

Research article

## Elevated antibody to D-alanyl lipoteichoic acid indicates caries experience associated with fluoride and gingival health

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**Background:** Acidogenic, acid-tolerant bacteria induce dental caries and require D-alanyl glycerol lipoteichoic acid (D-alanyl LTA) on their cell surface. Because fluoride inhibits acid-mediated enamel demineralization, an elevated antibody response to D-alanyl LTA may indicate subjects with more acidogenic bacteria and, therefore, an association of DMFT with fluoride exposure and gingival health not apparent in low responders.

**Methods:** Cluster analysis was used to identify low antibody content. Within low and high responders (control and test subjects), the number of teeth that were decayed missing and filled (DMFT), or decayed only (DT) were regressed against fluoride exposure in the water supply and from dentrifice use. The latter was determined from gingival health: prevalences of plaque (PL) and bleeding on probing (BOP), and mean pocket depth (PD). Age was measured as a possible confounding cofactor.

**Results:** In 35 high responders, DMFT associated with length of exposure to fluoridated water (F score), PL and BOP ( $R^2 = 0.51$ ,  $p < 0.001$ ), whereas in 67 low D-ala-IgG responders, DMFT associated with PL, age, and PD ( $R^2 = 0.26$ ,  $p < 0.001$ ). BOP correlated strongly with number of 7 decayed teeth (DT) in 54 high responders ( $R^2 = 0.57$ ,  $p < 0.001$ ), but poorly in 97 low responders ( $R^2 = 0.12$ ,  $p < 0.001$ ). The strength of the PD association with DMFT, or of BOP with DT, in high responders significantly differed from that in low responders ( $p < 0.05$ ).

**Conclusion:** Caries associates with gingival health and fluoridated water exposure in high D-alanyl LTA antibody responders.

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### Background

Over the last 50 years, the widespread usage of fluoridated water and fluoridated dentrifices have been cited as major

reasons for a decline in caries since the early 1970s [1], and for the appearance of a significant association between oral hygiene and caries experience [2–4]. An inverse

relationship exists between salivary fluoride concentration and caries experience in the deciduous and permanent dentition [5], but fluoride concentration is excluded from most caries prediction models [6,7]. Acids in bacterial plaques cause caries in pits, fissures and interdental regions of teeth, but they also enhance the inhibitory effect of fluoride on demineralization, confounding the ability to predict caries from the salivary fluoride concentration [8,9].

The greater the consumption of dietary sucrose, the greater the fall in pH and fraction of acidogenic, acid tolerant bacteria in tooth adherent plaques [10,11]. The number of these bacteria (mostly mutans streptococci and lactobacilli), and the fluoride content, discriminate between severe and mild caries in 12–15 year-old children [12,13]. Acid-tolerant bacteria require D-alanyl glycerol lipoteichoic acid (D-alanyl LTA) in their membranes and cell surfaces [14]. D-alanyl LTA is made by esterifying carboxyl-activated D-alanine to glycerol in membrane LTA by means of a D-alanyl-carrier enzyme, DCP [15]. Strains of *Streptococcus mutans* in which DCP is inactive do not initiate growth at below pH 6.5 and make glycerol LTA without D-alanine [14]. In the DCP active strains, soluble D-Alanyl LTA is extruded into culture fluid *in vitro* [16,17] or plaque *in vivo* [18]. The D-alanyl esters are stable at pH 6.0 at 37°C, but hydrolyze to free D-alanine and LTA with a half-life of 3.9 h at pH 8.0 [19]. Healthy gingival sulci have a pH of 6.5 – 7.5 and inflamed sulci a pH of 7.5–8.5 [20].

About 30% of young adults have serum IgG antibodies that precipitate with D-alanyl LTA, but not with D-alanine-free LTA [17,21]. It is likely that plaques induce these IgG antibodies from gingival sulci that contain more acid-tolerant bacteria. An elevated IgG antibody response to D-alanyl LTA may therefore indicate the subjects in whom an inhibitory effect of fluoride on caries is enhanced. The fluoride concentrations of plaque and saliva are related to whether the drinking water is fluoridated [13] and to oral hygiene, which nearly always involves using a fluoridated dentifrice. The aim of this study was therefore to determine whether elevated antibody responders to D-alanyl LTA show a association of DMFT with fluoride exposure and gingival health not apparent in low responders.

## Methods

### Subjects

Antibody was obtained from blood from four sources: 1) 105 dental students, 2) 147 patients seeking dental treatment, 3) 145 volunteer blood donors (volunteers), and 4) 37 siblings aged 5 through 25 from six Amish families. The dental students and patients were attending the University of Oklahoma Health Sciences Center between

1985 and 1988. The volunteers and Amish family members were attending centers elsewhere in the US at the same time. All subjects consented to provide blood for antibody analysis according to local Institutional Review Board procedures (see Acknowledgements). The student, patient and volunteer populations (397 subjects) were used to determine what IgG concentration constituted an elevated antibody response to D-alanyl LTA, to ensure that these antibodies were not unique to dental or Oklahoma populations and to examine whether the antibody concentration was sex or age-associated. The Amish family siblings were selected to determine the frequency of high antibody concentration in children and young adults. Each sibling had at least one parent high responder to increase the likelihood of exposure to an antibody-associated oral microbiota from birth.

The clinical study participants consisted of 87 dental students (88.4% male) and 64 patients (31.3% male) who were medically healthy. All had 18 or more natural teeth and were aged >22 and <38 years. Of these participants, 67 dental students and 35 patients provided information that permitted an estimate of exposure to fluoridated water: residence(s) from birth through age 14 in the 1980 Fluoridation Census. Subjects not using the public water supply, or resident outside of the US for more than 18 months, were excluded. Exposure to water fluoridation scored 1 for each of five 3-year age cohorts: 0–2, 3–5, 6–8, 9–11 and 12–14 to give a fluoride exposure score (F Score) of 0 (no exposure) to 5 (complete exposure) described previously [22].

Most dental students had mild caries and gingivitis and most patients had moderate to severe caries and gingivitis. Exceptionally healthy or exceptionally diseased subjects were therefore increased compared to a similar number of subjects obtained as a random sample. This wide distribution of clinical measurements provided more stable estimates (narrower confidence intervals) of regression coefficients ( $\beta$ ) than would be obtained from a similar number from a random survey of the general population. Regression lines are more robust when a greater range of measurements is used [23].

### Clinical measurements

Dental caries experience was the number of Decayed, Missing and Filled Teeth (DMFT), excluding third molars and teeth reported missing for other reasons. Decayed teeth (DT) were also enumerated separately from missing and filled teeth (MFT). DT indicates a combination of delay in seeking therapy and faster development of new cavities. Fluoridated dentifrice use is related to oral hygiene but not toothbrushing frequency in adults aged as in the present clinical study [3] and young enough to have likely used fluoridated dentifrices from early childhood. Sensi-

tive staining for plaque accumulation [24] was therefore used with measures of gingivitis and pocket depth at the mesio-buccal, buccal, disto-lingual and lingual surfaces of the six teeth employed for the simplified oral hygiene index [25], substituting adjacent teeth as necessary (24 sites sampled).

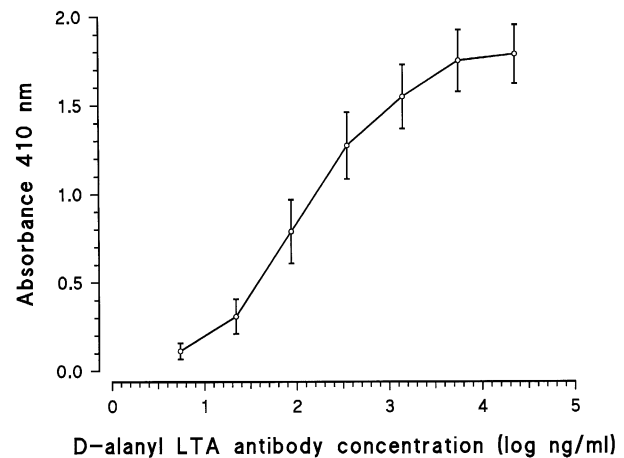
Gingivitis was determined by whether a site bled within 30 sec of gentle probing, BOP [26,27] and pocket depth by measuring the distance (mm) from the free gingival margin to the base of the sulcus or pocket. Finally, each subject was asked to suck an erythrosin tablet for 30 sec and the sites examined for stained plaque [24]. For each subject, the mean prevalences of plaque (PL) and bleeding on probing (BOP), and the mean pocket depth (PD), were calculated across all sampled sites. The clinical measurements were made by two experienced clinicians who were calibrated for this study. The clinicians agreed strongly with respect to all measurements (correlation coefficients,  $r > 0.85$ ;  $p < 0.001$ ) except gingival bleeding index, for which a weaker correlation was noted ( $r = 0.60$ ,  $p < 0.001$ ). The data reported are the mean measurements from the clinical examiners.

#### Antigen purification

D-alanyl LTA, but not D-alanine-free LTA, is present in culture filtrates of *Streptococcus mutans* GS5 [17]. Bacteria were grown at 37°C in trypticase soy broth to late stationary phase (96 h), when the maximal amount of antigen is extruded into the culture fluid [18]. After centrifugation to remove the bacteria, culture fluid (10 l) was concentrated 20-fold over a YM10 Diaflo Membrane filter (Amicon Corp., Beverly, MA). D-Alanyl LTA in the concentrate was detected by immunoelectrophoresis, using a standard human serum identified previously [16]. D-Alanine-free LTA does not react with this serum IgG [17,18,21]. The D-alanyl LTA was purified by passing the concentrated culture fluid over a 90 × 2.5 cm Sephacryl column in 0.4 M NaCl buffered with 0.05 M sodium acetate pH 5. Antigen in the fractions was collected. After equilibrating with 5 mM sodium acetate buffer pH 5.0, it bound to a short Sephacryl S-200 and eluted by adding 14 mM NaCl as described previously [16].

#### Measuring antibody content and determining high and low responders

IgG antibody content was measured by enzyme-immunoassay employing a Fast Assay Screening Test System at room temperature [28]. Pegs protruding from a lid were placed over a 96-well plate or trough containing 14 ml of 10 µg/ml D-alanyl LTA in acetate buffer pH 5 for 2 h (Becton Dickinson, Lincoln Park, NJ). The pegs were blocked with 14 ml of phosphate buffered saline (PBS) pH 7.0 in 1.0% Tween-20 and immersed in wells containing 0.1 ml serum. After overnight incubation, excess IgG antibody



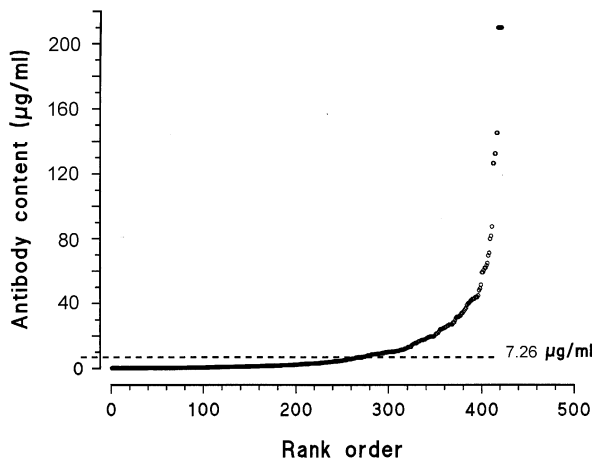
**Figure 1**  
Graph of absorbance at 410 nm against log ng/ml of antibody to D-alanyl LTA. Vertical lines indicate the standard deviation of the measurements.

from the serum was washed away by thrice transferring the pegs to troughs containing 14 ml of PBS containing 0.05 % Tween 20 (PBS-Tween) for 5 min each time. The pegs were then immersed for 2 h in a trough containing 14 ml of anti-human IgG F(ab'2) fragment conjugated to alkaline phosphatase in PBS-Tween and developed with nitrophenyl phosphate (Sigma Chemical Co. St Louis, MO).

The concentration of antigen-specific IgG in standard serum was obtained by measuring the optimal amount of protein immunoprecipitated [16], and a standard curve of absorbance against concentration was obtained (Fig. 1). The greatest range of absorbance occurred when sera were measured at a dilution of 1:200 [28]. Replicate antibody assays were performed on each serum and the concentrations read off the standard curve. The antibody concentrations are shown ranked in Fig. 2.

#### Data analyses

The amount of IgG antibody to D-alanyl LTA varies with no obvious cutoff (Fig. 2). However, the sera containing precipitating antibody should tend to have high IgG antibody contents. The IgG antibody measurements were alternatively divided into clusters, using the unweighted pair group method with arithmetic averages [29] and NT-SYS, a package of multivariate statistical computer programs [30]. A low response supremum was obtained by taking the antilog of the mean IgG content of the non-precipitating sera plus one standard deviation, or the antilog of the highest IgG content of the cluster grouping containing the least antibody.



**Figure 2**  
Graph of ranked antibody contents. The cut-off points separates high from low responders (see Methods).

The effect of age was determined after splitting the subjects into decile cohorts (Table 1) and comparing the fraction of high antibody responders in each cohort. The youngest cohort was composed of Amish family siblings who were younger than any dental students, patients or volunteers.

A multiple linear regression procedure was utilized to examine the relationship of caries (DMFT) with age, F score, and measures of gingival health obtained in this study: PL, BOP, and PD. The regression on DMFT was used: 1) to estimate the partial regression coefficients ( $\beta$  coefficients) within the high and low antibody response group; 2) to test each  $\beta$  coefficient for significance after accounting for the effects of the other four variables; and 3) to examine for significant differences in  $\beta$  coefficients between the antibody response groups. A  $\beta$  coefficient is interpreted as the change in disease response (DMFT) per unit change in one of the independent variables after adjusting for all the other independent variables in the model. Within each antibody response group, the multiple regression coefficient ( $R^2$ ) provided an estimate of the proportion of variance in DMFT explained by the combination of variables tested. Stepwise regression then identified the best estimate of the variance in DMFT that was explained by multiple variables in the separate and combined high and low responder groups. These multiple regression analyses were repeated using gingivitis (BOP) as the dependent variable and OHPI, PD, DT and MFT and age as independent variables. All of the clinically examined subjects were included because F score was not an independent variable for BOP.

**Table 1: Age decile cohorts for determining changes in fraction of high antibody responders**

Cohort No.	Decile	<sup>a</sup> Number in Cohort	High Responders
1	5-15	<sup>b</sup> 19	<sup>c</sup> 21.05%
2	15-25	<sup>b</sup> 97	35.05%
3	25-35	176	36.36%
4	35-45	76	40.79%
5	45-55	31	38.71%
6	55-65	13	<sup>c</sup> 23.08%
7	65-72	6	<sup>c</sup> 16.67%

<sup>a</sup>381 subjects after excluding all Amish family members and 16 of the 397 dental student, patient and volunteer subjects whose age was not recorded. <sup>b</sup>The Amish family siblings <15 years comprised Cohort 1 and those >15 years were included within Cohort 2. <sup>c</sup>Comparison of cohorts 1, 6 and 7 with the remainder:  $\chi^2 = 3.21$ ,  $p = 0.073$  (not significant).

To ensure that obtained relationships were robust, influential points (outliers) were identified using a statistic (DFFITS) which measured the change in coefficients caused by removing the data for each subject. If this change exceeded  $2\sqrt{(p/n)}$ , where  $p$  was the number of independent variables and  $n$  the number of samples [31], the point was influential. Repeating the regressions with all the influential points removed determined the degree to which these points had affected the results.

**Results**

**Definition of high antibody response**

IgG antibodies were initially detected in sera irrespective of whether D-alanyl LTA was immunoprecipitated. Excluding the Amish family group, there were 288 subjects whose sera failed to immunoprecipitate antigen (detected by immunoelectrophoresis). The mean IgG antibody concentration (log ng/ml) was 3.19 (0.64 standard deviation, s.d.) compared with 4.25 (0.57 s.d.) for 109 subjects whose sera did precipitate antigen ('t' test  $p < 10^{-6}$ ). High responders therefore had a log antibody content (ng/ml) that exceeded the mean plus standard deviation of non-precipitating serum (log ng IgG/ml >3.83). However, low IgG concentrations formed a cluster whose supremum (log ng IgG antibody/ml) was 3.861, which corresponds to 7.26 µg/ml (left side of Fig. 2). Subjects were therefore classified as low responders if their log IgG antibody content exceeded 3.86 rather than 3.83. The odds ratio for a serum from a high antibody responder immunoprecipitating D-alanyl LTA was 14 times greater than for a low responder.

**High antibody response, age and gender**

Of the 397 students, patients and volunteers, 16 did not have their age recorded. High responders had a mean age of 32.7 years ± 9.4 s.d. (136 subjects) and low responders a mean age of 33.0 years ± 10.7 s.d. (245 subjects). Table 1 shows the fraction of high responders in different age cohorts. There was a high responder frequency of 35–40% from age 15 through 54. The reduced frequencies of high response in childhood and old age were not significant. However, within the Amish family offspring high IgG responders were significantly older. Table 2 shows that the high responder offspring had a mean age of 17.1 years compared with 13.3 years for low responders. This significant difference ('t' statistic = 2.42, degrees of freedom, d.f. = 35; p < 0.03) was due to few high responder children and young teenagers and a slightly greater fraction of siblings aged 15–25 years who were high responders (50%) compared with the general population.

**Table 2: Age of siblings from 6 families with at least one high responder parent**

Family <sup>a</sup>	High responders Ages	Low responders Ages
F+M-	17	16,14,13,12,10,8
F+M+	20,13	21,18,16,8,5
F+M+	22,20,18	25,16,14
F?M+	None	12,11,10,9,8
F+M-	22,20,13,11	17
F+M'	17,16,13	20,18,10,7
<sup>b</sup> Mean age (s.d) <sup>c</sup>	17.08(3.68)	13.25(5.00)

<sup>a</sup>F, Father; M, Mother; +, high responder; -, low responder, ? Not known. <sup>b</sup>Mean age of the high and low responders <sup>c</sup>s.d., standard deviation.

**Table 3: Fraction of high antibody responders in or not in the clinical study.**

Subject group	Number	% high responders
Dental students in clinical study <sup>a</sup>	87	37.9
Patients in clinical study <sup>a</sup>	64	32.8
Same-age subjects not in clinical study	129	31.8
Other subjects not in clinical study <sup>c</sup>	117	39.7
All subjects	397	33.8

<sup>a</sup>See first section of Materials & Methods. <sup>b</sup>Same-age subjects not in the clinical study were 12.4% dental students, 17.1% patients and 70.5% volunteers. <sup>c</sup>Other subjects were a mixture of patients and volunteers: 70.9% older (ages >38 and <72 years), 15.4% younger (ages >15 and <22 years), and the remainder age unknown.

High antibody responders accounted for a similar fraction of subjects irrespective of whether they were in the clinical study, or dental students, or patients (Table 3). Men were 49.1% of the 395 students patients and volunteers whose sex was recorded. Men had also a greater frequency of high response 40.21% vs 31.34% and a greater mean log ng/ml IgG antibody content, 3.52 ± 0.76 standard deviation (s.d.) vs 3.44 ± 0.80 s.d. Neither of these differences were significant (X<sup>2</sup> = 3.00, p = 0.09; 't' statistic = 0.34, p = 0.73). In serum samples from 18 subjects aged between 22 and 38, the IgG antibody concentrations were essentially the same after 6 months as originally estimated (squared correlation coefficient, R<sup>2</sup> >0.95, p < 0.01). The results indicate that, for subjects aged 15 – 55, age and sex had little effect on the frequency of high D-alanyl LTA antibody response.

**Association of DMFT with gingival health and fluoride in high and low responders**

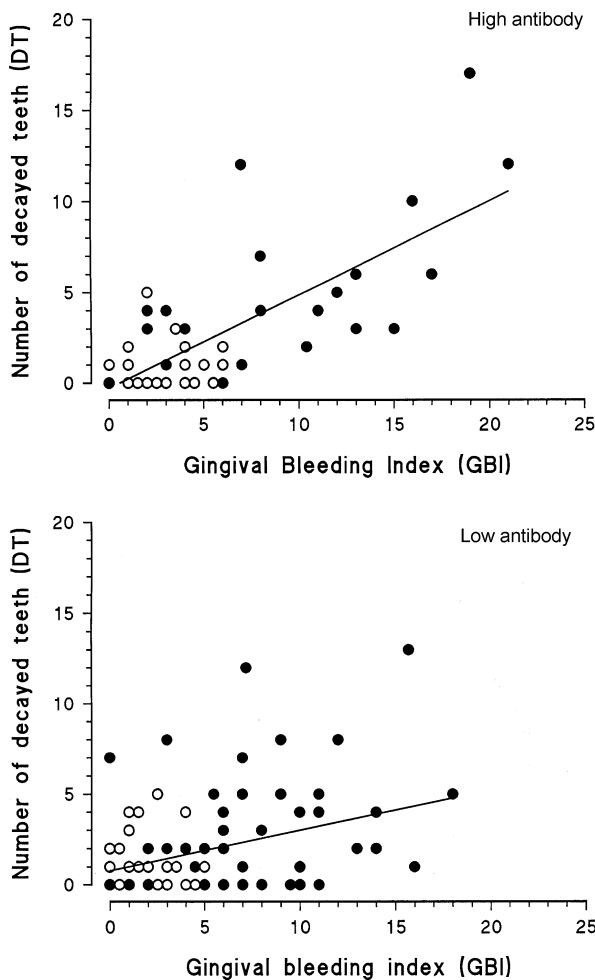
Table 4 lists the variables tested for association with DMFT and the observed β coefficients in high and low antibody responders. It was immediately apparent that the β coefficients from PL were similar in both groups, whereas those from BOP and F score were only significant in high responders and those from PD were only significant in low responders. Comparison of the differences in β coefficients between high and low responders, column 4 (column 2 – column 3), indicated relationships of DMFT to pocket depth that were significantly different and relationships of DMFT to F score that were almost significantly different, p = 0.062 (Table 4, column 4).

**Table 4: Changes in the equality of the partial β coefficients for association of tested variables with caries severity in high and/or low responders<sup>a</sup>.**

Variable <sup>a</sup>	High responders <sup>b</sup> n = 35	Low responders n = 67	Difference (Hi – Low)
F score	<sup>c</sup> -0.847	0.006	<sup>d</sup> -0.853
PL	<sup>d</sup> 0.259	<sup>d</sup> 0.234	0.025
BOP	<sup>c</sup> -0.447	0.167	0.280
PD	-1.903	<sup>c</sup> 4.249	<sup>c</sup> -6.152
Age	0.099	<sup>d</sup> 0.261	-0.162

<sup>a</sup>Variable names are defined under "Clinical Measurements" in the Methods <sup>b</sup>n = number of subjects <sup>c</sup>p <0.05 for value of constant or variable (β) or for all values in indicated model. <sup>d</sup>p > 0.05 & < 0.15. If no subscript, p > 0.15 <sup>e</sup>HLS: high response = 1, low response = 0.

Stepwise regression confirmed the similar associations of DMFT with plaque prevalence in both high and low IgG antibody responders, but significant associations with only F score and BOP prevalence in high responders and



**Figure 3**  
 Graph of number of decayed teeth against gingival bleeding index. Results are provided separately for the dental students (o) and patients (\*). Data from more than one subject are superimposed. Regression line equation, high responders (upper graph):  $DT = 0.123 \text{ BOP} - 3.65$  Regression line equation, low responders (lower graph):  $DT = 0.053 \text{ BOP} - 15.11$

with only PD and age in low responders. In high responders, DMFT increased as plaque and BOP prevalences increased and fell as fluoride exposure increased. The equation obtained was:  $DMFT = 4.60 + 0.28 \text{ PL} + 0.39\text{BOP} - 0.88\text{FScore}$  ( $R^2 = 0.51$ , F statistic = 10.75,  $p < 0.0001$ ). By contrast, in low responders, DMFT increased with plaque prevalence, pocket depth and age. The equation was:  $DMFT = -13.37 + 4.58 \text{ PD} + 0.27 \text{ Age} + 0.30 \text{ PL}$  ( $R^2 = 0.26$ , F statistic = 7.41,  $p < 0.0003$ ). Within each equation, the constant and the respective  $\beta$  coefficients were significant ( $p < 0.05$ ), except for the  $\beta$  coefficient of age in low responders ( $p = 0.062$ ). High responders receiving fluoridated drinking water for all 14 years of child-

hood (F score = 5), had a significantly lower DMFT than those never receiving fluoridated water (F score = 0):  $DMFT = 7.50 \pm 4.52$  (s.d.) vs  $11.60 \pm 4.06$  (s.d.); 't' test  $p < 0.04$ . This was not true of low responders in whom the difference between F score 5 and F score 0 was not significant ( $DMFT = 9.33 \pm 5.72$  vs  $12.07 \pm 5.85$ ; 't' test  $p = 0.14$ ).

When antibody was ignored in stepwise regression (control), DMFT increased with age, PL and BOP, and decreased with F score:  $DMFT = -2.11 + 0.17 \text{ Age} + 0.25 \text{ PL} + 0.27 \text{ BOP} - 0.39 \text{ FScore}$  ( $R^2 = 0.29$ , F statistic = 6.62,  $p < 0.0001$ ). PL and BOP were individually significant. F Score and age were borderline,  $p = 0.09$  and  $0.14$  respectively, and PD was not significant, being entirely replaced with age. Despite the subjects increasing to 102 (from 35 or 67) the strength of association was similar to that of low responders only.

**Association of DT with gingivitis in high and low responders**

Because caries experience associated with fluoride and gingival health in high responders, poor gingival health should increase the number of decayed teeth (DT) more than in low responders. When BOP was regressed against the variables in Table 5, only DT (column 4) differed significantly between the high and low responders. Although DT alone significantly correlated with BOP in both response groups ( $p < 0.01$ ), it associated with BOP much more strongly in high responders ( $R^2 = 0.57$ ) than in low responders ( $R^2 = 0.12$ ). Fig. 3 shows the respective correlations, and also the patient data (filled circles) skewed by few healthy subjects and the student data (unfilled circles) skewed by few moderate and no severely diseased subjects. Clearly, combining students and patients strengthened the respective associations ( $\beta$  coefficients). Stepwise regression indicated that, excluding DT, BOP associated with PL and PD similarly ( $BOP = -14.02 + 0.42 \text{ PL} + 4.8 \text{ PD}$  in high responders and  $BOP = -11.07 + 0.34 \text{ PL} + 4.10 \text{ PD}$  in low responders;  $R^2 = 0.40$  and  $0.41$  respectively;  $p < 0.001$ ). However, DT explained more variance ( $BOP = -1.46 + 0.24 \text{ PL} + 0.95 \text{ DT}$ ,  $R^2 = 0.62$ ,  $p < 0.001$ ) in high responders, and less variance ( $BOP = -3.25 + 0.41 \text{ PL} + 0.33 \text{ DT}$ ,  $R^2 = 0.31$ ,  $p < 0.001$ ) in low responders.

Influential points (outliers), whose presence might have affected the strength and significance of these complex regression analyses, were identified in five low responders and two high responders. When these subjects were omitted, the respective regression coefficients or their significance were little changed, indicating that the different, partial, linear regression coefficients in high or low responders were not artifacts of influential or outlying points.

**Table 5: Changes in the equality of the partial  $\beta$  coefficients for association of tested variables with gingivitis (BOP) in high and/or low responders.**

Variable <sup>a</sup>	High responders n = 54	Low responders n = 97	Difference (Hi - Low)
PL	<sup>d</sup> 0.182	<sup>c</sup> 0.321	-0.140
PD	<sup>c</sup> 2.529	<sup>c</sup> 3.588	-1.059
DT	<sup>c</sup> 0.906	0.202	<sup>c</sup> 0.703
MFT	0.154	0.054	0.099
Age	0.022	-0.053	0.074

<sup>a</sup>Variable names are defined under "Clinical Measurements" in the Methods <sup>b</sup>n = number of subjects. <sup>c</sup>p < 0.05 for value of constant or variable ( $\beta$ ). <sup>d</sup>p > 0.05 & < 0.15. If no subscript, p > 0.15.

## Discussion

This study has demonstrated that IgG antibodies to D-alanyl LTA are widespread in US adults. The fraction of high responders was essentially constant from early adulthood through middle age, but reduced in children (<15 years) and old age (>55 years). Within the adults (ages 15–55 years) a change from low to high response or *vice versa* was found unlikely from repeated measurements over 2–6 months. A similar lack of change in this IgG antibody concentration was reported 2–3 months after an additional 26 similarly aged patients had received oral hygiene therapy in another study [32]. Finally, the family studies established that a high antibody response was probably induced during the mid-teenage years. In order to apply the results of this study to children and young teenagers, longitudinal studies of the antibody response in relation to age and the clinical measurements in this study may need to be undertaken.

Despite few investigations of caries risk in 22–38 year old subjects compared with a younger or older group [7], the association of DMFT with PL in this study agrees with that obtained from 35 year old Norwegians [3]. Plaque (simplified oral hygiene index measurement) accounted for 15% of the variance in number of decayed/filled teeth surfaces in that study, and for 19% of the DMFT variance within all 151 clinically examined subjects in this study (ignoring age, antibody and all other variables). Other studies have shown that the amount of fluoride applied from dentrifices is measured better from oral hygiene or plaque accumulation, as in this study, and not from the reported frequency of dentrifice use [2,3]. Finally, because subjects aged more than 38 are unlikely to have used fluoridated toothpastes until later in life, they were omitted to avoid confounding the results.

The rationale behind this study was that acidic plaque environments increase the amount of D-alanyl LTA and promote its immunogenicity. Accordingly, caries-protection by fluoride in the water supply and dentrifices was strong in high IgG antibody responders, accounting for just over 50% of the variance in DMFT. In addition, gingivitis (BOP prevalence) associated strongly and significantly with the number of decayed (untreated) teeth, suggesting that preventing gingivitis increased fluoride exposure from dentrifices and reduced the number of decayed teeth. Increased exposure to fluoridated water, and dentrifices associated with good gingival health, may result in fluoride inhibiting enamel remineralization at the acidic plaque pH likely present in high responders.

In low responders, the fewer antibodies to D-alanyl LTA suggest less colonization by acid-tolerant bacteria and a weaker cariogenic attack. DMFT associated with age, as reported for other subjects whose sera did not precipitate D-alanyl LTA [22], and also with pocket depth. An increase in pocket depth is caused by periodontopathic bacteria that associate with an alkaline environment in the sulci over many years [20] and a microbiota that is neither acidic nor acid-tolerant [10]. The coefficient of DMFT association with PD in low responders therefore differed significantly from high responders within whom F score and gingival health were stronger covariates.

## Conclusions

An increased mutans streptococcal challenge accompanying low plaque pH (high antibody response to D-alanyl LTA) allows much of the variation in caries experience to be controlled by water fluoridation and by the use of fluoridated dentrifices associated with maintaining oral health. High IgG antibody responders are therefore better protected from caries in an optimally fluoridated environment. The concept that fluoride protects better from caries in a low pH environment [12] was recently used to explain why there is a poor association between caries experience and pH fall after a 10% sucrose rinse [33]. In low responders, increased fluoride exposure from dentrifice use to maintain oral health, or from water fluoridation, associate relatively poorly with caries experience. Although this study has indicated that the IgG antibodies to D-alanyl LTA do not become elevated until after age 17, when much caries may have already developed, they may be elevated to a lower level in children who eventually become high responders. The D-alanyl LTA antibody response is not detectable in saliva (unpublished studies), but it can be measured from only a thumb-prick of blood. Longitudinal studies of the D-alanyl LTA response in children could improve current efforts to predict caries susceptibility by relating it to fluoride or the fluoride ion product for fluoroapatite in saliva and the pH change after a sucrose rinse [5,12,13,33].

## Competing interests

None declared

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