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Immunoglobulin A (IgA) in Nasal Washings and Saliva of Leprosy Patients[1]

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Many infectious agents enter the body through the mucous membranes ([29]). The attachment of bacteria to epithelial cells is critical in the establishment of an infection, whether the infection colonizes the surface or results in penetration and spread ([17, 43]). Cells located in many mucosal tissues can secrete antibodies specific to the antigens applied to them and these secretory immunoglobulins may interfere with bacterial adherence to the epithelial surface and hence limit the colonization ([3, 8, 53]). Additional mechanisms by which secretory immunoglobulins may exert their function as a first line of defense have been reviewed by Tomasi ([49, 50]) and by Waldman and Ganguly ([51]).

A role for the mucosa in the defense against *Mycobacterium leprae* infection is not yet established. Although the main source of infectious *M. leprae* is now thought to be the nose of the lepromatous leprosy patient ([13, 14, 45]), the route by which the bacillus enters the human body is unknown. The pathology of early nerve involvement in leprosy suggested entry into cutaneous nerves through the skin ([4, 20, 47]). However, other studies indicate that the upper respiratory tract and the gastro-intestinal tract are possible routes of entry of *M. leprae* into the body ([2, 34, 52]). Interest in the respiratory tract as a route of entry has been strengthened by the experiments of Rees and McDougall ([35]) which showed that immunosuppressed mice could be infected by exposure to an *M. leprae* aerosol.

Although there have been numerous reports on serum immunoglobulin levels in leprosy patients ([5, 7, 16, 19, 22, 23, 25, 30, 39, 44, 48]), there is relatively little information on secretory immunoglobulins ([40, 41, 42]). In view of the possible importance of the respiratory tract as a route of infection, we have examined the impact of *M. leprae* infection on the level of immunoglobulins in the nasal washings and saliva of leprosy patients through the clinical and histopathological spectrum.

MATERIALS AND METHODS

Patients and controls. Saliva and nasal washings were obtained from 54 leprosy patients attending the New-Case Clinic at the All Africa Leprosy and Rehabilitation Training Centre (ALERT), Addis Ababa, Ethiopia. Sera for immunoglobulin determinations were available from 50 patients. All of the patients studied were newly diagnosed patients with no known previous antileprosy treatment. They included 35 males and 15 females. The ages varied from 8 to 60 years, with a mean age of 27 years. Diagnosis and classification was carried out clinically and by histopathological examination of skin biopsies, according to the system of Ridley and Jopling ([37]). The study included tuberculoid (TT), borderline tuberculoid (BT), borderline lepromatous (BL), and lepromatous (LL) patients. The borderline group (BB) were not found in sufficient numbers to be included in this study. The controls were nonleprosy patients of similar ethnic and socioeconomic groups attending the hospital out-patient clinic. They included ten females and six males. Leprosy was excluded in the control group by a physical examination. The ages

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TABLE 1. *Total protein and IgA concentration in nasal washings and saliva of normal subjects and leprosy patients.*

	Controls (16)[a]	Leprosy patients			
		TT (7)	BT (12)	BL (13)	LL (13)
Nasal washings					
IgA, mg/100 ml	10.0 ± 1.8[b]	10.2 ± 2.2 (n.s.)[c]	14.0 ± 2.6 (n.s.)	15.9 ± 0.8 ($p < 0.01$)	15.6 ± 1.1 ($p < 0.02$)
Protein, mg/ml	1.5 ± 0.2	2.4 ± 0.9 (n.s.)	3.2 ± 0.7 (n.s.)	4.3 ± 1.1 ($p < 0.01$)	7.4 ± 1.4 ($p < 0.001$)
IgA, mg/100 mg protein	9.1 ± 2.6	6.6 ± 2.4 (n.s.)	7.1 ± 1.7 (n.s.)	8.4 ± 2.5 (n.s.)	5.2 ± 2.2 (n.s.)
Saliva					
IgA, mg/100 ml	9.4 ± 1.8	8.6 ± 1.2 (n.s.)	10.0 ± 1.4 (n.s.)	11.8 ± 1.8 (n.s.)	13.4 ± 1.4 (n.s.)
Protein, mg/ml	2.6 ± 0.31	3.0 ± 0.9 (n.s.)	2.8 ± 0.3 (n.s.)	2.3 ± 0.4 (n.s.)	3.8 ± 0.54 (n.s.)
IgA, mg/100 mg protein	5.3 ± 1.5	6.8 ± 3.5 (n.s.)	5.0 ± 1.1 (n.s.)	6.7 ± 1.4 (n.s.)	4.6 ± 0.8 (n.s.)

[a] Total number of individuals in each group. Nasal washings were not available for nine patients due to blood contamination.
[b] Mean ± S.E.M.
[c] Not statistically significant ($p > 0.05$). Each patient group is compared to the control group.

of this group varied from 14 to 40 years, with a mean age of 25 years.

Collection and analysis of specimens. Nasal washings were collected by the technique described by Rossen, *et al.* ([38]). Briefly, following instillation of 10 ml of sterile physiological saline into the nostril of the patient, the solution was held in the nose for 10 sec and then blown energetically into a sterile beaker. Each nostril was so treated three times. The total return varied from 30 to 40 ml. The collected specimens were homogenized in a Sorvall Omni-Mixer and then centrifuged at 400 *g* to remove suspended materials. The supernatant was concentrated by vacuum dialysis using a colloidal bag (Sartorius GmbH, Göttingen, Germany) to a final volume of 3 ml and stored at −70°C until assayed. Some of the nasal wash specimens from the leprosy patients, mostly from the lepromatous group, were contaminated with blood and were discarded.

Saliva was collected from the parotid glands, after stimulation with citric acid, by a suction cup kindly provided by Prof. Morten Harboe, Institute of Experimental Medical Research, Ullevaal Hospital, Oslo, Norway.

The immunoglobulin levels were quantitated by the single radial diffusion technique ([26]). Rabbit antiserum to human IgG, IgM, and IgA (colostrum) specific for heavy chains were obtained from Nordic Immunological Laboratories, Tilburg, The Netherlands. Secretory IgA used as a standard (1.29 mg/ml) was kindly proved by Prof. Morten Harboe. IgG, IgM, and serum IgA standards were obtained from Behringwerke AG, Marburg, Germany. Protein concentrations were determined by the method of Lowry, *et al.* ([24]).

Statistical analysis was performed by Student's *t* test ([46]).

RESULTS

IgA was the dominant immunoglobulin in both nasal washings and salivas. The IgG and IgM levels were too low to measure by the single radial diffusion method. IgA data are summarized along with the data on protein content of nasal washings and saliva in Table 1. These data show a significant increase in IgA levels in the nasal washings of both the BL and LL groups of leprosy patients. This immunoglobulin was also elevated in the salivas of the BL and LL groups, but this increase was not statistically significant. In the TT and BT groups there was no deviation from the normal in either the nasal washings or the salivas.

Total protein was significantly elevated in the nasal washings of the BL and LL groups. There was also an increase in the protein

TABLE 2. *Serum protein and immunoglobulin levels in normal subjects and leprosy patients.*

	Control (16)[a]	Leprosy patients			
		TT (10)	BT (14)	BL (13)	LL (13)
Total protein (g/100 ml)	6.07 ± 0.16[b]	6.01 ± 0.48 (n.s.)[c]	6.61 ± 0.24 ($p < 0.01$)	6.70 ± 0.19 ($p < 0.01$)	7.04 ± 0.45 ($p < 0.01$)
IgG (mg/100 ml)	1309 ± 77	1333 ± 117 (n.s.)	1590 ± 101 ($p < 0.05$)	1701 ± 126 ($p < 0.02$)	1928 ± 114 ($p < 0.001$)
IgA (mg/100 ml)	179 ± 14	208 ± 15 (n.s.)	248 ± 24 ($p < 0.02$)	272 ± 26 ($p < 0.01$)	291 ± 40 ($p < 0.01$)
IgM (mg/100 ml)	154 ± 8.8	150 ± 39 (n.s.)	166 ± 9.8 (n.s.)	208 ± 31 (n.s.)	197 ± 13 ($p < 0.01$)

[a] Number of individuals in each group.
[b] Mean ± S.E.M.
[c] Not statistically significant ($p > 0.05$). Each patient group is compared to the control group.

content of the salivas in the LL group, but it was not statistically significant (Table 1). The increases in protein content of the nasal washings were greater than the IgA increases. Therefore, IgA expressed as mg/100 mg protein was actually less in the patient groups than in the control group, although the differences were not statistically significant.

The data were examined for correlations between IgA levels and protein levels in both nasal washings and saliva. No correlation was observed. IgA levels in the nasal washings of the patients were also not correlated with the IgA levels in the salivas.

The total serum protein and immunoglobulin levels in healthy individuals and different types of leprosy are shown in Table 2. The total serum protein was significantly above normal in BT, BL, and LL patients, but there was no significant difference between the TT patients and the healthy controls. The serum IgG levels were also significantly elevated compared to the normal values in BT, BL and LL patients, but not in the TT patients. Similarly, serum IgA levels were significantly elevated in the BT, BL and LL groups, but not in the TT group. Serum IgM levels were significantly elevated only in the LL group.

DISCUSSION

IgA is the predominant immunoglobulin in human parotid saliva and in nasal secretions ([12, 36]) due to local synthesis of IgA ([3]). The secretory IgA present in the external secretions has special characteristics which distinguish it from serum IgA ([49]). This study confirmed the predominance of IgA in the nasal washings and parotid saliva. The most important finding in this study was the increased IgA level in nasal washings of BL and LL patients which, as a group, corresponded with an increase in the protein content (Table 1). Since the production of secretory IgA antibodies in the mucosa depends on the local availability of antigen to the mucosal immunocompetent tissue ([31, 32]), an increased IgA level in the nasal washings of lepromatous leprosy patients may be the result of their bacillated nasal mucosa ([2, 13, 14, 18, 27, 33, 45]). Alternatively, the increased IgA levels may be attributable in part to the increase in protein synthesis which accompanies nasal infections. An increase in the total protein content of nasal washings has also been found during respiratory virus infections ([38]). Another possibility leading to increased protein content of the nasal fluid during infection would be exudation from the vascular compartment. However, when analyzing individual patients we could not show a correlation between IgA levels and the protein content of either the nasal washings or the saliva.

In the saliva, both protein and IgA were elevated in LL patients but the increase was not statistically significant. This finding contrasts with reports by Saha, *et al.* ([40, 41, 42]) of significantly lower IgA in the saliva, tears, and intestinal secretions of LL patients. The low IgA levels were attributed to alterations in the salivary and mucous glands.

With regard to serum protein levels, the increases observed in borderline lepromatous and lepromatous leprosy patients in this study (Table 2) are in agreement with the findings of other investigators ([5, 30, 48]). Our results also showed increased serum IgG and IgA levels in all the leprosy patients. The increase varied according to the type of disease, being insignificant in the TT patients and highly significant in the LL patients. Serum IgM was significantly increased only in LL patients. These findings are consistent with most reports by other workers ([7, 16, 23, 39]), but differ from that of Malaviya, *et al.* ([25]), where no significant difference in the immunoglobulin levels in LL patients and nonleprosy controls was detected. The generally high level of serum immunoglobulins in lepromatous patients has been attributed to chronic stimulation of the immune system by antigens of the leprosy bacillus ([23]). Alternatively, it has been suggested that the persistent increase in the immunoglobulin levels could be due to the loss of homeostatic control by the suppressor T lymphocytes regulating immunoglobulin synthesis ([39]).

Antibodies against many of the bacteria and viruses commonly found on the mucous membranes have been detected in the exocrine secretions ([6, 36]). Such antibody responses appear to be independent of those in the serum ([31]). In leprosy the existence of serum antibodies reacting with antigens of *M. leprae* is well documented ([15, 21, 28]), but the existence of antibodies in the secretions reacting with *M. leprae* has not yet been shown. If secretory antibodies directed toward antigens of *M. leprae* can be demonstrated in leprosy patients, it will be of interest to screen for such antibodies in family contacts exposed to patients with leprosy. It is clear that both the route of antigen administration and its physical state determine whether or not a secretory IgA response is produced ([9, 31]). Although nothing is yet known about the ability of *M. leprae* antigens to elicit IgA in the nasal secretions, it is possible that titers of these antibodies in healthy family or occupational contacts may reflect the level of exposure to aerosols of *M. leprae.* On the other hand, if colonization of the nasal mucosa is required to produce an anti-*M. leprae* antibody response in the nasal secretions, then elevated titers may be a more reliable index of leprosy infection than are serum antibody levels. Further, if colonization of the nasal mucosa is an early event in the pathogenesis of leprosy ([10, 11]), then anti-*M. leprae* antibodies in nasal secretions may be useful in the detection of early leprosy infections. The possibility that IgA reacting with *M. leprae* antigens is found in nasal secretions of leprosy patients requires further investigation.

Secretory IgA deficiency, which is the most common immunoglobulin deficiency encountered, has been associated with bronchopulmonary disorders ([1]). We have found no evidence of a deficiency of secretory IgA in leprosy. Nevertheless, if the respiratory tract is considered as a possible route of infection by *M. leprae, M. leprae*-reactive secretory IgA antibodies could prove to have an important role in immunity to leprosy.

SUMMARY

IgA levels in nasal washings and saliva were determined in leprosy patients and healthy controls. In addition, serum levels of IgA, IgG, and IgM and total serum protein were analyzed. IgA levels in the nasal washings, but not in the salivas, were elevated significantly in the borderline (BL) and lepromatous (LL) groups. In the tuberculoid (TT) and borderline tuberculoid (BT) groups, the IgA levels in both the nasal washings and salivas did not differ from the controls. Total protein was also elevated in the nasal washings in the BL and LL groups. Thus, the IgA expressed as mg/mg protein did not differ significantly from the control group. However, in individual patients the levels of IgA and total protein were not correlated in either the nasal washings or the salivas. The high serum immunoglobulin and protein levels were in accord with the findings of most other workers.

RESUMEN

Se midieron los niveles de IgA en lavados nasales y en saliva de pacientes con lepra y de controles sanos. También se midieron los niveles séricos de IgA, IgG, IgM y proteínas totales. En los grupos lepromatoso intermedio (BL) y lepromatoso (LL), los niveles de IgA se encontraron significativamente elevados en los lavados nasales pero no en las salivas. En los grupos tuberculoide (TT) e intermedio tuberculoide (BT), los niveles de IgA en los lavados nasales y en las salivas fueron similares a los encontrados en el grupo control. Las proteínas totales también estuvieron elevadas en

los lavados nasales de los grupos BL y LL. Así, la IgA expresada en mg por mg de proteína fue semejante a la del grupo control. En los pacientes individuales, sin embargo, los niveles de IgA y de proteína total no estuvieron correlacionados ni en los lavados nasales ni en las salivas. Los elevados niveles de las inmunoglobulinas séricas y de las proteínas totales simplemente estuvieron acordes con los datos de otros investigadores.

RÉSUMÉ

Chez des malades de la lèpre et chez des contrôles en bonne santé, on procédé à une détermination des taux de IgA dans des lavages nasaux et dans la salive. De plus, on a analysé les taux sériques d'IgA, d'IgG, d'IgM, ainsi que des protéines totales du sérum. Les taux de IgA dans les lavages du nez étaient significativement augmentés chez les malades souffrant de lèpre dimorphe (BL) et lépromateuse (LL), mais il n'en était pas ainsi pour la salive. Chez les sujets tuberculoides (TT) et tuberculoides dimorphes (BT), les taux d'IgA, tant dans les lavages nasaux que dans la salive, n'étaient pas différents de ceux notés chez les témoins. Chez les malades des groupes BL et LL, les protéines totales étaient également élevées dans les lavages du nez. Dès lors, le taux d'IgA exprimé en termes de mg/mg de protéine, ne différait pas significativement de celui relevé dans le groupe témoin. Toutefois, chez les malades individuels, les taux d'IgA et de protéines totales ne présentaient pas de corrélation, tant dans les lavages du nez que dans la salive. Les taux élevés d'immunoglobulines sériques et des protéines observées dans cette étude sont en accord avec les observations faites par la plupart des autres auteurs.

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