Immunologic Aspects of Lepromatous Leprosy as Related to the Immunoglobulins of the External Secretions: Salivary Immunoglobulins[1]

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Considerable interest has recently arisen concerning the immunoglobulins present in the various external secretions, which may be important local agents of the host defense mechanism in initial or recurrent bacterial, viral or other infectious processes ([1]). Recently immunoglobulin G, which is not plasma-derived but probably produced in the lower urinary tract, has been detected in the urine of lepromatous patients ([3]).

Although there are many reports on the levels of circulatory immunoglobulins in leprosy patients, little information regarding the status of immunoglobulins in various external secretions of these patients is available in the literature. The present study concerns local immunoglobulins of the oral cavity and an estimation of their levels in normal population and in lepromatous patients.

MATERIALS AND METHODS

Human materials. *Controls.* There were three types of controls, i.e., normal subjects, patients with oral malignancy, and undernourished individuals. In the normal controls, there were 23 healthy subjects including 7 females. Their ages ranged from 20 to 60 years, while their mean age was 38.2 years. Salivary secretions from ten cases of oral malignancies, including two females, served as the second control. Their mean age was 51.9 years and their ages ranged from 38 to 65 years. The oral malignancies included alveolar, postpharyngeal, tonsilar, retromolar and lingular carcinomas. One patient had a tonsilar lymphoma with bilateral metastases in his cervical lymph nodes. The third type of control consisted of 31 male subjects with severe undernutrition. Their ages varied from 25 to 65 years with a mean age of 37.5 years. Their nutritional status was assessed by the criteria of Jellife ([7]), including muscular wasting, diminished skin-fold thickness, diminished body weight, dietary history of low-protein calorie intake, total serum albumin level below 2.9 gm/100 ml, and low urinary creatinine excretion per 24 hours.

Leprosy patients. Twenty-one patients with lepromatous leprosy including six females formed the basis of the study. Their mean age was 31.6 years and their ages ranged from 22 to 58 years. They were selected from the Leprosy Home, Anand Gram, Shahdara, Delhi on the basis of clinical history, physical examination and skin biopsy ([14]). Patients with borderline lepromatous leprosy were included in the lepromatous group. All the patients were on the usual dapsone therapy.

Collection of saliva. The subjects were asked to wash their mouths with water and wait for a few minutes. Saliva was then collected as it was formed for 10 to 15 minutes. No attempt was made to accelerate the salivary flow by stimulation because Wallington *et al* ([20]) reported that any such attempt might lead to distorted results. Samples were collected from the floor of the mouth by a sterile pipette. This included the secretions of all salivary glands including mucous glands of the oral cavity. Immediately after sample collection, a trypsin activity inhibitor (Trasylol, Bayer, Germany) in a concentration of 500 KI units/ml ([11]) was added and the mixtures were stored at -20°C. On the following day the samples were concentrated ten times by dialyzing against crystalline sucrose at 4°C for 24 hours.

Immunoglobulin estimation. *Single radial immunodiffusion.* IgA, IgG and IgM were quantitated by a single radial immunodiffusion method ([12]). Monospecific goat antisera against heavy chains of human IgA, IgG and IgM and reference standards of IgG and IgM were obtained commercially from

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Meloy Laboratories, U.S.A. Secretory IgA which was purified from human colostrum by Dr. R. Thompson, Birmingham, England was used by us for reference standard for secretory IgA. Also ten samples of colostrum from Indian mothers were taken to compare their IgA levels with those in the saliva.

Rocket immunoelectrophoresis. The sensitivity of the single radial immunodiffusion technic was insufficient to estimate salivary IgG. Rocket immunoelectrophoresis was employed for this purpose ([12]). Briefly, 0.1 ml anti-IgG antiserum was mixed with 0.6% agar in veronal buffer, pH 8.6. Reference IgG standards of 426 mg, 212 mg, 106 mg, and 53 mg, along with test samples were put in several wells, and were allowed to diffuse under 80 volts and 8 mA for 17 hours. The lower limit of accurate measurement was 2 mg/100 ml.

RESULTS

IgA was the dominant immunoglobulin in all the normal salivary samples. Its mean ± (SD) level was 11.5 ± 6.5 mg/100 ml (Table 1). IgM was not detected in any sample and the mean IgG level was found to be 2.8 ± 2.0 mg/100 ml.

IgA level in 21 cases with leprosy was 5.6 ± 3.6 mg/100 ml which showed significant reduction in comparison to the normal level ($t = 3.19$; $p < 0.001$). IgM was not detected in any of the samples. IgG was detected in only 12 of 19 cases. Among the 12 samples in which it was detected, the level was less than 2 mg/100 ml in ten cases, while it was 2.0 and 3.5 mg/100 ml in the remaining two cases. Thus, the salivary IgG levels in these patients were also reduced.

DISCUSSION

The salivary immunoglobulin level is an important criterion of the status of the secretory immunoglobulins ([21]). Thus, McClelland and his associates observed raised IgA and IgM levels in jejunal juice in dermatitis herpetiformis and similar alteration of IgA level in saliva, from which they pointed to a

TABLE 1. *Immunoglobulin levels in salivary secretions of normal subjects and patients with lepromatous leprosy.*

Type of samples	Mean level IgA	IgM	IgG
	mg per 100 ml (range)		
Normal subjects (15)	11.5 ± 6.5 (2 — 47)[c]	Not detected in any sample[b]	2.8 ± 2.0 (< 2 — 11)[c]
Lepromatous leprosy patients (21)	5.6 ± 3.6 (2 — 19)[c]	Not detected in any sample[b]	Detected in 12 patients[a]

[a] In ten the IgG level was below 2 mg/100 ml; out of the remaining two, in one IgG level was 2 mg/100 ml, and in the other the level was 3.5 mg/100 ml.
[b] No precipitin line was observed when saliva and antihuman IgM antiserum were allowed to diffuse in agar.
[c] Range levels of various immunoglobulins.

TABLE 2. *Immunoglobulin levels in the salivary secretions of patients with diseases other than leprosy.*

Types of samples	Mean level IgA	IgM	IgG
	mg per 100 ml (range)		
Malnutrition (31)	21.8 ± 13.3 (6.5 — 59)[d]	Found in 2 samples[a]	3.7 ± 1.9 (< 2 — 6.5)[d]
Oral malignancies (10)	24.3 ± 4.0 (19 — 33)[d]	Not detected in any sample[c]	Found in 2 samples[b]

[a] Below 2 mg per 100 ml.
[b] IgG levels were below 2 mg per 100 ml.
[c] No precipitin line was observed when saliva and antihuman IgM antiserum were allowed to diffuse in agar.
[d] Range levels of various immunoglobulins.

widespread abnormality in the secretory immune apparatus in this disease ([11]).

The mean IgA level in the salivary secretion of 15 normal Indian subjects was 11.5 ± 6.5 (SD) mg/100 ml (Table 1), which agrees well with the studies of others. Thus McClelland *et al* ([11]) found that the average IgA in their 16 controls was 7.0 ± 2.3 mg/100 ml. The normal level of salivary immunoglobulin obtained by Wallington *et al* ([20]) was 7.1 mg IgA per 100 ml, by Lindstrom ([10]) 8.0 mg IgA per 100 ml, and those reported by Brandtzeag ([2]) were 19.4 mg IgA per 100 ml and 1.44 mg IgG per 100 ml. The mean sIgA and IgM concentration in ten samples of colostrum from Indian mothers were 301 ± 106 mg/100 ml (108 - 406 mg/100 ml) and 304 ± 57 mg/100 ml (200 - 393 mg/100 ml) respectively. Thus, the IgA content in colostrum from Indian mothers was about 30 times that of normal saliva, and this agrees with that (265 mg/100 ml) of the colostrum of the Guatemalan mothers ([19]). Therefore, our estimation of sIgA seems reliable.

The major immunoglobulin of the oral cavity is IgA which is derived from saliva. Small quantities of IgG and IgM may also originate from cervicular (represents the anatomical region between teeth and gums) fluid ([9]). In addition, IgG is found inconsistently in lower lip salivary gland secretion. In our study, the significantly decreased levels of the major secretory immunoglobulin in lepromatous leprosy patients might be due to pathologic alteration of the salivary as well as mucus glands. Also the significant reduction of IgG in them shows the diminished production of minor immunoglobulins by the periodontal tissue, which is the source of IgG and IgM. Similarly in the presence of carious lesions, the salivary antibody titers were found to be significantly lower than in caries-free subjects, and thus the onset of active caries might possibly be related to a fall in salivary antibody titer ([10]). However, it is not known precisely how the diminished production of salivary immunoglobulins is related to oral lepromatous infection.

In contrast to lepromatous leprosy, the study of immunoglobulin levels in the saliva of the patients with severe undernutrition as well as with oral malignancies showed raised IgA levels in both (Table 2). In undernutrition, the raised levels of salivary IgA may be due to the proliferation of the local lymphoid tissue in response to associated infections from which these individuals often suffer ([17]). This view is also supported by Chisholm and Mason ([5]) who found that the synthesis of salivary IgA antibody can be specifically stimulated by microorganisms proliferating in the mouth. In malignant diseases of the oral cavity, the increased levels of salivary IgA may be due to the immune response to antigens leading to the breakdown of tumor surveillance ([9]). In fulminant untreated lepromatous leprosy, the soft palate, uvula and arypiglottic folds, as well as the fauces are often involved ([18]). Recent quantitative data indicate a major excretion of *M. leprae* in the secretions from the nose, mouth and upper respiratory tract from untreated lepromatous patients ([13]). It is possible that the observed reduced salivary immunoglobulin levels in lepromatous patients might be due to the atrophy of the local lymphoid tissues.

Impairment of cellular immunity has been associated with severe undernutrition ([4, 16]) as well as with malignant diseases ([6]). Thus, the observed raised levels of the salivary immunoglobulins in these two above disorders (Table 2), which are invariably associated with the depression of cell-mediated immunity, suggest that the reduction of IgA level in the saliva of lepromatous leprosy patients is probably not related to their associated T cell functions.

Many previous studies have shown diffuse polyclonal hyper-immunoglobulinemia in lepromatous leprosy ([15]). The present study on salivary immunoglobulins in such patients demonstrated reduction of the major as well as minor secretory immunoglobulin levels. These striking opposite profiles of these two immune systems also indicate that the origin and mode of synthesis of the plasma-derived and local secretory immunoglobulins are different.

SUMMARY

A significant reduction in salivary immunoglobulins in lepromatous leprosy is recorded as compared to normal subjects. Saliva of undernourished subjects and patients with oral malignant tumors, which were studied as controls, showed an appreciable rise in IgA levels in both. It is suggested that impairment of T cell function, which is associated with lepromatous leprosy, is not

responsible for the observed low level of salivary immunoglobulins in lepromatous leprosy.

RESUMEN

Los pacientes con lepra lepromatosa presentan, en comparación con individuos normales, una importante disminución en sus niveles de inmunoglobulinas salivales. En la saliva de individuos desnutridos y en la saliva de pacientes con tumores orales malignos, los cuales se usaron como controles, se encontró una elevación apreciable en sus niveles de IgA. Se sugiere que la alteración en la función de las células T, la cual está asociada con la lepra lepromatosa, no es la responsable de los bajos niveles de inmunoglobulinas salivales observados en los pacientes con lepra lepromatosa.

RÉSUMÉ

Lorsqu'on les compare à des sujets normaux, on constate que les malades atteints de lèpre lépromateuse présentent une réduction significative des immunoglobulines dans la salive. La salive de sujets malnourris, ou de malades souffrant de tumeurs buccales malignes, étudiés comme témoins, ont montré une augmentation appréciable des niveaux d'IgA. On suggère que l'altération de la fonction des cellules T, associée a la lèpre lépromateuse, n'est pas responsable pour les taux faibles d'immunoglobulines salivaires qui ont été observés chez les malades souffrant de lèpre lépromateuse.

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