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Immunoglobulin A1 and A2 subclass of salivary antibodies to *Candida albicans* in patients with oral candidosis

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SUMMARY

Immunoglobulin A antibody titres to a cytoplasmic protein extract of *Candida albicans* were determined by ELISA in saliva from 20 patients with oral candidosis and 21 controls. Patients had significantly increased levels of salivary IgA anti-*Candida* antibodies when compared with controls ($P < 0{\cdot}001$). Antibody levels were associated with IgA1 subclass in 90% of the patients; in contrast, IgA2 subclass was predominant in 67% of the controls. Antifungal therapy resulted in significantly decreased IgA1 titres ($P < 0{\cdot}05$) whilst the mean IgA2 antibody titre remained unchanged. The results indicate that *Candida* infection may change the subclass pattern of salivary IgA antibodies.

Keywords salivary antibodies IgA subclasses oral candidosis

INTRODUCTION

Chronic atrophic candidosis of the denture-bearing area of the palate (denture stomatitis) is the commonest form of oral candidosis. IgA antibodies were demonstrated in saliva from infected carriers of *Candida albicans* and non-infected controls (Lehner, 1966; Epstein *et al.*, 1982).

Human IgA consists of two subclasses, IgA1 and IgA2, which differ in the primary structure, carbohydrate composition, antigenic determinants and sensitivity to microbial IgA proteases (Kornfeld & Plant, 1981; Kilian *et al.*, 1983). IgA1 comprises 75 to 90% of the serum IgA, whilst IgA2 is a major component of secretory IgA comprising 50% of the total IgA in secretions including saliva (Delacroix *et al.*, 1982). However, the distribution of specific salivary IgA1 or IgA2 antibodies to *c. albicans* in health and disease has not been previously investigated.

The aims of this investigation were to compare salivary IgA antibody levels and their subclasses to cytoplasmic protein extract from *C. albicans* in individual patients with oral candidosis and in controls, and to determine the relationship, if any, between *C. albicans* colony counts and salivary IgA antibody levels. Furthermore, the effect of antifungal treatment on the IgA subclass pattern of anti-*Candida* salivary antibodies was investigated in individual patients.

MATERIALS AND METHODS

Human subjects. Group I contained 20 patients (seven males and 13 females; age range 24–76 years) with denture stomatitis. Diagnosis was made on the basis of clinical signs and symptoms and positive culture of *C. albicans*. Patients with a history of drug use or medical conditions known to

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predispose to oral candidosis were excluded. Group II consisted of 21 control subjects with clinically healthy oral mucosa (11 males and 10 females; age range 17–82 years).

Saliva samples. Saliva samples (2 ml) were collected at the same time of day, immediately placed on ice and transported to the laboratory for study. From each sample, 0·1 ml of uncentrifuged saliva (undiluted, diluted 1:10 and 1:100) was poured onto Sabouraud's dextrose agar plates containing crystomycin (0·35 mg/ml). The plates were incubated aerobically at 37°C for 48 h and the number of colony-forming units per ml of saliva was counted. Cytological examinations for the presence of hyphae were also performed on each saliva specimen. All saliva samples were clarified by centrifugation and the supernatants were stored at −20°C until assayed for the determination of specific antibodies and their subclasses.

In order to study the effect of antifungal treatment on IgA antibody levels, saliva from eight patients with oral candidosis was also collected after 4 weeks of treatment with Fungilin lozenges (10 mg amphotericin B, four times a day).

Antigens. The cytoplasmic protein extract of *C. albicans* used was a gift from Dr A. Ford (Institute of Biological Standards and Controls) and was prepared according to the method described by Longbottom *et al.* (1976). The total protein of the extract was determined by the method of Lowry *et al.* (1951) and the antigen was used at an optimal concentration of 50 μg protein/ml.

The oral bacterial antigens used were sonicated supernatants of *Actinobacillus actinomycetemcomitans* NCTC 10979, *Bacteriodes gingivalis* W50 and *Actinomyces viscosus* WVU 627 and they were prepared as described in detail previously (Farida *et al.*, 1986). These antigens were used at an optimal concentration of 25 μg protein/ml.

Enzyme-linked immunosorbent assay (ELISA). The method used for measurement of antibody titres to the above antigens was based upon a modified solid phase ELISA (Engvall & Perlmann, 1972). Briefly, 200 μl of each antigen was absorbed onto disposable flat-bottomed microtitre plates (Sterilin) followed by 3 h of incubation at 37°C and overnight at 4°C. Subsequently, the unbound antigen was removed, wells were thoroughly washed with PBS, 0·5% bovine serum albumin (BSA) and 0·05% Tween 20 and then blocked by incubation with PBS and BSA for 2 h at 37°C. After blocking, 200 μl of saliva (neat, 1:5, 1:25, 1:125) were added to each well, incubated for 45 min at 37°C and washed three times. Two hundred microlitres of horseradish peroxidase conjugate antihuman IgA (Miles Yeda Ltd) were added to each well, incubated for 30 min at 37°C and washed three times as before. Freshly made up substrate (1,2-phenylenediamine) was then added to each well and incubated for 1 h at 37°C. The substrate reaction was terminated by adding 50 μl of sulphuric acid (4 N H_2SO_4) to each well. The optical density of each well was measured at 492 nm using a Titertek multiscan spectrophotometer, converted into relative binding values (100% = binding to human Ig coated wells) and the saliva dilution giving 20% of the binding (ABT_{20}) was then calculated. The results were evaluated statistically using the Mann Whitney *U*-test and Kendall's rank correlation test.

Determination of IgA subclasses. The flat-bottomed microtitre plates (Sterilin) were coated with 100 μl of the antigens (*C. albicans*, *A. actinomycetemcomitans*, *B. gingivalis* and *Actinomyces viscosus*) and blocked as in the double antibody assay. Subsequently, they were incubated with 100 μl of saliva diluted in PBS-BSA-Tween 10 (neat, 1:5, 1:25) for 45 min at 37°C. The plates were then washed three times, each wash for 3 min, and coated with 100 μl of anti-IgA1 or anti-IgA2 monoclonal antibody (Nordic) at optimal dilutions of 1:1000 and 1:500 respectively. The monoclonal antibodies have been shown to be specific for human IgA1 and IgA2, regardless of light-chain type or IgA2 allotype as previously described (Crago *et al.*, 1984). The plates were further processed as described in the preceding paragraph.

The assay was standardized in parallel in a manner which simulated antigen binding. Wells were coated with serial 2-fold dilutions of purified human IgA1 or IgA2 myeloma proteins obtained as a gift from Dr Jefferies (University of Birmingham). The standard wells were developed with the anti-IgA subclass hybridoma antibody. The results of unknowns are expressed as nanograms per millilitre relative to these standards by using the logit-log method of interpolation (Brown & Mestecky, 1985). The absolute concentrations of IgA protein determined on the basis of this calibration may, however, be overvalued in view of a recent report that 10 times more IgG1

Table 1. Comparison and relationship of salivary IgA antibody levels to *Candida* antigen and *Candida* counts in patients and controls

Subjects (No.)	Salivary IgA antibody titre mean ± s.e.	*C. albicans* colony count per ml mean ± s.e.	*P* value
Patients (20)	40 ± 7·59	13705 ± 4478·60	< 0·05
Controls (21)	2 ± 0·63	94 ± 48·12	< 0·01

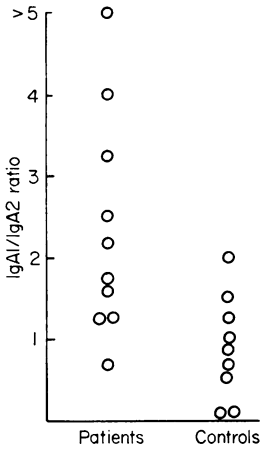

Fig. 1. Ratio of IgA1 to IgA2 antibodies to *C. albicans* antigen in saliva from patients and controls.

myeloma protein then specific antibody is needed for the same level of binding of the anti-isotype antibody (Mattila, 1985).

RESULTS

Salivary IgA antibody levels and C. albicans *colony counts.* Salivary IgA antibody levels and *Candida* counts were compared in patients and controls (Table 1). Control subjects with clinically healthy oral mucosa had low salivary IgA antibody levels to *Candida* antigen with a mean of 2 ± 0·63. In contrast, patients with oral candidosis had significantly increased antibody levels to *Candida antigen* ($P < 0·001$) with a mean of 40 ± 7·59. A statistically significant difference ($P < 0·001$) was also found between *C. albicans* colony counts in patients (mean 13705 ± 4478·6) when compared with controls (mean 94 ± 48·12).

Furthermore, a statistically significant relationship was found between salivary anti-*Candida* IgA antibody levels and *C. albicans* colony counts in individual patients ($P < 0·05$) and controls ($P < 0·01$) (Table 1).

Ratio of IgA1 to IgA2 salivary antibodies to microbial antigens. The distribution of IgA1 and IgA2 antibodies to *Candida* antigen was determined in saliva from 10 patients with oral candidosis and nine controls (Fig. 1). In nine out of 10 patients examined, antibodies occurred predominantly in the IgA1 subclass. In contrast, six out of nine control subjects with clinically healthy oral mucosa had a predominance of IgA2 antibodies to *Candida* antigen. Out of these, only IgA2 was detected in two salivas.

Table 2. Ratio of salivary IgA1 to IgA2 antibodies to oral microbial antigens

	C. albicans		*A. actinomycetem-comitans*		*B. gingivalis*		*A. viscosus*	
Subject no.	IgA antibody titre*	IgA1 / IgA2	IgA antibody titre	IgA1 / igA2	IgA antibody titre	IgA1 / IgA2	IgA antibody titre	IgA1 / IgA2
1	4	0·54	2	A2	3	0·75	4	A2
2	2	0·67	2	0·63	2	A2	4	0·94
3	3	2·00	3	0·94	2	0·54	8	1·50
4	2	A2	2	0·63	4	0·94	3	1·64
5	18	5·89	2	2·00	4	1·88	7	A1
6	44	3·24	2	0·54	2	0·67	5	1·25

* Dilution of saline giving 20% binding to antigen (ABT_{20}).
A2, only IgA2 detected.
A1, only IgA1 detected.

Table 3. Comparison of salivary IgA antibody titres and ratio of IgA1 to IgA2 in patients with oral candidosis before and after antifungal therapy

Patient no.	Total IgA titre*		IgA1 (ng/ml)		IgA2 (ng/ml)		IgA1/IgA2 ratio		
	Before	After	Before	After	Before	After	Before	After	Change
1	39	23	600	375	280	300	2·14	1·25	−0·69
2	25	16	150	75	240	200	0·63	0·38	−0·25
3	52	4	150	75	60	60	2·50	1·25	−1·25
4	16	2	300	225	240	300	1·25	0·75	−0·50
5	42	15	600	120	150	120	4·00	1·00	−3·00
6	10	4	375	150	300	320	1·25	0·47	−0·78
7	18	0	825	NT	140	NT	5·89	NT	—
8	18	0	300	NT	176	NT	1·70	NT	—
Mean ± s.e.	28 ± 5·29	8 ± 3·09	362 ± 83·10	153 ± 51·09†	194 ± 39·69	216 ± 44·24‡	1·96	0·65	−1·31

* ABT_{20}.
† $P < 0·05$.
‡ Not significant.
NT, not tested.

In order to find out if individuals responded predominantly in one subclass to a given antigen, the distribution of specific IgA1 or IgA2 salivary antibodies to three other oral bacterial antigens were investigated (Table 2). The predominant subclass response to all oral antigens was found to be IgA2 in subjects Nos 1 and 2, and IgA1 in subject No. 5. Subjects Nos 3, 4 and 6 had a predominance of IgA2 antibodies to *A. actinomycetemcomitans* and *B. gingivalis*, and a predominance of IgA1 antibodies to *Actinomyces viscosus*. The results also show that antibodies to *A. actinomycetemcomitans* and to *B. gingivalis* were mainly of the IgA2 subclass (five out of six subjects) whilst antibodies to *A. viscosus* were associated with IgA1 subclass (four out of six subjects).

Effect of antifungal therapy on IgA antibody levels. The salivary IgA antibody levels and subclass distribution to *Candida* antigen were compared in patients with oral candidosis before and after antifungal therapy (Table 3). The results show a significant decrease in mean salivary IgA anti-

Candida antibody titre following antifungal treatment (from $28 \pm 5{\cdot}29$ to $8 \pm 3{\cdot}09$: $P < 0{\cdot}01$). Following therapy, the mean IgA1 antibody titre decreased significantly ($P < 0{\cdot}05$) from 362 ng/ml to 153 ng/ml, whilst the mean IgA2 antibody titre remained unchanged (194 ng/ml and 216 ng/ml). Subclass shift from IgA1 to IgA2 predominance was found in two out of six patients after antifungal therapy.

DISCUSSION

The results of this study demonstrated a positive correlation between salivary IgA anti-*Candida* antibody levels and *Candida* colony counts in patients with oral candidiosis and in control subjects. These findings are in agreement with Epstein *et al.* (1982) who reported a direct correlation between salivary antibody levels and the frequency of isolation of *C. albicans* from the oral cavity.

Analysis of IgA subclass distribution revealed that salivary antibodies to *C. albicans* were predominantly of the IgA2 subclass in controls with clinically healthy oral mucosa. In contrast, the majority of patients with oral candidosis had predominantly IgA1 salivary antibodies to *C. albicans*. These results indicate that an infection may result in a change of subclass pattern. Similarly it was reported that infection with live influenza virus stimulated a predominantly IgA1 antibody response in nasal washes (Brown *et al.*, 1985). Even though IgA2 antibodies were found before infection, they did not increase during the course of the infection.

Antifungal therapy in patients with oral candidosis resulted in a significant decrease in IgA1 antibody titres, whilst the IgA2 antibody titres remained unchanged. Furthermore, a shift from IgA1 to IgA2 predominance after therapy was observed in two out of six patients. The change to IgA2 predominance following successful antifungal therapy may indicate a return to the 'resting state' subclass pattern as found in healthy controls. These results indicate that IgA1 subclass antibodies are produced selectively in response to clinically manifested *Candida* infection. This could be due to increased antigenic load, antigen presentation by different accessory cells or other yet unknown factors which may be relevant for the pathogenesis or defect in host resistance.

Naturally occurring salivary antibodies to protein antigens from the oral bacteria *A. actinomycetemcomitans* and *B. gingivalis* were found to be associated with IgA2, whilst antibodies to *A. viscosus* were predominantly of the IgA1 subclass. These results are somewhat surprising in view of a report that protein antigens from *Streptococcus mutans* elicited mainly IgA1, whilst lipopolysaccharides induced mostly IgA2 salivary antibody responses (Brown & Mestecky, 1985). However, Persson *et al.* (1986) demonstrated IgA2 predominance of antibodies to *Salmonella* lipopolysaccharide and IgA1 predominance of antibodies to *Shigella* lipopolysaccharide. These findings suggest that the pattern of IgA subclass cannot be clearly linked with either protein or lipopolysaccharide antigens. The predominance of IgA1 subclass more likely reflects the state of active immunisation. This view is supported by the presented findings in oral candidosis, by the stimulation of antibodies to *A. viscosus* as a result of mild gingivitis (Moore *et al.*, 1982) and by the stimulation of antibodies to *S. mutans* as a result of caries (Challacombe, 1980).

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