Serum antibodies to commensal oral and gut bacteria vary with age

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Abstract

This study examined the relationship between serum antibody levels to selected bacteria from the commensal oral and gut flora with increased age in a healthy adult population. A total of 116 healthy subjects were studied consisting of the following age groups: 20–39 years (group A), 40–59 years (group B), 60–79 years (group C) and 80 + years (group D). Only significantly lower mean IgM antibody levels to Streptococcus mutans strain Guy’s serotype c were observed in older age groups (P < 0.001). With Actinomyces viscosus NCTC 10951 significantly reduced IgM levels (P < 0.02) and significantly elevated IgA levels were observed with increased age (P < 0.05). IgA and IgG antibodies to Escherichia coli NCTC 10418 were increased significantly in the older age groups (P < 0.001), whilst a trend toward lower levels of IgM antibodies was recorded with age. No changes in IgA antibodies to Streptococcus faecalis NCTC 775 were observed but the lowest level of IgM antibodies were detected in the oldest age group (P < 0.05). Mean specific activity was decreased with age with IgM antibodies to the oral bacteria and increased with age with IgG and IgA antibodies to E. coli. Overall, our results suggest a general reduction in serum IgM antibody responses. This impairment in the circulatory IgM immune response may contribute to the increased occurrence of infections in the elderly.

Keywords: Specific antibody; Serum; Gut flora; Oral flora; Ageing

1. Introduction

There is much evidence to suggest that the immune response declines with advancing age, but it would appear that components of the response decline at different rates [1]. Although the number of B cells remains relatively stable, the size of certain sub-populations of B cell appears to change with age causing fluctuations in levels of individual serum immunoglobulin classes [2]. With increased age the most obvious change is a reduction of about 15% in the number of circulating lymphocytes, mostly involving the population of T cells [3]. Studies of age-associated changes in the number of CD4 and CD8 subtypes, have given contradictory results, and either an increase or decrease in cell numbers has been demonstrated in individual mouse strains or human subjects [4]. The immunoglobulin levels in serum directly reflect the activity of B-cells and also

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indirectly that of T-cells [5]. It is generally agreed that IgG and IgA levels increase with age while IgM levels decrease [6–8].

The relationship between age and specific antibody levels is less clear. Positive correlations between increased age and increased prevalence and level of specific serum antibody classes A, G and M to Porphyromonas gingivalis have been observed [9]. In contrast the levels of serum IgG and IgA antibodies against P. gingivalis were found to be similar in elderly and young subjects [10]. An earlier study indicated a significant reduction in levels of IgM antibodies to a gut bacterium, Salmonella adelaide, in elderly individuals [11].

An increase in the occurrence of certain diseases characterises the approach of old age, but it is not clear whether this is related to waning of functional activity of antibodies in the defence against bacterial diseases especially those occurring on mucosal surfaces. Commensal bacteria offer an antigenic challenge throughout life, and immune responses to the selected examples allow an analysis of age-related immune responses to be made. Consequently we have examined serum isotype specific antibodies to four resident gut and oral Gram-positive and Gram-negative bacteria in a healthy adult population of different ages.

2. Materials and methods

2.1. Study population

A total of 116 healthy unmedicated Caucasian subjects took part in this study and were divided into the following age groups. Group A were aged between 20–39 years (mean age 26.7 years, S.E.M. = 1.06, n = 29); group B between 40–59 years (mean age 50.4 years, S.E.M. = 1.08, n = 30); group C between 60–79 years (mean age 71.7 years, S.E.M. = 1.07, n = 28); and group D were aged 80 years and over (mean age 84.0 years, S.E.M. = 0.61, n = 29). The subjects in group A and B included staff and patients of Guy’s Dental Hospital, London, who were attending routine dental examinations. Group C and D were healthy elderly people of no particular social group obtained from a unique college (Morden College, Blackheath, London) which was a mixture of houses, flats and sheltered accommodation. Subject to infirmity, these subjects also had normal life styles outside the residence and formed an ideal population to study. All participants that took part in this study were assessed clinically and satisfied the following requirements for inclusion within this and previous studies on microbiology of plaque and saliva: (a) the presence of a minimum number of teeth (seven) including one molar; (b) the absence of active oral disease; (c) no history of antimicrobial therapy or other drug treatment including immunosuppressives within the previous six months; and (d) no history of diabetes [12,13]. The mean number (±S.D.) of remaining teeth in each age group were 27 (1.9), 23 (6.5), 15 (8.2) and 13 (6.8) in groups A, B, C and D, respectively; the respective mean gingival indices (±S.D.) were 0.41 (0.49), 0.77 (0.81), 1.38 (1.07) and 1.42 (0.73) [14]. Mean gingival indices were calculated from the sum of all the individual gingival indices for each subject and the mean calculated for each age group.

2.2. Collection of blood samples

Venous blood samples were taken from all subjects and after centrifugation aliquots of serum were stored at −70°C until used in ELISA assays.

2.3. Antigen preparation

The following bacteria were used as antigens in the ELISA solid phase: Streptococcus mutans strain Guy’s serotype c [15]; Escherichia coli NCTC 10418; Streptococcus faecalis NCTC 775 and Actinomyces viscosus NCTC 10951. All the above bacteria were kept in liquid nitrogen and, when required, were cultivated in appropriate liquid media and their purity checked by Gram-stain. For antibody analyses, bacteria were plated on Columbia blood agar; S. mutans and A. viscosus cultures were incubated anaerobically under H₂/CO₂ atmosphere at 37°C, whilst E. coli and S. faecalis cultures were incubated aerobically at 37°C. Bacterial growth was transferred from the plates and resuspended in buffered formalin and incubated at room temperature overnight. The organisms were washed three times in a phosphate buffer saline (PBS) with azide (0.01
g/100 ml) and the optical density of the cultures adjusted to give cell counts of approximately $4 \times 10^9$ cells/ml prior to storage at 4°C.

2.4. ELISA assays for quantification of specific antibodies

Levels of specific antibodies IgA, IgG and IgM to the four types of bacteria in serum were quantified by ELISA by a modification of methods described previously [16,17]. Briefly, wells of Dynatech Immunolon II (Chantilly, VA) microtitre plates were coated with whole bacteria suspended in 0.3% methyl glyoxal (Sigma, Poole, UK), at a cell concentration of $4 \times 10^9$ cells/ml and incubated at 37°C for 2 h. Four doubling dilutions (1:100–1:800) were prepared for each subject. A reference standard was generated from pooled sera of subjects having high antibody titres to the selected microorganisms and the positive standard was arbitrarily assigned 100 000 ELISA units (EUs) and doubling dilutions prepared. The plates were then incubated overnight at 4°C. The detection stage and the rest of the steps were followed as described previously [8]. Values of antibodies were calculated as ELISA units (EU)/ml and the value for each individual sample was taken as the mean of each of the doubling dilutions falling within the standard curve.

2.5. Absorption assays for determination of antibody specificity

The specificity of serum antibodies for the four types of bacteria was examined in cross-absorption tests. Diluted serum samples from six subjects were mixed at a ratio of 1:5 vol of packed formalin-killed bacterial cells previously washed in PBS + azide [17]. The mixtures were then incubated for 1 h at 37°C on a cell mixer followed by overnight incubation at 4°C. The microorganisms were removed by centrifugation and the resulting supernatant was used to test for residual antibody activity by ELISA assays.

2.6. Statistical analysis

Data are expressed as mean ± S.E.M. Differences among means were analysed for statistical signific-
group B. Specific antibodies to *S. faecalis* are presented in Fig. 4. IgA antibodies were not obviously related to age, IgG antibodies were maximal in group C whereas IgM antibodies significantly (*P* < 0.05) declined with age.

The specific activities of antibodies to *S. mutans*, *A. viscosus*, *E. coli* and *S. faecalis* in relation to age are shown in Table 2. These were calculated by dividing the values of the specific antibody class (EU/ml) by the corresponding immunoglobulin concentration (µg/ml) with the resulting value expressed as (EU/µg). There was a significant decrease in IgM specific activity to *S. mutans* in the older age groups C and D compared with the youngest age group A (*P* < 0.001). Similarly the IgM specific activity to *A. viscosus* was significantly reduced with increased age (*P* < 0.001). Both IgA and IgG specific activities to *E. coli* were significantly increased when older age groups were compared with the younger group A (*P* < 0.01).

![Fig. 1. Specific antibodies to *S. mutans* in serum in relation to age.](image1)

![Fig. 2. Specific antibodies to *A. viscosus* in serum in relation to age.](image2)

4. Discussion

This investigation has demonstrated significant changes in relation to age of antibodies of isotypes IgA, IgG and IgM to commensal oral and gut flora. IgM antibody titres to the selected oral and gut bacteria were at lowest levels in the most elderly groups C and D, whilst IgA antibody titres to *A. viscosus* and *E. coli* were increased in relation to age. IgG antibody titres were elevated with increased age in the case of the two gut bacteria *E. coli* and *S. faecalis*. No age-related changes in IgA antibody levels to *S. mutans* were observed and it is interesting to note that microbiological studies have suggested no obvious changes in numbers of *S. mutans* with age [12].

In contrast, IgA antibodies to *A. viscosus* increased with age. *Actinomyces* spp., like *S. mutans,*
Table 2
Mean specific activity in serum of isotype specific antibodies to gut and oral bacteria

<table>
<thead>
<tr>
<th>Age group</th>
<th>S. mutans</th>
<th>A. viscosus</th>
<th>E. coli</th>
<th>S. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgA</td>
<td>IgG</td>
<td>IgM</td>
<td>IgA</td>
</tr>
<tr>
<td>A</td>
<td>10.0 (7.7)</td>
<td>3.8 (3.1)</td>
<td>44.3 (21.2)</td>
<td>8.8 (8.4)</td>
</tr>
<tr>
<td>B</td>
<td>9.1 (6.0)</td>
<td>4.0 (4.4)</td>
<td>54.9 (30.0)</td>
<td>7.9 (5.0)</td>
</tr>
<tr>
<td>C</td>
<td>8.3 (5.2)</td>
<td>3.9 (3.6)</td>
<td>25.6 (11.9)</td>
<td>10.5 (5.4)</td>
</tr>
<tr>
<td>D</td>
<td>7.7 (4.2)</td>
<td>4.0 (2.0)</td>
<td>28.3 (13.7)</td>
<td>9.1 (4.2)</td>
</tr>
</tbody>
</table>

Group A = 20–39 years (n = 29).
Group B = 40–59 years (n = 30).
Group C = 60–79 years (n = 28).
Group D = 80+ years (n = 29).
Values are means with S.D. in parentheses.
* Statistically significant differences compared with group A.
are part of the predominant cultivable microflora of the oral cavity and the numbers of *A. viscosus* have been demonstrated to increase with age [12,18,19]. This may indicate the presence of persistent antigenic stimulation which may have resulted in the observed increased antibody level. Antigens from oral bacteria gain access to the systemic immune system possibly via the gingival crevice in dentate individuals or probably via the gut when swallowed [20,21].

Other investigators have reported variable observations with respect to antibody levels [22–24]. The levels of serum IgA, IgG and IgM antibodies to a surface protein antigen of *S. mutans* in edentulous aged subjects were generally similar to, or greater than, those of younger individuals [22]. Similar levels of IgA and IgG antibodies to oral bacteria in elderly and young (3 to 81 years) individuals have also been reported, whereas impaired IgG antibody production to flagellin antigen of *Salmonella adelaide* in elderly subjects (> 60 years) was observed [23,24].

In the present study antibody IgA, IgG and IgM classes to *E. coli* and *S. faecalis* were also detected. Both of these organisms are regarded as common inhabitants of the human intestinal microflora, although *S. faecalis* and enteric rods have also been isolated from subgingival flora in approximately 5% of severe adult periodontitis patients [25–27]. Increases in circulating antibodies to members of the gut flora may suggest continuous stimulation or changes in permeability of mucosal membranes in the gut. Studies have described anatomical changes in mucous membranes especially with increased age, and decreased numbers of Peyer’s patches with fewer lymphoid follicles have been noted [28]. It was also noticed that, as gastric mucosa ages, it becomes progressively less active in secreting acid, therefore it is possible that bacteria such as *E. coli* which is
less acid-tolerant will increase in number [29]. It has also been reported that the incidence of gut-associated disease increases with age [30,31]. This may lead to increased access of antigens via the gut to circulation and result in elevated antibody levels. Increased antibody titres to *S. bovis* and *S. faecalis* have been reported in patients with colonic cancer [32].

In the present study reduced IgM antibody levels were observed in the oldest age groups both in response to the gut flora, *E. coli* and *S. faecalis*, and also in responses to oral flora. In agreement with our findings, an earlier study has demonstrated a significant reduction in levels of IgM antibodies to gut bacteria *Salmonella adelaide* in older subjects when compared with younger individuals and more recently reduced levels of serum IgM antibodies to dietary proteins have been observed in elderly subjects (68–91 years) [11,33]. The observed decline in IgM antibody levels may be explained by changes in the ageing immune system where, for example, impaired B cell differentiation involving aberrant terminal differentiation into IgM-immunoglobulin secreting cells has been observed [7,34]. This is in agreement with frequently reported alterations in levels of serum IgM [5,6,8].

In this study, we have expressed antibody activity as specific activity (EU/μg; Table 2) which is more appropriate in assessing the degree to which the immune system responds to a particular antigen [35]. Interestingly, specific activity to both oral organisms showed a decrease with age whilst gut flora did not. This suggests the possibility that the antigen handling of localised antigens in the oral cavity may differ from the more extensive system in the gut. Similarly the increased specific activity of IgA and IgG antibodies to *E. coli* with age may reflect chronic intestinal challenge. It is not clear whether there are comparable changes in the gut flora with age.

The results of this study indicated that the titre of specific IgM antibody was consistently low in the most elderly which may be due to a reduced levels of IgM immunoglobulin synthesis with age and which could account for the observed decrease in the specific antibody titre [8]. IgM antibodies represent the initial host response to antigenic challenge and a decrease in antibody titre may reflect an impairment in the circulating immune responses leading to increased incidence of disease and susceptibility to infections in the elderly [36].

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References

[12] Percival, R.S., Challacombe, R.S. and Marsh, P.D. (1991) Age-related microbial changes in the salivary and


