serial dilutions (from 100 μg to 1 ng/ml) of leprosin ([3]) kindly supplied by Dr. J. Bahr, Department of Bacteriology, Middlesex Hospital, London. Dr. J. Brostoff kindly provided laboratory facilities for this research in the Department of Immunology, Middlesex Hospital Medical School, London. With the technique used no specific IgE could be shown, high c.p.m. values possibly being due to non-specific binding.

Our results demonstrate that serum IgE levels are increased in leprosy, as previously reported ([2, 4]), even when compared with IgE values of subjects living in the same areas and matched for ethnic and socioeconomic conditions. This would imply that the increase in IgE is linked to the disease itself and does not depend only on the presence of parasitic infections, mainly ascariasis, which are very common in tropical zones.

In previous works, IgE values were not found to discriminate between the two polar forms (LL and TT) of leprosy. However, some authors ([1, 6]) reported an increase in IgE in the LL forms, although not significant. In our study, patients with the LL form of leprosy showed significantly higher IgE levels than those with the TT form.

It is well recognized that cell cooperation is necessary for IgE synthesis and it has been suggested ([5]) that a T cell imbalance could be relevant to explain IgE overproduction in leprosy. Since we were not able to detect specific IgE antibodies to leprosin, a defect of the cells involved in the non-specific control of IgE should be suspected. This would be more evident in the LL forms of the disease, in which a T cell deficiency is more marked.

—Mario Nuti, M.D.
—Guido Rasi, M.D.
—Carla Rosa, M.D.
—Sergio Bonini, M.D.

University of Rome
Policlinico Umberto I
00161 Rome
Italy

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Salivary Immunoglobulins in Leprosy

To the Editor:

Leprosy has been known for its involvement of the oral and nasal mucosa. Recently considerable interest has been shown regarding the role of immunoglobulins in the external secretions which could be an important local agent of host defense.

In the present study, salivary immunoglobulins have been estimated in 50 patients with polar leprosy. An attempt has been made to find out their relationship, if any, with the duration of disease and tobacco intake. The diagnosis in all the patients was confirmed by clinical examination and histopathological evaluation of skin biopsies. Those individuals who had any periodontal disease were excluded from the study. A history of tobacco consumption was recorded in all cases. As controls, ten healthy,

THE TABLE. *Salivary immunoglobulins in polar leprosy.*

Groups	Number of cases	Immunoglobulins in mg percent (mean ± S.D.)		
		IgG	IgA	IgM
Control	10	15.26 ± 1.43	13.63 ± 2.83	Nil
Tuberculoid leprosy	25	4.61 ± 2.32	8.63 ± 1.63	Nil
Lepromatous leprosy	25	4.22 ± 2.18	8.65 ± 2.44	Nil

age-matched male individuals from the same socioeconomic strata were also studied.

After a mouth wash, saliva was collected from the floor of the mouth using a clean, sterilized pipette. Immediately after the collection of saliva, a trypsin activity inhibitor (Trasylol®-Bayers, West Germany) in a concentration of 500 K.I. units per ml was added. The saliva was concentrated in a dialysing bag surrounded by crystalline sucrose at 4°C. The concentrated fluid was used for qualitative detection of various immunoglobulins (IgG, IgA, and IgM) by immunodiffusion and counter-immuno-electrophoresis. Those samples which showed precipitation lines by these techniques were further subjected to quantitative assay by a radial immunodiffusion method using low standards ([1]). Colostral IgA was used as a standard for the measurement of salivary IgA. The immunoglobulin concentration of the test samples was read from the standard curve and appropriately computed to account for the concentration factor.

Qualitative detection and quantitative estimation of secretory IgA, IgG, and IgM in salivary secretions revealed that none of the samples from either the control or the leprosy subjects were positive for IgM. McClelland, *et al.* ([2]) have demonstrated IgM in salivary secretions in a concentration of 2.55 ± 0.51 mg percent. In the leprosy patients, secretory IgA and IgG were found to be significantly decreased in comparison to the control subjects ($p < 0.05$), although no significant differences in these immunoglobulins were observed between the two polar types of leprosy (The Table). The duration of the disease also did not significantly affect the levels of secretory immunoglobulins in saliva. Saha and Chakraborty ([3]) also reported decreased levels of secretory immunoglobulins in lepromatous leprosy. Scrimshaw, *et al.* ([4]) have reported high levels of secretory IgA in malnutrition which they thought to be due to proliferation of local lymphoid tissue in response to associated infections from which these individuals often suffered. Leprosy patients are frequently chronically malnourished and therefore one would expect an increase in secretory IgA instead of decreased levels. This suggests some additional factor or factors in leprosy which are responsible for decreased secretory IgA and IgG. Since leprosy bacilli do involve the oral mucosa, the decreased levels of secretory immunoglobulins might be due to involvement of salivary and mucus glands leading to pathological alterations, or these decreased levels could be due to atrophy of the local lymphoid tissues.

Tobacco with lime (Surti) is a known carcinogenic agent which not only destroys the mucosa but also alters the physiological activities of the mucosal epithelium. It has been found in the present study that the secretory immunoglobulins are not affected by tobacco intake in either the control or the leprosy subjects. Variable results were observed in individuals who consumed moderate to heavy amounts of tobacco. The changes in secretory immunoglobulins in leprosy seem therefore to be due primarily to the disease process and not related to tobacco intake.

—S. C. Gupta, M.D., F.R.C.P.
—B. Chhabra
—T. N. Mehrotra, M.D., Ph.D.
—A. K. Bajaj, M.D.
—S. N. Sinha, Ph.D., D.Sc.

Department of Pathology
M.L.N. Medical College
Allahabad (U.P.)
India

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Serological Activity of Purified Glycolipid from *Mycobacterium leprae*

TO THE EDITOR:

A readily available and chemically characterized leprosy-specific antigen would be a valuable diagnostic and taxonomic tool. Brennan and Barrow [1] found serological activity in lipid fractions from *Mycobacterium leprae*. Hunter and Brennan [3] subsequently identified a major component of the active fractions as a glycolipid resembling mycoside A from *Mycobacterium kansasii* but possessing an unique trisaccharide. Unfortunately the pure lipid had no serological activity, as measured by immunodiffusion experiments using sonicated suspensions of glycolipid.

To show immunological activity in lipids is technically difficult since they are effectively insoluble in aqueous systems. Sonicated suspensions consist of aggregates of lipid molecules, not necessarily having their antigenic determinants exposed (though hydrophilic groups would be expected to be outermost in lipids sonicated in aqueous media). In the case of the unique glycolipid derived from *M. leprae*, the antigenic determinant is presumably the carbohydrate moiety.

We have incorporated purified glycolipid from *M. leprae* into liposomes following the technique of Six, *et al.* [4] and have shown in a limited series of experiments that these liposomes are aggregated by, and form precipitates in agarose gels with, sera from leprosy patients.

Liposomes were prepared with sphingomyelin-cholesterol-dicetyl phosphate-glycolipid (2:1.5:0.2:0.1 in molar ratios) and swollen in Tris-buffered saline (0.15 M NaCl, 20 mM Tris adjusted to pH 8 with HCl). They were sonicated to produce vesicles small enough to diffuse in agarose. Agarose was 0.8% w/v in Tris-buffered saline. Control liposomes were prepared in the same way but without glycolipid. Two glycolipid preparations were used, one of about 95% purity and the other without detectable contaminants (assessed by thin-layer chromatography).

Both were obtained from supernatants of homogenates of livers and spleens of experimentally infected nine-banded armadillos [6] and were purified by column- and thin-layer chromatography. The glycolipid had identical physical and chemical properties to the material described by Hunter and Brennan [3].

Control liposomes produced no precipitation lines with any of the sera used. Liposomes with glycolipid produced precipitates with undiluted sera from three patients with active lepromatous (LL) leprosy, a pool of serum from several lepromatous patients, and serum from an experimentally infected armadillo. No precipitates were formed with 2 sera from patients with tuberculoid leprosy (BT and TT), sera from 2 mice heavily infected with *M. lepraemurium* or with sera from 2 patients with active pulmonary tuberculosis. The LL serum showing the strongest precipitation line produced a visible line when diluted 1:8 with Tris-buffered saline, with an amount of liposomes containing 9 μg of pure glycolipid. Nearly pure and pure glycolipid produced precipitates of equal intensity.

In a single experiment one serum from a