

Ocular Immunoglobulins in Lepromatous Leprosy¹

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Considerable interest has recently arisen concerning the immunoglobulins of various body fluids, which may be important local agents of the host defense mechanism (4). Although there are many reports on the levels of circulatory immunoglobulins in leprosy patients (15), little information regarding the status of immunoglobulins in the ocular fluids of these patients is available. The anterior segment of the eye is most commonly affected in lepromatous leprosy (1, 5, 6, 11) but the exact role of the immunoglobulins in the lachrymal fluid and the aqueous humor in the etiopathogenesis of these ocular disorders and their levels are not known. We have, therefore, turned our attention to the study of the local immunoglobulins of the eye in lepromatous leprosy and have investigated their levels in tears and aqueous humor in control and lepromatous patients. These immunoglobulins have also been partially characterized.

SUBJECTS AND METHODS

Tears. Tears were collected from 100 control subjects without any ocular or other systemic disease. These individuals had a mean age of 33.4 years (range 1-70 years). There were 55 males and 45 females.

Tears were also collected from 34 cases of lepromatous leprosy (25 males and 9 females). Their mean age was 38.5 years (range 17-70 years). The cases were selected on the basis of clinical history, physical examination and skin biopsy (10). Patients with borderline lepromatous leprosy were included in the lepromatous group. All patients were on the usual dapsone therapy and none

were taking any corticosteroids. Tears were collected according to the method described elsewhere (12) and were stored at -20°C.

Aqueous humor. Samples of aqueous humor were collected from 44 control patients with senile cataracts. Their mean age was 55.9 years (range 35-85 years). Eight patients having lepromatous leprosy, including two borderline lepromatous cases, were studied. Of these, seven had clinically healed anterior uveitis and one was a case of active anterior uveitis with multiple nodules in the iris. Six cases with active endogenous anterior uveitis were taken as another type of uveitis. Their mean age was 30.5 years (range 14-35 years). These cases were not under steroid therapy or other immunosuppressive drugs prior to the collection of samples. Diagnosis of anterior uveitis was established by detailed clinical examination including biomicroscopy.

The samples were collected from the eyes under local anesthesia. A small stab incision was made with a keratome at the limbus and about 0.2 ml of aqueous humor was drawn into a tuberculin syringe and stored at -20°C.

Immunoglobulin estimation. IgA, IgG and IgM in tears were quantitated by a single radial immunodiffusion method (9). Monospecific goat antisera against heavy chains of human IgA, IgG and IgM and the reference standards of IgG and IgM were obtained from Meloy Laboratories, U.S.A. Purified human colostral IgA was used as IgA standard.

In one experiment, the IgA in tears and aqueous humor were characterized by using an immunodiffusion technic against anti-IgA (alpha chain) obtained from WHO and anti-IgA antisera (Nordic, Denmark). IgG in the aqueous humor was characterized against anti-IgG (gamma chain) obtained from WHO, anti-Fc (Nordic), and anti-Fab (Nordic) antisera. The precipitin lines thus obtained were compared with those shown by human myeloma IgG and IgA (Meloy Laboratories).

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RESULTS

Tears. IgA was found in all control samples. The mean IgA level was 27.8 ± 15.3 mg/100 ml in normal cases as compared to 21.4 ± 6.8 mg/100 ml in leprosy patients (Table 1). There was a highly significant difference statistically ($t = 3.3$; $p < 0.001$). In 19 cases of the leprosy group the eyes were found to be normal, clinically as well as by slit lamp examination; while 12 cases had iridocyclitis and three cases just had mild flare. The mean tear IgA level in cases without iridocyclitis was 20.7 ± 9.6 mg/100 ml. Among the 12 cases with iridocyclitis, the tear IgA level was 20.3 ± 16.1 mg/100 ml. There was statistically no significant difference between cases with or without iridocyclitis ($p = 0.40$; $t = 2.69$). IgM was detected in one normal sample while IgG was detected in eight such cases. Neither IgG nor IgM could be detected in any of the leprosy patients.

Aqueous humor. It was not possible to collect aqueous humor of normal subject for

obvious reasons. Samples were therefore obtained from patients with senile cataracts to serve as controls. IgA could not be detected in any aqueous humor samples. The mean IgG level was 7.0 ± 4.2 mg/100 ml (Table 2). In the lepromatous group only two patients who had healed uveitis had spectacularly high levels of IgA and IgG; while in six patients with anterior endogenous uveitis all had a very high IgG level, and high levels of IgA were also observed in five cases. IgM was detected in only two cases of anterior endogenous uveitis.

Immunologic characterization. Figure 1 shows that while tears gave sharp precipitin lines with anti-sIgA antiserum, they gave broad and multiple precipitin lines only with anti-IgA antiserum. However, myeloma IgA showed multiple precipitin lines only with anti-IgA antiserum but did not show any reaction with anti-sIgA antiserum. A similar pattern of precipitin reactions was obtained with IgG of the aqueous humor, wherein it

TABLE 1. Immunoglobulin levels in tears of normal people and patients with lepromatous leprosy.

| Types of samples | Mean levels in mg/100 ml (\pm SD; range) | | |
|-----------------------|---|--|---------------------------------------|
| | IgA | IgM | IgG |
| Normal subjects (100) | 27.8 ± 15.3 (1-87) | Detected in one sample ^a | Detected in 8 samples ^a |
| Leprosy subjects (34) | 21.4 ± 6.8 (1-46.2) | Detected in none | Detected in none |

^aLess than 1 mg/100 ml.

TABLE 2. Immunoglobulin levels in the aqueous humor of patients with cataracts, uveitis associated with leprosy, and anterior uveitis not associated with leprosy.

| Types of samples | Mean levels in mg/100 ml (\pm SD; range) | | |
|--|---|---------------------------------|---------------------------------|
| | IgA | IgM | IgG |
| Cataract cases (¹³) (44) | Detected in none | Detected in none | 7.0 ± 4.2 (1.5-17.6) |
| Lepromatous leprosy with uveitis (8) | Detected in two ^a | Detected in none | Detected in two ^b |
| Active endogenous uveitis cases without leprosy | 45.0 ± 8.3^c (35-55) | Detected in two ^d | 63.5 ± 23.7 (50-81) |

^a 50.0 and 52.0 mg/100 ml.

^b 94.0 and 104 mg/100 ml.

^c Detected in five cases.

^d Detected but could not be estimated due to lack of sufficient samples.

gave precipitin reactions with anti-IgG (gamma chain), anti-Fc and anti-Fab antisera (Fig. 2). Myeloma IgG gave precipitin reactions with anti-IgG and anti-Fc antisera. Figure 3 shows the presence of Fab fragment in the IgG of the aqueous humor.

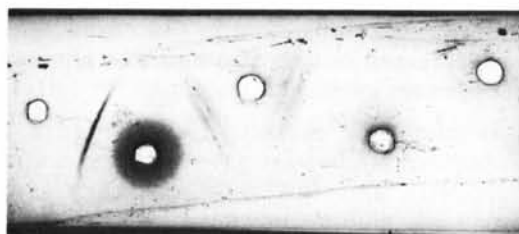


FIG. 1. The wells in the upper row contained, from left to right, anti-sIgA, anti-IgA (alpha chain), and anti-sIgA antisera. The left and right wells in the bottom row contained tear and human myeloma IgA, respectively.

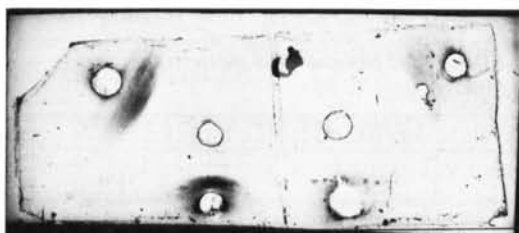


FIG. 2. Both wells in the upper row contained anti-IgG (gamma chain) antisera. The left well in the middle row contained aqueous humor; the right well in the same row contained human myeloma IgG. Both bottom wells contained anti-Fc antiserum.

DISCUSSION

IgA has been found to be the major immunoglobulin in normal tears. On the contrary, IgG is the only immunoglobulin detected in the aqueous humor of patients with senile cataracts. These findings agree fairly well with observations of most authors (2,3). Audain *et al* (3) studied the contents of immunoglobulins in the human aqueous humor from five senile cataractous eyes of five patients and found IgG in all samples, which ranged from 5 to 8.5 mg/100 ml, IgA in two samples (4 and 4.5 mg/100 ml), and IgM in none. Allansmith *et al* (2) also studied the normal concentration of the immunoglobu-

lins in the aqueous humor. Their average values were 13.6 mg/100 ml IgA, 4 mg/100 ml IgG, and 3.9 mg/100 ml IgM. IgA is the major immunoglobulin in the lachrymal fluid but the predominant immunoglobulin in the aqueous humor is IgG, which suggests that the origins of the immunoglobulins in these ocular fluids are different. The recent studies of Zirm *et al* (16) have shown that the secretory IgA is not detectable in the normal aqueous humor, which corroborates our results.

Pathologic alteration of the lachrymal glands might be responsible for the low IgA level in tears of leprosy patients (Table 1). Similar low IgA levels were also found in the tears of trachoma patients (14). We have noticed strikingly high levels of IgA and IgG in the aqueous humor of most patients with endogenous uveitis (Table 2), which might indicate increased production of these local immunoglobulins in the aqueous humor due to antigenic stimulation. Audain *et al* (3) found a similar rise of immunoglobulins in the aqueous humor of endogenous uveitis patients. The average levels of IgG, IgM and IgA in their study were 67 mg/100 ml, 11 mg/100 ml, and 16 mg/100 ml, respectively. They observed, in one patient, an IgG level of 87 mg/100 ml at the time of illness and 98

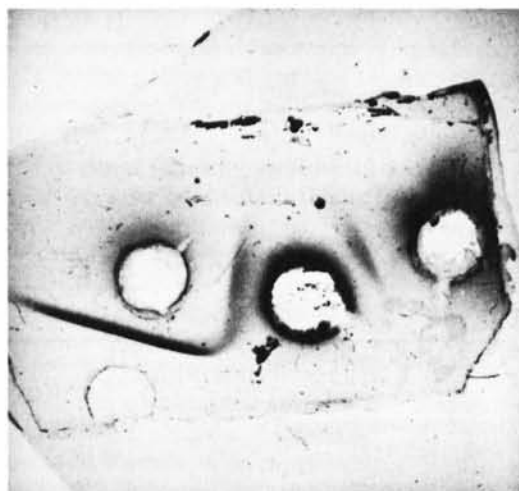


FIG. 3. The left well in the top row contained aqueous humor, while the right well in the same row contained human myeloma IgG. The central well contained anti-Fab antiserum. The bottom well in the left corner had anti-IgG (gamma chain) antiserum.

mg/100 ml one year thereafter. This might suggest continuous production of IgG in the aqueous humor of this patient. Our findings are also similar because in two lepromatous patients, who had clinically healed uveitis at the time of the study, there were raised IgA and IgG levels. It is suggested that the high levels of immunoglobulins might have been induced due to the repeated insults occurring during the course of illness and thus might inflict further damage to the uveal tract by type III reaction (8). Recently, Hobbs (7) also suggested that leprotic iritis is due to the effect of increasing quantities of bacterial protein upon the uveal tissue, which are released in the circulation through the greater efficacy of the antileprosy drugs presently employed. Thus, this study showed that there is a significant decrease of IgA and IgG levels in both ocular fluids, at least in some cases of lepromatous leprosy. It is not known precisely how this is related to the ocular lepromatous pathologic change.

SUMMARY

Immunoglobulin levels in the ocular fluids have been estimated in normal subjects and lepromatous leprosy patients. In the normal tear, IgA is the major immunoglobulin while IgG is the only immunoglobulin detected in the aqueous humor. The immunoglobulin profiles in the tear and the aqueous humor in normal subjects are different. The mean IgA level in the tears of the lepromatous leprosy group is significantly lower than in the control patients. IgA and IgG levels are raised in the aqueous humor of some leprosy cases who had suffered from uveitis in the past and also in all cases with active endogenous uveitis. Therefore, in lepromatous leprosy the pattern of immunoglobulin alteration in the tear and the aqueous humor is not parallel.

RESUMEN

Se midieron los niveles de las inmunoglobulinas en los fluidos oculares de individuos sanos y en aquellos de pacientes con lepra lepromatosa. En los individuos normales, la IgA es la inmunoglobulina más abundante en las lágrimas mientras que la IgG es la única inmunoglobulina detectable en el humor acuoso. El nivel medio de la IgA en las lágrimas de los pacientes con lepra lepromatosa es significativamente más bajo que el de los individuos control. En algunos de los pa-

cientes con lepra que han tenido uveitis en el pasado y en aquellos con uveitis endógena activa, los niveles de IgA y de IgG en el humor acuoso se encuentran elevados. Por lo tanto, en los pacientes con lepra lepromatosa, la alteración en los niveles de las inmunoglobulinas de las lágrimas no es paralela a la alteración encontrada en el humor acuoso.

RÉSUMÉ

Chez les sujets normaux et chez les malades atteints de lèpre lépromateuse, on a procédé à la détermination des taux d'immuno-globulines dans les liquides oculaires. Dans les larmes normales, l'IgA constitue l'immuno-globuline principale que l'on peut détecter dans l'humeur aqueuse. Les profils d'immuno-globuline dans les larmes et dans l'humeur aqueuse chez des sujets normaux sont différents. Le taux moyen d'IgA dans les larmes de malades appartenant au groupe de la lèpre lépromateuse est significativement plus bas que celui noté chez les malades témoins. Chez certains cas de lèpre ayant souffert d'uvéite dans le passé, de même que chez tous les cas qui présentent une uvéite endogène active, on observe une élévation des taux d'IgA et d'IgG dans l'humeur aqueuse. Dès lors, on peut en déduire que les profils d'altération des immuno-globulines dans les larmes et dans l'humeur aqueuse, chez les malades souffrant de lèpre lépromateuse, ne sont pas semblables.

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