberculosis ([6]) and leprosy ([13, 16]).

A possible immunoregulatory role for these antibodies has been postulated since LCAs directed toward specific T-cell subsets have been reported in systemic lupus erythematosis (SLE) ([7]). LCAs directed against B cells have been reported to have a beneficial effect on the course of renal transplants ([4]). Although the biological stimulus for LCA production is obscure, some evidence indicates that the anti-B cell LCAs are autoantibodies to self IgM ([1, 17]). Alternatively, the increased incidence of LCAs in nonconsanguinous relatives of patients with SLE ([2]) and in inflammatory bowel disease ([5]) suggests that the stimulus may be an environmental agent, i.e., virus.

In light of the known immunoregulatory disturbances in leprosy ([9, 12]), we investigated the LCA activity of sera from tuberculoid and lepromatous leprosy patients.

MATERIALS AND METHODS

Patients. Fifty-seven consecutive patients (27 lepromatous and 30 tuberculoid) attending the Hansen's clinic of our hospital were studied. Both groups included the polar and borderline cases. Patients were classified on clinical criteria and their bacillary status as seen in slit-skin smears. The lepromatous (LL/BL) group had 16 untreated new cases and three cases with erythema nodosum leprosum (ENL). The tuberculoid (TT/BT) group included 15 untreated cases. Sera were collected from the patients and their clinical status was charted. The sera were coded and stored at −70°C until testing. Decoding was done at the end of the study.

Thirty-three control sera were collected from sex- and age-matched patients attending the surgical outpatient clinic for minor procedures. This was done in order to ensure a similar socioeconomic and ethnic group as the patients. Each control was carefully questioned, and any person with a recent history of fever or viral illness was excluded.

Methods. The sera were tested for the presence of LCAs by a modification of the standard two-stage NIH microcytotoxicity assay ([10]). The modifications included incubation at 15°C instead of 25°C and for a total of 180 min instead of 90 min. All sera were also tested under standard conditions at 25°C. Pre-tested, nontoxic rabbit serum was used as the complement source. Each serum was tested against peripheral blood lymphocytes (PBL) of 30 normal, HLA-typed individuals.

Cytotoxicity was scored as: 0 = <20% cell death, 1 = 20%–50% cell death, 2 = 51%–75% cell death, 3 = 76%–90% cell death, and 4 = >90% cell death. The individual scores of a serum against each of 30 panel cells were added, and the means were designated as the cytotoxic scores.

Thirty-five of the 57 patients were HLA typed for the A, B, C, and DR locus antigens, and their sera were tested for the presence of HBsAg by a reverse-passive hemagglutination method.

Statistical analysis was done by the Chi-squared test and the Wilcoxon rank sum test, as applicable.

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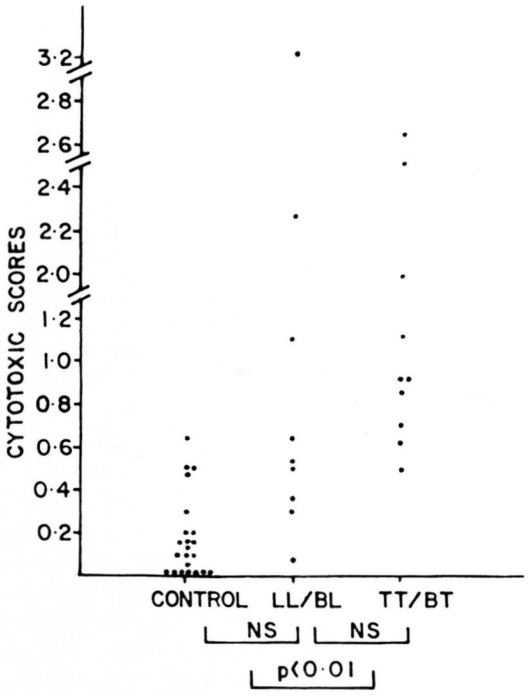
[2] S. Naik, M. D., Reader, Department of Pathology, Seth G. S. Medical College, Parel, Bombay 400012, India. B. Kumar, M.D., Assistant Professor; S. Kaur, M. D., Professor, Department of Dermatology; S. Sehgal, M.S., F.A.M.S., Professor of Immunopathology, Post-Graduate Institute of Medical Education and Research, Chandigarh 160012, India.



THE FIGURE. Distribution of individual lymphocytotoxic scores in controls and patients.

RESULTS

Eighteen of the 57 (31.6%) leprosy patients' sera had LCA activity; whereas a significantly larger proportion of control sera (22 of 33, 66.7%) had LCA activity (χ^2 = 10.42, $p < 0.05$) (The Table). There were no significant differences between the positivity rates of the tuberculoid and lepromatous sera. The cytotoxicity scores of the leprosy sera were higher than those of the control sera ($p < 0.01$) (The Figure). There was no difference in scores between tuberculoid and lepromatous sera. All the sera were nonreactive at 25°C.

The LCA positivity was similar in treated and untreated cases. There was no correlation between the cytotoxic score and the bacillary load of LL/BL cases. The three LL/BL cases with ENL did not have any LCA activity. Ten of 57 patients and 6 of 33 controls were females. Of these, only three showed LCA activity. Reactive sera did not show any correlation with any HLA antigen of the cell donor panel. Thirty-five of 57 leprosy patients were HLA typed, but the occurrence of LCA activity did not correlate with any HLA antigen of the serum donors.

Two of 35 leprosy sera tested were positive for HBsAg, and both were nonreactive for LCAs.

DISCUSSION

Our results show that low-titer, cold-reactive LCAs were present in a significant proportion of the control sera. Fewer leprosy sera had cold-reactive LCAs but when present, they had significantly high titers.

Since a large number of viral infections have been shown to stimulate LCA activity ([3, 8]), special care was taken not to include subjects with a recent history of viral illness in our control population. Similar low "background" levels of LCA activity reported in the control population from Papua New Guinea ([16]) were attributed to the higher frequency of infective episodes in tropical and subtropical climates. This explanation could be valid for our population.

The cytotoxicity was not directed against HLA determinants because: a) cytotoxicity was not seen at 25°C, at which temperature HLA antibodies of the IgG type usually

THE TABLE. *Distribution of cytotoxic scores among controls and leprosy patients.*

Score[a]	Controls (N = 33)		BL/LL (N = 27)		TT/BT (N = 30)	
	No.	%	No.	%	No.	%
0	11	33.3	19	70.4	20	66.7
0–0.19	13	39.4	0	0	0	0
0.2–0.39	5	15.2	2	7.4	0	0
0.4–0.59	3	9.1	2	7.4	1	3.3
0.6–0.79	1	3	1	3.7	2	6.7
0.8–0.99	0	0	0	0	3	10
1.0 and more	0	0	3	11.1	4	13.3

[a] (Cytotoxic score against each of 30 cell targets)/30. Individual scoring as in Serjeantson and Dry ([16]).

react; b) the majority of reactive sera were from nontransfused males with no source of allostimulation; and c) the reactivity pattern did not correlate with any of the HLA antigens in the cell panel.

The strength of reactivity of the patients' sera was significantly greater than that of the control sera. The reactivity was independent of the HLA antigen profile in the 35 patients tested. This was specifically determined in order to look for any genetic predisposition to LCA formation. The reactivities of the TT/BT and the BL/LL groups were comparable, and could not be correlated with the bacillary load or duration of treatment. LCA activity in leprosy was observed by Serjeantson and Dry ([16]) to correlate with the HBsAg positivity of LL cases. We could not attribute our results to HBsAg status, since the only two positive sera were negative for LCA.

LCA activity in a number of autoimmune conditions, especially SLE and rheumatoid arthritis, has been reported to be directed against T-lymphocyte subpopulations ([18]) and, hence, an immunoregulatory role for LCAs has been suggested. Disturbances in the T-cell subpopulations with resultant suppressor T-cell overactivity have been reported in lepromatous ([9]) and tuberculoid ([12]) leprosy. The nature of the LCAs in leprosy and their target lymphocyte have to be further defined.

SUMMARY

Fifty-seven sera from leprosy patients and 33 sera from age- and sex-matched hospital controls were tested for the presence of cold-reacting lymphocytotoxic antibodies (LCAs) at 15°C against a panel of 30 HLA-typed normal lymphocytes. Eighteen of 57 (31.6%) leprosy sera and 22 of 33 (67%) control sera showed reactivity, but the strength of reactivity of the patients' sera was significantly more than that of the control group ($p < 0.01$ by Wilcoxon rank sum test). Within the leprosy group, there was no significant difference in the reactivity of 30 tuberculoid and 27 lepromatous sera. The occurrence of LCAs was independent of the sex or the HBsAg status of the serum donor. LCA activity was not correlated with treatment status, bacillary load, or reaction state.

RESUMEN

Cincuenta y siete sueros de pacientes con lepra y 33 sueros de controles de hospital apareados en edad y sexo, se probaron para buscar la presencia de anticuerpos linfocitotóxicos frios (ALC) reactivos a 15°C contra un panel de linfocitos de 30 individuos tipificados para HLA. Diez y ocho de 57 (31.6%) sueros de los pacientes con lepra y 22 de 33 (67%) sueros control mostraron reactividad pero la potencia de reactividad del suero de los pacientes fue significativamente mayor que la del grupo control ($p < 0.01$ por la prueba de Wilcoxon). Dentro del grupo de pacientes con lepra, no hubieron diferencias significativas en la reactividad de 30 sueros tuberculoides y 27 sueros lepromatosos. La ocurrencia de ALC fue independiente del sexo o de la presencia de HBsAg en el suero del donador. La actividad de ALC no estuvo relacionada con el estado de tratamiento, la carga bacilar o el estado reaccional.

RÉSUMÉ

On a étudié des échantillons de sérum obtenus chez 57 malades de la lèpre, et chez 33 individus témoins hospitalisés, assortis pour l'âge et le sexe, en vue de déceler la présence d'anticorps lymphocytotoxiques réagissant au froid (LCAs) à 15°C, contre une batterie de 30 lymphocytes normaux déterminés pour HLA. On a observé une réaction chez 18 des 57 échantillons de sérum de lèpre (31,6%) et chez 22 des 33 échantillons de sérum de sujets témoins (67%). Néanmoins, l'intensité de la réaction était significativement plus marquée chez les malades que dans le groupe témoin ($p < 0{,}01$ par la méthode de Wilcoxon "test desrangs"). Parmi le groupe atteint de lèpre, on n'a noté aucune différence significative dans l'intensité de la réaction chez les 30 malades tuberculoïdes et chez 27 lépromateux. L'existence d'anticorps lymphocytotoxiques était indépendante du sexe, ou du statut HBsAg du sérum donneur. L'activité LCA ne présentait pas de corrélation avec le traitement, la charge bacillaire, ou l'état réactionnel.

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