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The influence of local antichlamydial antibody on the acquisition and persistence of human ocular chlamydial infection: IgG antibodies are not protective

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SUMMARY

In order to study the effect of antichlamydial antibodies in ocular secretions on resistance to ocular chlamydial infection and clearance of this infection, we have performed linked longitudinal studies in a Gambian village in which trachoma is endemic. We have measured IgG and IgA antibody levels to a local serotype B isolate of *Chlamydia trachomatis* by amplified enzyme immunoassay, and chlamydial antigen levels in conjunctival swabs using a commercially available immunoassay which detects chlamydial glycolipid. Having previously demonstrated that sharing a bedroom with a case of active trachoma is a risk factor for acquisition of the disease, we have analysed the effect of IgG and IgA antibody on the acquisition and persistance of clinical trachoma after controlling for age, sex, exposure to infection and for the presence of chlamydial antigen using a Poisson regression model. We have found that the presence of antichlamydial IgG in ocular secretions of disease-free subjects is associated with an increased incidence of trachoma. IgA antibody shows an opposite trend, but this is not statistically significant. One possible explanation of these findings is that antichlamydial IgG antibodies enhance the infectivity of *C. trachomatis* for the human eye; this could have major implications for the development of a chlamydial vaccine.

INTRODUCTION

Trachoma is a chronic follicular conjunctivitis caused by *Chlamydia trachomatis*. It is believed to affect more than 400 million people, of whom some 7 million are blind as a result [1]. The age specific prevalence of active trachoma, which is a disease of childhood, suggests that protective immunity develops after one or more cycles of infection; it is rarely possible to isolate *C. trachomatis* from the eyes of adults in communities with endemic trachoma [2, 3].

The immunological basis of protective immunity to ocular chlamydial infection has been studied in a variety of animal models. In the Taiwan monkey, immunity was shown to be short-lived and serotype specific [4]. In the guinea-pig, immunity

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to the guinea-pig inclusion conjunctivitis agent (GPIC, a strain of *C. psittaci*) depends on the presence of chlamydia-specific secretory IgA antibody in ocular secretions. Passive transfer experiments have shown that serum IgG antibody is not protective in this model [5]. Similar results have been obtained with *C. trachomatis* in the owl monkey (*Aotus trivirgatus*) [6].

The presence of chlamydia-specific antibody in the ocular secretions of humans with trachoma was first demonstrated by Bernkopf and colleagues [7]. Nichols and colleagues showed that tears from children with trachoma would partly neutralize homologous strains of chlamydia inoculated into the owl monkey eye [8], but since chlamydia were present in conjunctival scrapings from these children it was clear that they themselves were not protected from infection. Barenfanger demonstrated that neutralization was mediated in this case by immunoglobulin [9]. Others have found a positive correlation between anti-chlamydial antibody levels and the presence of chlamydia in the conjunctiva of individuals in trachoma endemic communities; indeed the presence of such antibodies has been used as a diagnostic marker of active trachoma [10–12].

In order to demonstrate conclusively a relationship between local antibody levels and protective immunity to human chlamydial eye infections, it is necessary to conduct longitudinal studies in which the relationship between antibody level and the risk of acquisition of disease can be measured, preferably after stratifying for the degree of exposure to infection. We report here such a study, carried out in the Gambian village of Jali.

METHODS

Field studies

This study was carried out in the village of Jali in the West Kiang district of The Gambia. The geography and social anthropology of this village have been previously described [13, 14]. Well over 95% of the population of the village (approximately 900) was examined on three occasions: December 1985, July 1986 and August 1987.

Examinations were all carried out by a single observer (D.C.W.M.) using a $\times 4$ illuminated monocular loupe, and findings recorded according to the criteria of Dawson and co-workers [1]. Active trachoma was diagnosed when five or more follicles were found in the central area of the subtarsal conjunctiva, or when papillary hypertrophy obscured the blood vessels over more than half the subtarsal conjunctiva, and follicles were present.

After the survey in August 1986, all active cases were treated with a 4-week course of twice daily supervised 1% tetracycline ointment to both eyes, and a 10-day course of oral tetracycline twice daily (erythromycin in the case of children aged less than 6 years).

This study was approved by the joint Gambia Government/Medical Research Council Ethical Committee.

Sample collection

Samples were collected for the detection of chlamydial antigen and chlamydia specific antibodies in ocular secretions as previously described [15]. They were

placed immediately on ice and frozen at −20 °C within 6 h of collection. Blood was collected by finger prick or venepuncture and stored on ice for up to 6 h before separation. Serum was frozen at −20 °C until assayed.

In December 1985, ocular secretions and swabs for chlamydial antigen detection were collected from all clinically active cases, and from a one in three randomized sample of the population stratified by age. Age groups selected were 0–1 years; 2–3 years; 4–7 years; 8–11 years; 12–15 years and > 15 years. Specimens were collected from a total of 450 individuals. Serum was also obtained from 412 of these subjects.

In August 1986, swabs and ocular secretions were collected from all subjects examined ($n = 843$).

Laboratory methods

Chlamydial antigen was assayed as previously described, using the Chlamydial IDEIA kit (NovoBiolabs, UK) [3]. This monoclonal antibody-based assay includes an amplification step, bound alkaline phosphatase splitting NADP to NAD, which enters a redox cycle, reducing colourless oxidized tetrazolium to red formazan.

Since this redox-amplified EIA is between 10 and 100 times more sensitive than conventional EIA, it was also used for the measurement of chlamydia specific IgG, IgA and sIgA antibody in ocular secretions. The EIA coating antigen used was a mixture of two serovar B isolates of *C. trachomatis* obtained from eye swabs taken in Jali villages (B/Jali 5/OT and B/Jali 20/OT), and was used at 10 μg per well. Duplicate tear samples were tested at a dilution of 1 in 50.

Serum antibodies were measured at a dilution of 1 in 100 by conventional EIA, using the antigen described above. Peroxidase-conjugated rabbit anti-human IgG or IgA was used to detect bound antibody (Dako-Patts, Copenhagen, Denmark). Substrate was hydrogen peroxide (BDH, Poole, England) in a solution of tetramethylbenzidine (Sigma Co. Ltd) and dimethyl sulphoxide (BDH).

The results of serum and tear antibody assays were corrected to minimize variation between 96-well plates. Quadruplicate twofold dilutions of two specimens of known optical density (OD) were included in each tray, and eight replicates of diluent were used as an indicator of assay background for that tray.

The mean OD for the positive controls was calculated for each tray, and divided by the tray's mean negative control OD to derive a correction factor for each tray. Assay results for each tray were multiplied by the appropriate correction factor to ensure inter-tray comparability. The mean background of the eight negative controls was calculated for each tray. This figure plus three standard deviations was subtracted from the result for each well. Thus any result greater than zero was taken as positive.

Statistical analysis

Only individuals from whom there were complete data at two successive surveys were considered in the analysis. Rate ratios for outcome were calculated for the individual antibody classes using the EGRET software package. The effects of exposure to infection (sharing a room with an active case), antigen positivity, and of differences between the two pairs of surveys were examined using crude rate

ratios. We explored the influence of these variables on outcome using two kinds of regression model. A Poisson regression model, in which the ELISA ODs were modelled as continuous variables and the rate multiplier was set to be the interval between surveys, was used to control for confounding due to differences in exposure (sharing a room with an active case) and to antigen positivity, and to further explore differences between the two pairs of surveys, in those becoming clinically active at the subsequent survey. This model gave a much better fit than modelling antibody as a binary discrete variable, but is likely to underestimate the true rates. A logistic regression model was also used in which odds ratios were estimated rather than rate ratios, but the conclusions were not materially different. A similar method was used to explore the effects of treatment and antibody status on outcome in those initially clinically active.

RESULTS

The age specific prevalence of active trachoma in the three surveys is shown in Fig. 1. It is similar for the December 1985 and July 1986 surveys, no treatment having been given in the intervening period. In 1987 the prevalence was lower, since all active cases identified in July 1986 had been treated.

The final data set contained 771 individuals who were initially clinically negative, of whom 37 became active at the next survey. There were 184 initially clinically active, of whom 64 remained active at the next survey.

The relationship between chlamydia specific IgG antibody levels in ocular secretions and levels of chlamydial antigen found in conjunctival swabs at the December 1985 and July 1986 surveys is shown in Fig. 2. Most subjects in whom antigen was detectable had high levels of IgG antibody in ocular secretions. By contrast, the relationship between anti-chlamydial IgA levels in ocular secretions and chlamydial antigen is also shown n Fig. 2. Most subjects with high levels of chlamydial antigen had low levels of tear IgA antibody.

The prevalence of anti-chlamydial IgG in ocular secretions of subjects with and without clinical evidence of active trachoma was 68 and 29% respectively; the corresponding figures for anti-chlamydial IgA were 46 and 20%.

In order to evaluate the importance of local antibody in resistance to reinfection with *C. trachomatis* and in the clearance of ocular chlamydial infection, linked longitudinal studies were performed.

Role of local antibody in resistance to reinfection

Combining the results for all three surveys and considering only those without active trachoma at baseline, 14/231 of those with antichlamydial IgG antibody in tears acquired disease (6·1%), compared with 23/392 of those without IgG antibody (4·3%) (rate ratio 1·45, 95% CI 0·69–3·06). Individuals with high levels of tear IgG had a greatly increased rate of infection and this was reflected in the results of the multivariate analysis below. There is a clear trend in the rate ratios as tear IgG increases (Table 1); modelling tear IgG as a continuous variable resulted in an improved fit.

Combining the results for all three surveys, 7/163 of those with antichlamydial IgA antibody in tears acquired disease 4·2%), compared with 30/608 of those

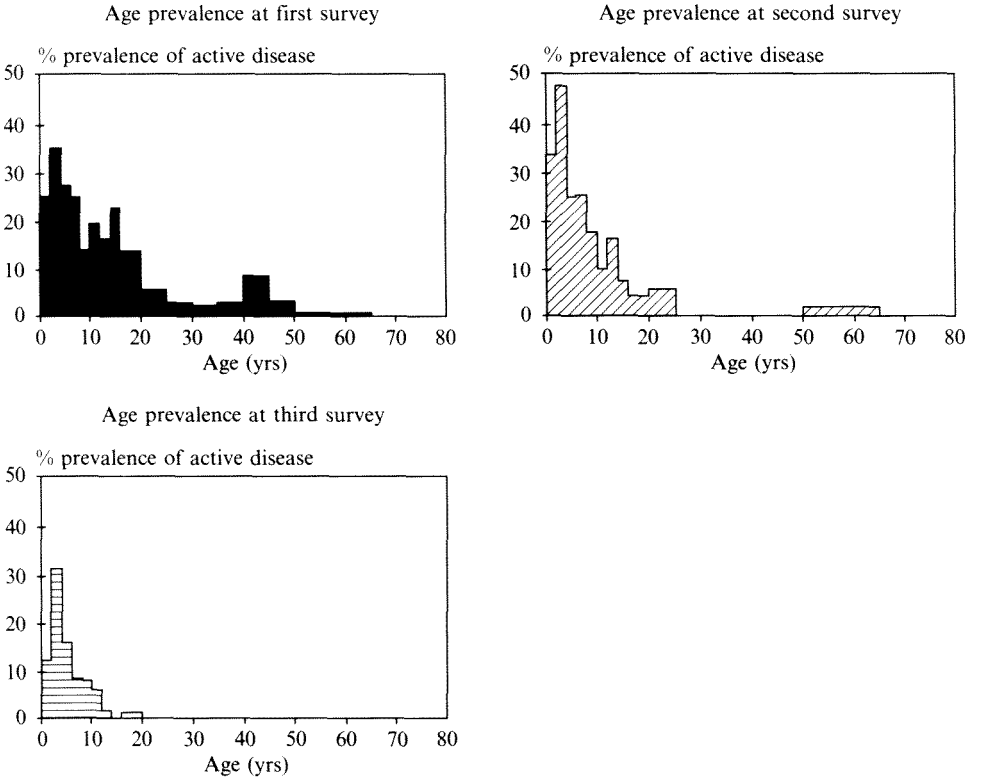

Fig. 1. Age prevalence rates for the three surveys.

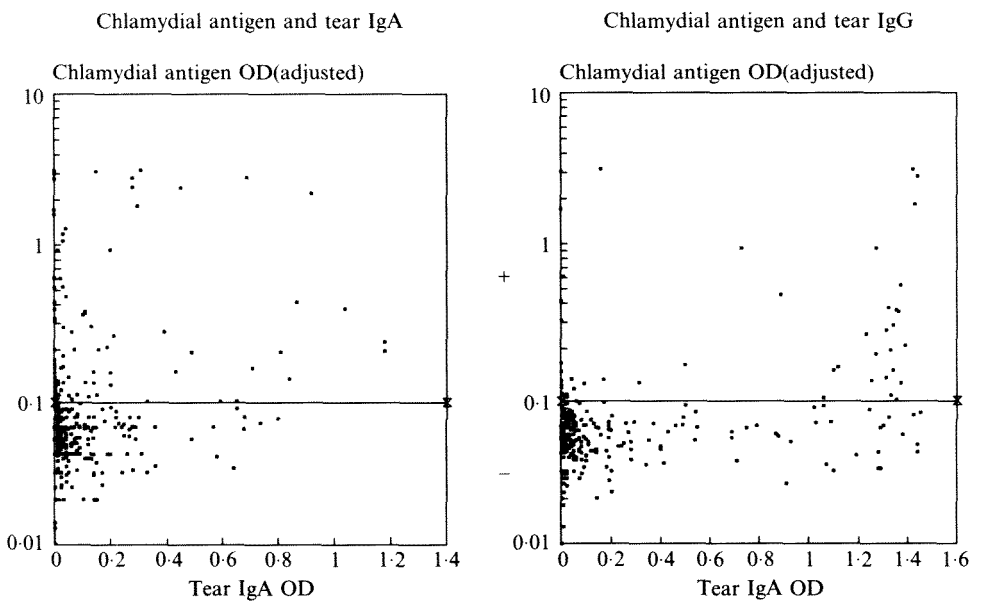

Fig. 2. The cut-off is adjusted to allow the data from two surveys to be combined. There are 800 values on the vertical axis below the cut-off on both diagrams.

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Table 1. *Influence of tear IgG level on acquisition of new disease*

Tear IgG ELISA OD	Rate ratio
Less than 0·4	1·0
0·40–0·799	3·33
0·8–1·199	4·89
1·2 or more	5·00

Chi square for trend: 9·383; $P = 0·002$.

Table 2. *Crude rate ratios for factors influencing acquisition of new disease*

Protective factors	Crude RR	*P* value
Female sex	0·82	0·55
Tear IgA OD	0·63	0·86
Age > 15 yrs	0·08	< 0·001
Treatment (Survey 2–3 *v.* 1–2)	0·38	0·003
Risk factors	**Crude RR**	***P* value**
Tear IgG OD	3·73	< 0·001
Sharing room with case	2·11	0·033
Antigen + ve	2·97	0·010
Serum IgG OD (219 obs. only)	1·69	0·72
Serum IgA (219 obs. only)	6·99	0·31
Age < 4 yrs	6·60	< 0·001
Age 4–7 yrs	1·87	0·116

Pooled data from three surveys, adjusted to take account of different time intervals between surveys.

Table 3. *Adjusted rate ratios for factors influencing acquisition of new disease*

Protective factors	Adjusted RR (95% CI)	*P* value
Female sex	0·91 (0·46–1·78)	0·78
Tear IgA OD	0·001 ($1·5 \times 10^{-7}$–8·1)	0·13
Age > 15 yrs	0·12 (0·03–0·42)	< 0·001
Treatment (survey 2–3 *v.* 1–2)	0·40 (0·20–0·78)	0·007
Risk factor	**Adjusted RR (95% CI)**	***P* value**
Tear IgG OD	4·83 (1·92–12·1)	< 0·001
Sharing room with case	1·57 (0·76–3·22)	0·22
Age < 4 yrs	3·39 (1·65–6·98)	< 0·001
Antigen + ve	1·20 (0·46–3·13)	0·71

RR adjusted for effects of confounding by the other variables.

without antichlamydial IgA antibody in tears (4·9%) (rate ratio 0·87 95% CI 0·31–2·06).

The crude and adjusted rate ratios for acquisition of active disease, calculated from the Poisson regression model, are shown in Table 2 and 3. In the final model the adjusted rate ratio for tear IgG of 4·83 (95% CI 1·92–12·11), estimated for an increase in tear IgG OD of 1·00 (actual range 0–1·6) appears to be the dominant determinant of acquisition of disease. The effect does not vary significantly between surveys; weak enhancing effects of antigen positivity and exposure, and a weak protective effect of tear IgA are still apparent.

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Table 4. *Adjusted rate ratios for factors influencing persistence of disease*

Protective factor	Adjusted RR (95% CI)	*P* value
Treatment (survey 2–3 *v.* 1–2)	0·20 (0·11–0·36)	< 0·001
Female sex	0·73 (0·43–1·25)	0·26
Tear IgA OD	0·53 (0·11–2·51)	0·43
Sharing room with case	0·82 (0·46–1·48)	0·51
Risk factor	**Adjusted RR (95% CI)**	***P* value**
Age < 4 yrs	3·06 (1·45–6·47)	0·003
Age 4–7 yrs	2·58 (1·17–5·71)	0·019
Antigen + ve	1·51 (0·81–2·78)	0·190
Tear IgG OD	1·33 (0·77–2·29)	0·306

RR adjusted for effects of confounding by the other variables.

Role of local antibody in clearance of infection

Combining the results of surveys 1, 2 and 3, 46/122 of those with active trachoma and detectable tear IgG antibody had persistent disease at the following survey (38%), compared with 18/62 of those without tear IgG antibody (29%) (rate ratio 1·30 95% CI 0·69–1·53).

Combining the results for surveys 1, 2 and 3, 30/85 of those with active trachoma and detectable tear IgA antibody had persistent disease at the following survey (35%), compared with 34/99 of those without tear IgA antibody (34%) (rate ratio 1·03 95% CI 0·69–1·53).

The effect of tear IgG and IgA antibody on the persistence of active trachoma, analysed by a Poisson regression model, is shown in Table 4. The rate ratio given is for an increase in OD of 1·0 units (observed range 0–1·6). The effect of antigen positivity is also shown in Table 1. The effect of treatment on the persistence of active disease is estimated by comparing rates of persistence between surveys 1 and 2 with those between surveys 2 and 3, treatment having been given to all active cases after survey 2. After adjusting for the other variables included in the model, only treatment has a significant effect on the persistence of active trachoma. However, it is of interest that there is a trend towards persistence associated with increased IgG antibody levels, and an opposite effect is seen with IgA antibody.

DISCUSSION

The hypothesis that antichlamydial IgG antibodies might enhance chlamydial infectivity for the human eye was first proposed by Hathaway and Peters in 1971 [16]. Their proposal was based on the finding of a high prevalence of antichlamydial IgG antibody in ocular secretions from children with active trachoma, and the fact that higher titres of antichlamydial IgG antibody were found in serum and tears from isolation positive than isolation negative subjects [10–12]. On the other hand, ocular secretions from children with trachoma showed neutralizing activity in the owl monkey model [8]. Brunham and colleagues have shown an inverse correlation between endocervical antichlamydial IgA levels and

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the shedding of *C. trachomatis* from the endocervix [17]; it is therefore possible that the class or subclass of antichlamydial antibody in local secretions is of critical importance in determining whether infection is enhanced or inhibited.

It is not possible in a cross-sectional survey to determine whether a particular class or subclass of antibody is associated with increased or reduced susceptibility to infection. For example, the positive correlation between IgG antibody levels and isolation positivity observed in the above studies may simply reflect the fact that a large inoculum of *C. trachomatis* is more likely to induce an IgG antibody response. We have therefore conducted a longitudinal study in a trachoma endemic community in order to assess the effect of antichlamydial IgG and IgA antibody in ocular secretions on the susceptibility to and the persistence of ocular chlamydial infection.

We have found a positive correlation between the level of antichlamydial IgG in ocular secretions and the acquisition of ocular chlamydial infection. Since this effect might be due to the confounding effect of exposure to infection on tear IgG antibody levels, we have adjusted for exposure in the form of sharing a bedroom with an active case; a previous study in the same village showed that this was a significant risk factor for the acquisition of disease [18]. The positive correlation persists after adjusting for differences in exposure. There are several possible explanations for our findings.

It is possible that subjects with antichlamydial antibody in ocular secretions in the absence of clinical evidence of trachoma were in the early (subclinical) stages of infection, and that the higher incidence of active trachoma in those with IgG antibody merely reflects the natural progression of the disease. There was a group of individuals in this study who were antigen positive without signs of active disease and were thus subclinically infected. However, within this group high tear IgG levels were still associated with an increased rate of infection, as indeed they were in those without evidence of subclinical infection (data not shown). Thus subclinical infection does not by itself account for the findings. Furthermore, if raised tear antibody levels are a surrogate marker of an inflammatory process itself associated with new infection, then we would not expect the opposite trend for IgG and IgA seen here.

A second possibility is that there has been a change in the predominant serotype of *C. trachomatis* circulating in Jali. Serological studies at the time of the second survey showed a predominance of serotype B specific antibody (11/14 positive sera), with serotype A accounting for only 3/14 [19]. At a survey conducted in 1990, in which ocular infections were directly serotyped by polymerase chain reaction amplification of the major outer membrane protein, serotype A was found in 41/51 subjects (80%) [20]. It is possible that the ocular IgG antibody measured in this study was predominantly serotype B specific, and that this failed to neutralize a serotype A challenge but rather enhanced its infectivity. The data presented here cannot substantiate or refute this hypothesis, since the EIA which we have used is not serotype specific.

Trachoma vaccine field trials carried out in Taiwan using a mineral oil-based vaccine showed that whereas a bivalent vaccine was protective, a monovalent vaccine led to a higher incidence of disease in the vaccinated group [21]. The hypothesis that local antibody directed at the wrong serotype might enhance

infectivity would be supported by these results, although unfortunately no ocular isolates were serotyped at the time of those field trials.

By analogy with other infectious diseases in which enhancing antibodies are found, there appear to be two possible mechanisms by which IgG antibodies might enhance the infectivity of *C. trachomatis* for mucosal surfaces: by blocking the binding of neutralizing antibody (cf. *Neisseria meningitidis*) [22] and by enhancing the attachment and internalization of elementary bodies by epithelial cells via binding to Fc receptors (cf. dengue [23]).

Su and co-workers have shown that the human epithelial cell line HeLa 229 expresses the FcgammaIII receptor, and that this has important implications for the neutralization of *C. trachomatis* infections [24]. An intact monoclonal antibody, which neutralizes infection in other (non-FcR expressing) cell lines, fails to neutralize the infection of HeLa 229 cells, whereas the F(ab) fragment will do so by preventing attachment. A recent study by Peterson and colleagues has shown that a murine monoclonal of the IgG2b subclass enhances the infectivity of *C. trachomatis* for FcgammaRIII expressing HeLa cell lines but not for non-FcR expressing lines, whereas an IgG1 subclass monoclonal was neutralizing for both cell types [25].

There is as yet no evidence that human conjunctival epithelial cells express Fc receptors. The expression of FcgammaI receptors on human neutrophils can be induced by interferon gamma, which is secreted by sensitized lymphocytes in response to stimulation by chlamydial antigens and has been implicated in the pathogenesis of trachoma [26, 27].

Whatever the explanation for our findings, they clearly have important implications for the development of a chlamydial vaccine, and the relationship between antibody class, subclass and affinity and protection from mucosal chlamydial infection should be studied in detail.

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