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Low salivary IgA activity to cell-surface antigens of mutans streptococci related to HLA-DRB1*04

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*Wallengren MLL, Hamberg K, Ericson D, Nordberg J. Low salivary IgA activity to cell-surface antigens of mutans streptococci related to HLA-DRB1*04.*
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Background/aims: Mutans streptococci are found in almost all individuals, though there are large differences in colonization levels between individuals. These differences are not readily explained, though several factors are believed to influence the colonization. One factor is the immune response to mutans streptococci, mainly provided by salivary immunoglobulin A (IgA). In a previous study, differences in salivary IgA reactions to oral streptococci were observed between human leukocyte antigen (HLA)-DR4-positive and DR4-negative individuals. A lower salivary IgA activity to *Streptococcus mutans* in particular was most pronounced for two DR4 subgroups, DRB1*0401 and *0404. The main purpose of this study was to further investigate, in a larger study group, the salivary IgA activity to antigens of three oral streptococci in relation to different HLA-DRB1*04 alleles.
Methods: Stimulated saliva was collected from 58 HLA-DRB1*04-positive individuals. Whole cell antigen extracts from *S. mutans*, *Streptococcus sobrinus* and *Streptococcus parasanguis* and the streptococcal antigen (SA) I/II were separated in SDS-PAGE, transblotted and detected with diluted saliva (Western blot), and analyzed in a computer program. All distinct immunoblot bands over 100 kDa were recorded and compared in relation to DRB1*04.
Results: The immunoblots revealed lower salivary IgA reactions to *S. mutans*, *S. sobrinus* and SA I/II, but not to *S. parasanguis,* for the DRB1*0401- and *0404-positive individuals compared to other DRB1*04 types. For the *0401 subgroup there was a significant association with a lower IgA response to *S. mutans*.
Conclusion: The results confirm earlier observations and may also support previous demonstrated association between colonization by mutans streptococci and the serologically defined HLA-DR4.

Key words: cell-surface antigen; HLA; oral streptococci; salivary IgA; Western blot

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Mutans streptococci are considered the principal bacteria causing dental caries in humans (9, 18). They are found in almost all individuals, though there are large variations in levels of colonization (2, 37). These interindividual variations in colonization are believed to depend on several factors (4–7, 13, 24, 35, 36). Also the immune factor has been intensely investigated, especially in the search for a caries vaccine (8, 34).

The specific immune response of the individual to mutans streptococci is believed to be provided mainly by salivary immunoglobulin A (IgA) (30), which is generated by the common mucosal immune system (21). However, studies aimed at correlating salivary IgA antibodies against mutans streptococci with resistance or susceptibility to dental caries have produced variable and conflicting results (30). One reason for this might be that IgA has often been assessed against whole cells of mutans streptococci instead of antigens relevant for colonization, and therefore specific antibodies were not analyzed. Widerström and coworkers (41) observed

that salivary IgA reacts with a number of proteins separated from mutans streptococci, and that the reaction pattern often was unique for a person, though a certain familial association was observed (42).

The human leukocyte antigen (HLA) complex is an inherent system of alloantigens that are the products of genes of the human major histocompatibility complex (MHC). Many studies have looked for associations between HLA and diverse diseases, since the HLA cell surface molecules perform an essential function in the immune response, and several studies have reported an important role for both inherited (HLA) as well as environmental factors in the induction of many diseases (10). It has also been suggested that the MHC alleles might be crucial in the resistance or susceptibility to dental caries (14, 16, 32).

In 1991, the present study group reported a possible relationship between colonization by mutans streptococci and a HLA class II allele, DR4 (39). In a later study by the same group, it was observed that HLA-DR4-positive, compared to DR4-negative, individuals demonstrated a weaker salivary IgA activity to antigens separated from *Streptococcus mutans* (19). This lower salivary IgA activity was the most pronounced among individuals expressing certain HLA–DR4 subgroups, such as DRB1*0401 and 0404, though the number of HLA–DR4-positive subjects, a total of 19, was too small for additional analyses on a subgroup level. Another shortcoming was that the immunoblot analyses were extensive and intricate to analyze, since as many as 12–15 immunoblot bands were detected for each bacterial extract. However, the significance of five to six high molecular weight bands, i.e. bands with a molecular weight over 100 kDa, has been demonstrated in a recent study of ours (20). These bands were found to likely represent important cell-surface antigens and it was concluded that, in future studies with larger study groups, it would be relevant to focus on these high molecular weight bands.

The main purpose of this study was to further investigate, in a larger study group, a possible correlation between different HLA–DRB1*04 alleles and the salivary IgA activity to important cell-surface antigens of mutans streptococci.

Material and methods

Subjects

Fifty-eight healthy HLA-DR4-positive subjects (23–75 years old, 36 females and 22 males of northern European heritage) agreed to participate in the study. Thirty-eight of the subjects had already participated in a previous study by the present group (39). The new participants were employees at the Blood Bank in Lund. HLA–DR4 subtyping, DRB1*04, was performed using standard techniques (26, 27) at the Blood Bank, Lund University Hospital, Sweden. Also five HLA–DR4-negative subjects participated as saliva donors for a negative saliva pool. The Ethical Committee at Lund University, Sweden, approved the study.

Saliva samples

New saliva samples were obtained from all participants. Approximately 10 ml of whole paraffin-stimulated saliva was collected from each subject, and the collecting time measured. One hour prior to the collection, the subjects had refrained from eating, drinking and smoking.

The saliva was stored at −20°C until analyzed. Before use, the samples were thawed, clarified by centrifugation at $1520 \times g$ for 10 min at 4°C and then diluted (1 : 4) using Tris-buffered saline (TBS), pH 7.4. The total amount of salivary IgA was measured by the modified method (3) for immunobead enzyme-linked immunosorbent assay (ELISA) of Sack and coworkers (31). The protein concentration of the saliva samples was determined using Bio-Rad Laboratories protein assay (Richmond, CA).

For estimation of the salivary levels of mutans streptococci, the 'Strip mutans®' test (Orion Diagnostica, Espoo, Finland) was performed for each subject and the salivary levels of mutans streptococci were estimated according to the manufacturer's

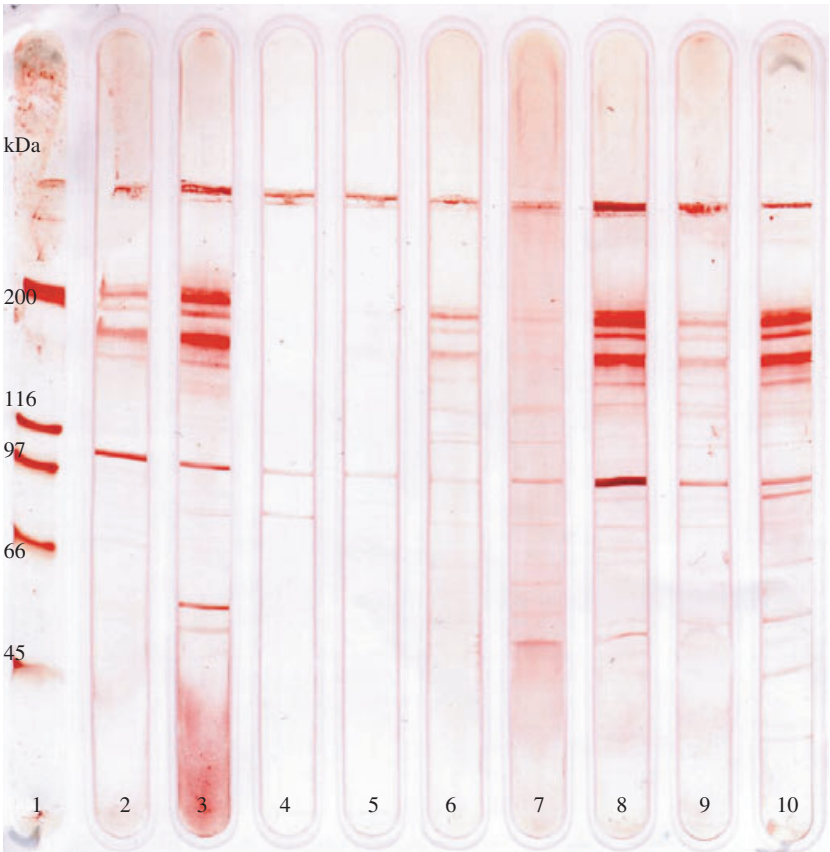

Fig. 1. Representative immunoblot of salivary IgA activity in different DRB1*04 salivas (lanes 2–8), DRB1*0401 positive pool (lane 9) and DR4-negative pool (lane 10) to antigens separated from *S. mutans*. In lane 1 is a high molecular weight standard.

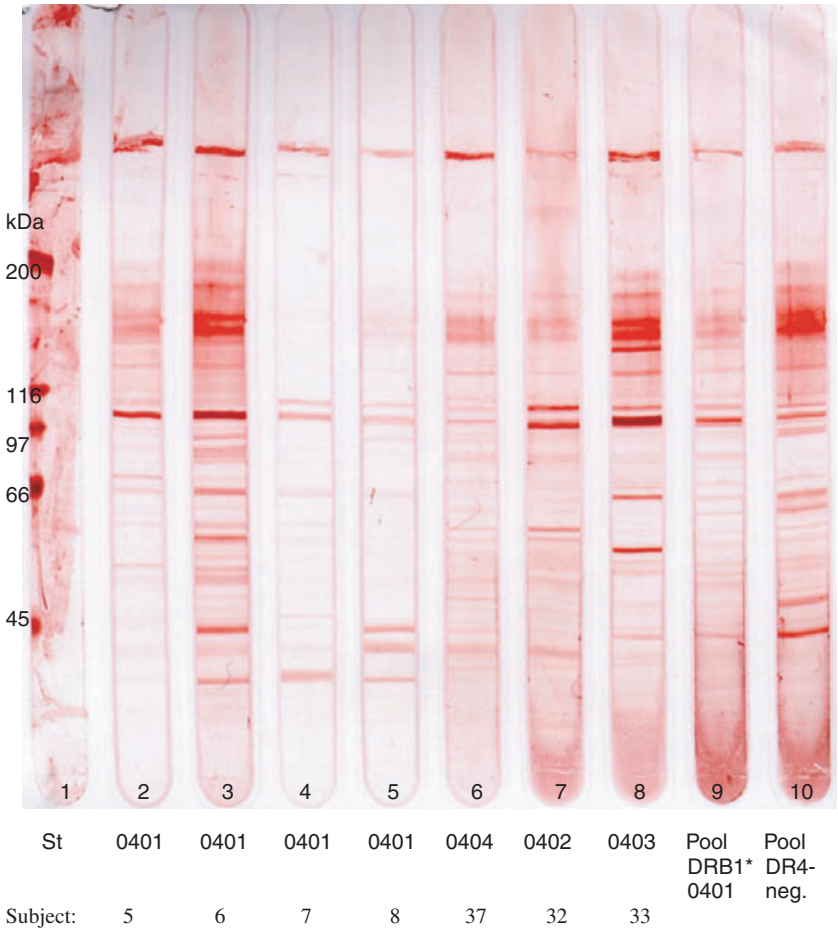

Fig. 2. Representative immunoblot of salivary IgA activity in different DRB1*04 salivas (lanes 2–8), DRB1*0401-positive pool (lane 9) and DR4-negative pool (lane 10) to antigens separated from *S. sobrinus*. In lane 1 is a high molecular weight standard.

instructions (11). The test strips were then stored at −20°C.

Two saliva pools were prepared for the study. Equal amounts of saliva from the five HLA–DR4-negative subjects were mixed together for a DR4 negative saliva pool, and saliva samples from all DRB1*0401-positive subjects were mixed together for a DRB1*0401 pool.

Antigen preparation, SDS-Page and Western blot analysis

The preparation of antigen extracts from whole cells of *S. mutans* serotype c (strain KPSK2), *Streptococcus sobrinus* serotype g (strain OMZ65) and *Streptococcus parasanguis* (strain NT62) was performed in accordance with the methods described by Renneberg et al. (28) and as modified by the Widerström group (41). Briefly, the cells were washed in phosphate-buffered saline, pH 7.2, suspended in HEPES in Tris and mechanically broken by sonication three times for 5 min each, at 80 W in an ice-chilled cup and thereafter centrifuged for 15 min at 5000 × *g*. The streptococcal antigen (SA) I/II (variously designated as SpaP, PAc, protein B, P1) from *S. mutans* was also included in the study. Purified SA I/II was the kind gift of Professor Lehner and colleagues (29). The protein concentration of the prepared antigen extracts was determined by Bio-Rad Laboratories protein assay. The extracts were thereafter stored at −20°C until analyzed.

The antigen extracts were thawed and adjusted, using TBS, to the same protein concentration, 1 mg/ml, before the electrophoretic separation of the antigens was carried out in a 7.5% SDS-PAGE for 55 min at 190 V (15). As molecular weight markers, Bio-Rad Laboratories high molecular weight markers were used. The proteins were transblotted to Immobilon membrane (Millipore Intertech, Bedford, MA) (38).

Western blot analysis (38) was performed to detect salivary IgA antibodies to the antigen extracts. Briefly, the membranes were rinsed repeatedly with TBS and nonspecific binding sites were blocked with 5% nonfat milk in TBS for 1 h. Thereafter the blots were washed three times for 5 min each in 0.1% nonfat milk in TBS and incubated with the appropriate diluted saliva overnight. A new wash, as described above, followed and peroxidase conjugated rabbit alpha-chain specific anti-human IgA HRP (Dako, Copenhagen, Denmark) was added for 2 h. The membranes were then rewashed as previously and stained for 15 min with peroxidase substrate in a buffer with sodium acetate. Finally, the membranes were repetitively washed with distilled water. The Western blot was performed at room temperature on an Orbital shaker.

Immunoblot analysis

For all 58 subjects, the salivary IgA activity to each of the four antigen extracts was revealed. For each antigen extract, nine immunoblot assays were performed, and the salivas were always present together with the same samples on each blot irrespective of antigen extract. One DRB1*0401-positive and one DR4-negative saliva pool was also included in each immunoblot, as a control and reference, in order to be able to evaluate any divergences between different assays.

The developed immunoblot membranes were scanned into a computer program for analysis (The Discovery Series™, Quantity One® SW, PC, Bio-Rad). First, all lanes, and then all bands in each lane, were defined. The molecular weight and intensity of all defined bands were determined. Reports of all lanes in each immunoblot were made and the total trace intensity of identified bands in each lane was thereafter calculated. The trace intensity of a band corresponds to the area under its curve. Graphs were also printed of each lane, and distinct bands, i.e. bands with peak intensity $\geqslant$ 10 units in the graphs, were recorded and the total number of distinct bands calculated for each subject. The trace intensity as well as number of distinct bands was recorded primarily for bands with a molecular weight over 100 kDa, likely representing cell-surface antigens and therefore relevant (20). All analyses were done by one of the authors without knowledge of the identity of current salivas.

Comparisons between lanes with different DRB1*04 salivas were made for each bacterial extract as well as for SA I/II. The trace intensity was used for ranking within

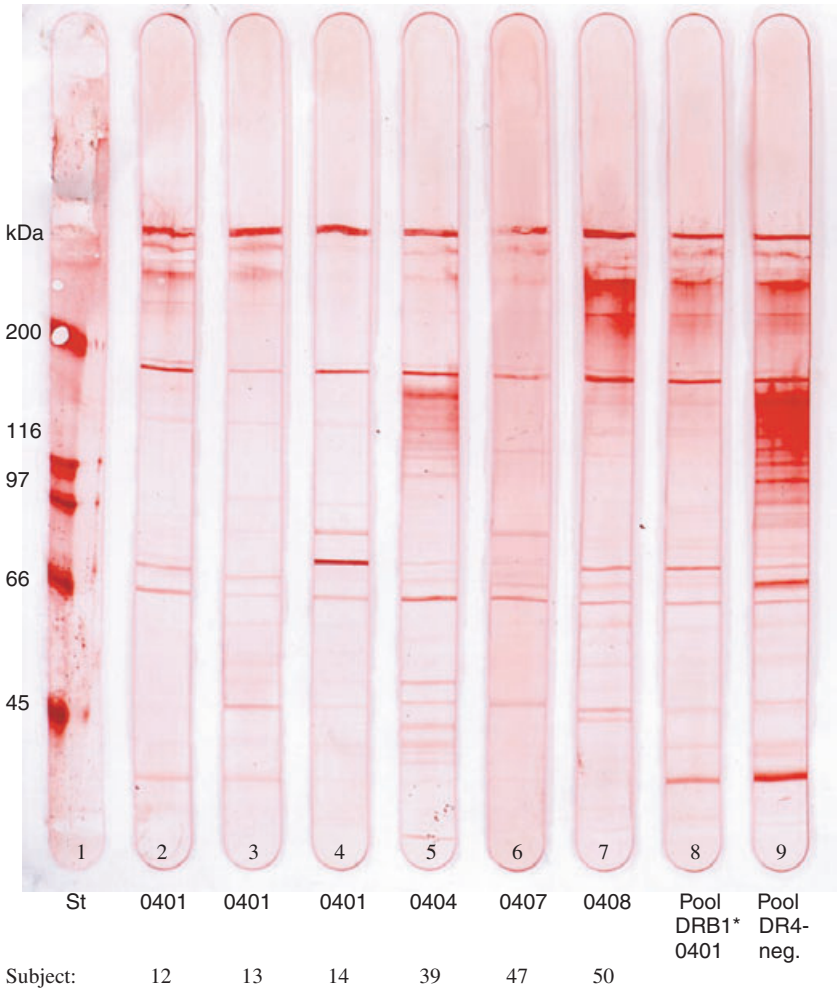

Fig. 3. Representative immunoblot of salivary IgA activity in different DRB1*04 salivas (lanes 2–8), DRB1*0401-positive pool (lane 9) and DR4-negative pool (lane 10) to antigens separated from *S. parasanguis*. In lane 1 is a high molecular weight standard.

each blot. For possible comparisons between blots, a 'relative trace intensity' ratio was introduced. This ratio was calculated by dividing the trace intensity of each lane by the trace intensity measured for the reference DR4-negative pool present on the same blot. The median 'relative trace intensity' was then compared between three DRB1*04 groups – *0401, *0404, and 'Other' – i.e. the remaining subgroups, using the Kruskal–Wallis test for overall comparisons. The Mann–Whitney *U*-test with Bonferroni correction for multiple comparisons was used for paired analysis between the groups.

Results

In Table 1, the main results obtained from the 58 participants are presented. These include the distribution of DRB1*04 alleles, Strip mutans class, total IgA, percent IgA in relation to total protein, and the number of distinct bands with a molecular weight > 100 kDa recorded for each antigen and individual. Three individuals were HLA–DR4 homozygotes, though heterozygote at subgroup level.

The most significant observations from the *entire* immunoblot analysis of each antigen extract are briefly described below. In addition, the results are illustrated by printouts of representative immunoblots, one of each antigen (Fig. 1–4) and by one intensity graph, visualizing observed dissimilarities between the two saliva pools (Fig. 5).

S. mutans

The most significant reactions, as well as the major differences between diverse DRB1*04 alleles, were noted for bands with a molecular weight > 100 kDa, i.e. likely cell-surface antigens. One intense, and so far unidentified, band at ~ 100 kDa was observed for almost all saliva. A couple of bands, often weak, with a molecular weight below 100 kDa were also usually detected for most of the individuals. In five individuals, four DRB1*0401- and one DRB1*0404-positive, no distinct, high molecular weight bands were found. Moreover, for one *0401-positive individual no distinct bands at all were seen (Table 1 and Fig. 1).

S. sobrinus

The most intense reactions for *S. sobrinus*, as well as the most significant differences between different DRB1*04 alleles, were noted in the high molecular weight region of the blots. In addition, two to three quite strong bands were often detected around 100 kDa. For most individuals, several bands with a molecular weight below 100 kDa were observed. In six individuals, all DRB1*0401-positive, no distinct, high molecular, bands at all were seen (Table 1 and Fig. 2).

S. parasanguis

For almost all subjects, there was a cluster of intense bands with a molecular weight over 200 kDa, as well as one intense band, often together with one weak band, at ~ 190 kDa. Some saliva samples also revealed five to six very tight bands around 150–180 kDa. For almost all individuals, several bands with differing molecular weights below 100 kDa were observed. The individual immunoblot profiles of the different salivas to *S. parasanguis* seemed to diverge more than did the immunoblot profiles to the other antigen extracts, and all individuals revealed at least one distinct band to the antigen extract (Table 1 and Fig. 3).

SA I/II

In nearly all individuals, one wide, intense, or rather intense, stained band was visualized around 200 kDa. Often, this intense band was together with two weaker bands around 180–190 kDa. In a few lanes, one weak band at ~ 100 kDa was also noted. However, three DRB1*0401-positive individuals showed no reactions at all to the antigen. The same individuals also showed very low IgA activity to *S. mutans* and/or *S. sobrinus* (Table 1 and Fig. 4). Thirty-six immunoblots in all were performed. All recordings and calculations were made for distinct bands with a molecular weight

Table 1. HLA-DRB1*04 type, Strip mutans class, total IgA, percent IgA in relation to total protein, total number of distinct bands observed for each antigen extract and 'relative trace intensity'

Subject	DRB1*04	Strip mutans class[a]	Total IgA mg/100 ml	%IgA/ protein	No. of all distinct bands *S. mutans*[b]	No. of high distinct bands *S. mutans*[c]	No. of high distinct bands *S. sobrinus*[c]	No. of high distinct bands *S. parasanguis*[c]	No. of high distinct bands SA I/II [c]	Trace intensity/ Neg Pool trace int. for *S. mutans*
1	0401	2	2.80	10.25	2	0	0	2	1	0.01
2	0401	0	4.32	9.39	7	6	5	8	1	0.21
3	0401	2	2.75	9.37	3	2	1	3	1	0.09
4	0401	0	1.76	5.93	1	3	2	5	1	0.06
5	0401	2	2.32	5.68	5	5	6	4	2	0.36
6	0401	3	4.16	16.54	7	6	7	7	3	1.07
7	0401	3	1.91	6.62	2	0	0	2	0	0.00
8	0401	1	1.44	6.45	1	0	0	1	1	0.00
9	0401	1	2.82	13.55	6	5	7	5	3	0.44
10	0401	1	2.00	8.11	0	0	1	1	0	0.00
11	0401	2	3.40	11.33	4	1	0	4	0	0.02
12	0401	1	2.89	8.69	4	6	4	3	2	0.32
13	0401	2	1.45	10.88	2	1	2	2	1	0.02
14	0401	2	3.41	13.35	2	1	4	1	1	0.03
15	0401	1	3.69	16.50	1	1	4	2	1	0.16
16	0401	0	2.30	7.27	5	5	2	5	3	0.46
17	0401	3	4.87	16.08	11	7	5	3	2	0.64
18	0401	1	3.20	19.77	5	3	5	5	2	0.26
19	0401	2	4.93	14.39	14	6	6	4	3	1.14
20	0401	0	2.90	15.84	5	5	4	5	3	0.55
21	0401	ND	3.02	10.32	4	3	4	4	2	0.17
22	0401	1	1.78	9.62	6	3	0	2	1	0.07
23	0401	0	2.01	6.93	7	4	4	3	1	0.26
24	0401	0	5.54	29.42	13	10	5	1	2	0.38
25	0401	0	2.53	10.41	8	2	0	1	1	0.05
26	0401	0	2.02	6.76	12	7	4	1	2	0.44
27	0401	1	4.86	9.86	3	2	2	1	1	0.07
28	0401	0	2.23	9.75	7	5	4	2	1	0.65
29	0401	0	1.72	7.99	5	2	4	2	1	0.05
31	0402	2	1.35	8.88	8	7	2	2	2	0.33
32	0402	2	3.98	11.90	7	3	5	1	1	0.07
33	0403	2	9.28	27.26	9	7	6	5	3	0.94
34	0403	1	1.53	10.19	5	6	9	6	3	0.62
35	0404	0	1.51	7.50	8	6	7	1	4	1.14
36	0404	0	2.05	6.78	6	4	3	5	1	0.33
37	0404	3	1.60	10.86	4	6	4	4	1	0.22
38	0404	2	1.22	5.87	3	1	4	5	1	0.03
39	0404	3	3.46	11.11	6	6	7	6	4	1.18
40	0404	2	2.41	10.79	1	0	4	3	1	0.02
41	0404	0	2.20	10.14	4	4	5	3	1	0.09
42	0404	0	1.18	5.37	9	1	3	4	1	0.09
43	0404	0	2.14	14.19	7	8	3	2	1	0.15
44	0404	0	2.11	10.08	7	3	4	1	1	0.33
59	0404	2	5.42	16.43	17	7	4	ND	1	0.76
45	0405	1	1.07	5.25	4	5	7	4	1	0.10
46	0407	0	1.56	6.14	4	4	7	3	1	0.14
47	0407	2	2.81	16.62	4	5	5	2	3	0.80
48	0407	2	2.03	12.66	9	6	8	3	2	0.44
49	0407	1	4.29	13.69	7	6	4	4	3	0.73
50	0408	1	4.17	11.17	6	4	7	6	1	0.31
51	0408	ND	4.16	12.39	7	6	5	6	4	1.27
52	0408	2	3.65	9.60	3	1	2	4	1	0.53
53	0408	1	2.50	8.23	9	6	4	6	2	0.75
54	0408	3	2.03	11.00	9	5	4	ND	1	0.48
55	0414	2	2.16	8.63	7	3	1	2	1	0.16
56	0401, 0405	1	2.09	7.29	9	5	4	4	1	0.14
57	0401, 0404	3	2.35	3.73	15	4	3	3	1	0.12
58	0401, 0404	3	6.48	13.49	18	6	5	9	2	0.80

ND: not determined.

[a]Strip mutans class 0 corresponds to about < 10^4 colony-forming units (CFU)/ml saliva; class 1: 10^4–10^5 CFU/ml saliva; class 2: 10^5–10^6 CFU/ml saliva; and class 3: > 10^6 CFU/ml saliva.

[b]Total number of distinct bands (bands with an intensity $\geqslant$ 10 units from the graphs).

[c]Total number of distinct bands (bands with an intensity $\geqslant$ 10 units from the graphs) with a molecular weight > 100 kDa.

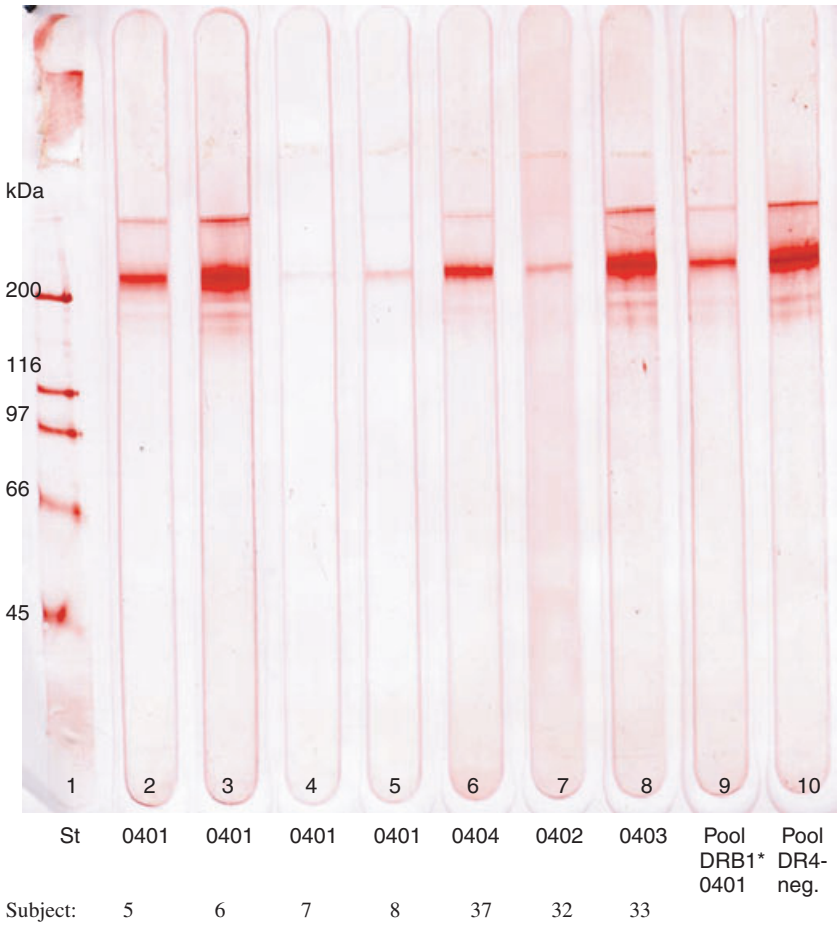

Fig. 4. Representative immunoblot of salivary IgA activity in different DRB1*04 salivas (lanes 2–8), DRB1*0401-positive pool (lane 9) and DR4-negative pool (lane 10) to a purified SA I/II. In lane 1 is a high molecular weight standard.

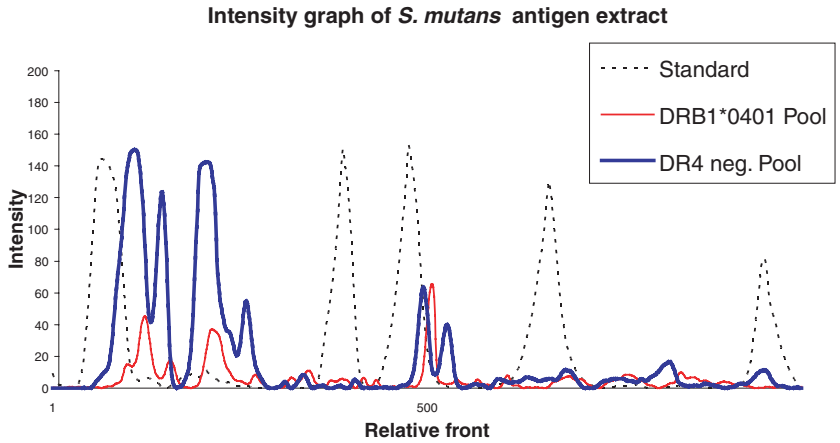

Fig. 5. Intensity graph for comparison of the salivary IgA activity in the DRB1*0401-positive and DR4-negative saliva pools to antigens separated from *S. mutans*. The high molecular weight standard is also included.

over 100 kDa. When comparing blots, the number of distinct bands, as well as the molecular weights noted for each band, were fairly similar for the two pools from one blot to another. However, the trace intensity for the pools varied more among the different blots. This variation made straight comparisons between different immunoblot difficult. Consequently, the 'relative trace intensity' ratio was applied, and the trace intensity was only used for comparisons within the blots.

Comparisons between samples/lanes *within a blot* included ranking the trace intensity value for each lane on a scale. Noteworthy is that, for *S. mutans*, the DRB1*0401 and *0404 alleles, though to a smaller extent, were the only alleles always present in the lowest intensity rank on the scale. This observation was comparable for *S. sobrinus* and SA I/II, but not for *S. parasanguis*.

To help illustrate the comparisons made between *all* samples/lanes on the nine blots that were performed for each antigen, the median of the 'relative trace intensity' was calculated and is presented in Fig. 6. The individuals were initially divided into four groups – DRB1*0401, *0404, 'Other', and the homozygotes. A significant difference was noted in the reactions to *S. mutans*, though the homozygotes (three individuals) were excluded ($P = 0.039$, Kruskal–Wallis). A significant difference was also observed between *0401 and 'Other' ($P = 0.031$, Mann-Whitney U, with Bonferroni correction).

The number of distinct, high molecular weight bands to *S. mutans*, i.e. bands with a molecular weight > 100 kDa, in relation to the different DRB1*04 alleles, is presented in Fig. 7. All but one of the 16 individuals in whom a small, i.e. < 3, number of bands was seen, were DRB1*0401- or *0404-positive. The same pattern was noted also when calculating the total number of *all* distinct bands detected for each individual to *S. mutans*.

Discussion

The results of this study are clearly in accordance with previous observations from both the present study group and other groups, suggesting that the immune response of the individual to mutans streptococci is influenced by MHC class II genes (1, 12, 19, 39, 40). The Western blot assays revealed that not only the DRB1*0401 allele, but also the *0404 allele, was often associated with low salivary IgA activity to antigens separated from mutans streptococci.

For *S. mutans,* the most significant reactions, as well as the major differences distinguished between diverse DRB1*04 alleles, were noted for five to six bands with a molecular weight of 170–190 kDa. In a recent study it was demonstrated that this cluster of bands represents cell-surface antigens (20), likely the SA I/II (molecular

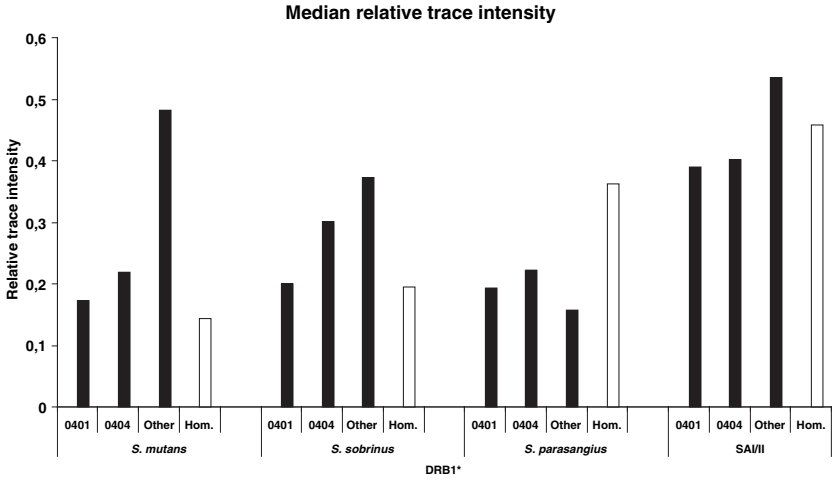

Fig. 6. The median 'relative trace intensity' calculated for bands with a molecular weight > 100 kDa, to the different antigen extracts. The individuals are divided into four groups: *****0401 ($n = 29$), *****0404 ($n = 11$), 'Other' (= remaining subgroups together, $n = 15$), and the homozygotes ($n = 3$).

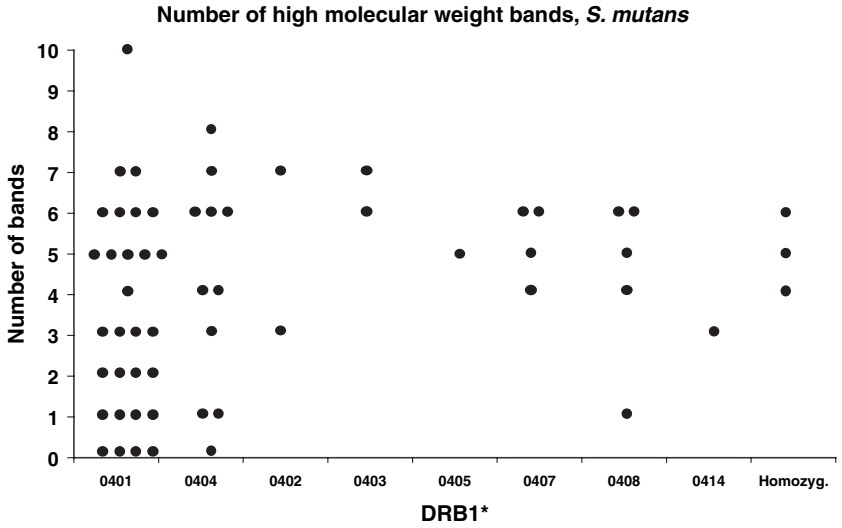

Fig. 7. Plot of total number of distinct immunoblot bands with a molecular weight > 100 kDa noted for *S. mutans* vs. each DRB1*****04 subgroup and the homozygotes.

weight of approximately 190 kDa) and glycosyltransferase (often described with a molecular mass of approximately 170 kDa). Both antigens have been implicated in the initial adherence of *S. mutans* and as primary candidates for a human caries vaccine (8). A comparable immunoblot pattern was observed for *S. sobrinus* and the different DRB1*****04 alleles, but not for *S. parasanguis.*

The Western blot technique has been used in many studies at our laboratory, and there have been no end of discussions while analyzing the results. Often, numerous bands are visualized, weak or strong, wide or narrow, some lanes dark and others pale, some immunoblots dark some faint. What do these differences signify? In this study, the results from the immunoblots were presented as the number of distinct bands detected for each individual, as described in a previous study by the same group (19). However, the trace intensity, i.e. the area under the curve, for all recorded bands on each lane was measured as well, since it did not seem sufficient only to calculate the numbers of distinct bands – the quality of the bands must be considered important, too. Over the years, however, we have also realised that it is dangerous to make comparisons between different immunoblots. Therefore we primarily chose to rank, within each blot, the trace intensity recorded for each sample. It turned out that number of distinct bands corresponded well to the ranking recorded for each subject, i.e. a low ranking also implied a low number of distinct bands.

In the present Western blot assays, the different saliva samples were always present together with the same samples on each immunoblot, independent of antigen extract. This implied that an immunoblot profile was always compared and ranked in relation to the same five or six other immunoblot profiles, as well as the two saliva pools. Since 29 subjects were DRB1*****0401-positive, three or four *****0401-positive saliva were always present and were thus compared with each other on the same immunoblot, so that, even if their IgA activity was very weak, one could be ranked as a 5, for example. An interesting observation in this context was that the results were quite similar when the ranking order for each sample/individual was compared on different antigen extracts. This means that if an individual was ranked a low responder to *S. mutans*, he was also ranked a low responder to *S. sobrinus* and SA I/II, though often not to *S. parasanguis*.

For all antigen extracts, the weakest trace intensity ranking number possible on each blot was 8, and for *S. mutans*, it was always a DRB1*****0401- or a *****0404-positive saliva. In addition there were five individuals, four DRB1*****0401 and one DRB1*****0404, in whom there were no observed reactions at all to *S. mutans*. For SA I/II, three salivas showed no distinct reactions, and they were all DRB1*****0401 positive. These results were well in line with the results from a previous study of ours (19). In that study, using Western blot analyses as well, the same two subgroups, DRB1*****0401 and *****0404, revealed low IgA activity, in particular to antigens separated from *S. mutans*. However, there were only 19 DR4-positive individuals subtyped for DRB1*****04. Another interesting observation from that study was that the number of distinct bands detected for an individual corresponded to the total salivary IgA; this finding was not supported in the present study.

Comparison of the relative trace intensity values must be interpreted with great caution, due to the inherent variability that is observed in Western blots. However, using the same reference sample on all blots might help the evaluation of the noted variations. Further, the statistical analysis unequivocally supports the results of the different methods used in this study

for comparisons, i.e. the number of bands and ranking, in that the *0401 subtype, in particular, demonstrated a lower salivary IgA response pattern to *S. mutans*.

DRB1*0401 and *0404 were the low responders, indicating likely similarities between these two alleles. Numerous studies on the genetic disposition for rheumatoid arthritis have shown that DRB1*0401 and *0404 alleles encode similar amino acids, a so-called shared epitope. This shared epitope is located in or close to the peptide binding region of the HLA molecule, suggesting that it influences the antigen presentation. The presence of the shared epitope has been considered a higher risk for rheumatoid arthritis as well as a prognostic marker for the severity of the disease, though it is not known how it supports the development of rheumatoid arthritis (22). An interesting observation, however, was that two of the DR4 homozygote subjects in this study, DRB1*0401/0404-positive at subgroup level, were not among the low responders, as might have been suspected. On the other hand, there is ambiguous support in the literature for a relationship between a higher susceptibility or resistance to, for example, infectious disease, and HLA homozygosity or heterozygosity (17).

The results diverged more for the other DRB1*04 alleles present in this study, which might partly explain the varying results observed in previous studies that analyzed a possible relation between the serologically defined HLA–DR4 and colonization of mutans streptococci (39, 40). In our first study, comprising 76 renal transplant subjects, a statistically significant correlation between DR4 and colonization of mutans streptococci was observed. However, in subsequent studies, including this study, no such significant correlation between levels of mutans streptococci and DR4 was found, only a trend in the same direction (19, 40). A further conceivable cause for the varying results is that colonization of mutans streptococci is influenced by several other, rather well defined, environmental factors, such as diet, oral hygiene, and fluoride exposure as well as nonimmune factors of the individual. In addition, no absolute association has yet been found between HLA alleles and disease, indicating not only the contribution of environmental factors but probably also the influence of other genes (25).

Other study groups have also reported findings comparable to ours. In 1998, Senpuku and his group (33) studied the binding of a PAc antigen peptide to different HLA-DR molecules and found that the PAc, or SA I/II, peptide bound more strongly to the DR5, DR6 and DR8 molecules. The year after, Acton and coworkers (1) found a positive association between high levels of *S. mutans* and DRB1*3 and DRB1*4, though no association with the DMFT index. In 2002, Nomura et al. (23) reported several peptides from glycosyltransferase and PAc that bound strongly to the DRB1*0802 molecule.

To conclude, certain subtypes of HLA-DR4 seem to be related to low salivary IgA activity to mutans streptococci. A likely explanation might be that these alleles have an amino acid sequence that binds poorly to the antigenic peptides of the mutans streptococcus. In future studies it seems therefore relevant to focus on specific IgA reactions to significant cell-surface antigens in relation to known peptide binding region of the HLA molecule, as peptide binding has been shown to be important for the role of HLA molecules. Searching for associations that groups alleles in categories based on peptide binding region motifs might therefore be more successful than with alleles in a classical genetic sense.

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