Short Communication

HLA-DR4 and number of mutans streptococci in saliva among dental students and staff

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Our aim was to corroborate previous findings that HLA-DR4 carriers are characterized by higher levels of mutans streptococci in saliva than are individuals expressing other HLA-DR types. Of 68 subjects (dental students, staff, and faculty) who were sampled for salivary counts of mutans streptococci, 13 subjects with the lowest counts of mutans streptococci and 15 subjects with the highest counts were selected for HLA-typing. Of the 13 who expressed HLA-DR4, 8 were heavily colonized by mutans streptococci. Although a trend towards a relationship was found between HLA-DR4 carriage and high levels of mutans streptococci, it was not statistically significant. In this selected population, knowledge of how to minimize the risk of caries and mutans streptococci level may have influenced the results. ☐ *Dental caries; HLA-DR-antigens; oral microbiology*

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Human leukocyte antigen (HLA) surface molecules are cell membrane glycoproteins controlled by loci mapping in the major histocompatibility complex (MHC), a region on the short arm of human chromosome 6 (1). The linkage of specific HLA antigens with particular disease states is sought, and relationships have been found to exist between HLA and various human disorders (2), although in very few such conditions is compelling evidence available of the involvement of HLA in the etiology.

The most important group of microorganisms to be identified as a causative agent of dental caries is the mutans group of streptococci (3–5). More than 85% of the adult population is colonized, although there are wide individual differences in the level of colonization (3, 6, 7). These individual differences cannot be fully explained simply by environmental factors such as retention sites, oral hygiene, and diet, although it has been shown, for example, that the level of sucrose intake can somewhat affect the level of bacteria in saliva (8).

Streptococcus mutans possesses a cell surface protein antigen of 190 kDa that has been variously called SAI/II, PAc, B, or P1; this protein antigen has been the focus of widespread attention. In 1980, Lehner et al. (9) reported that the antigen SAI/II induces protection against dental caries after immunization of rhesus monkeys. Caries-prone subjects were found to have T cells requiring 1000-fold higher doses of SAI/II to respond with helper factor activity, as compared with the T cells of caries-free subjects. It has been suggested that a class-II antigen, HLA-DR4, may be one of the gene products controlling this response (9). This suggestion derived support from the results of an earlier study of ours comprising 170 subjects (76 renal transplant patients and 94 healthy blood donors) (10), in which we found the frequency of the DR4 antigen to vary inversely with the level of colonization by mutans streptococci among the renal transplant patients; the healthy blood donors manifested the same tendency, although among them the relationship was not statistically significant.

For the present study, undertaken to further evaluate the possible relationship between HLA-DR4 antigens and levels of mutans streptococci, we selected subjects characterized either by very low or very high levels of mutans streptococci.

Materials and methods

Altogether 68 persons volunteered for the study. The wooden spatula method (11) was used for enumeration of mutans streptococci. The 13 subjects with the lowest and the 15 with the highest levels were selected for the study. Their levels of mutans streptococci were then determined in paraffin-stimulated saliva samples (12, 13) to confirm the results of the wooden spatula method. The tissue typing was performed at the Blood Bank, University Hospital, Lund. HLA-A, -B, and -C antigens were typed using the anti-lymphocytotoxicity test described by Kissmeyer-Nielsen & Kjerbye (14). HLA-DR and DQ antigens were typed in accordance

Table 1. Distribution of age, sex, DMFS, and salivary levels of mutans streptococci among DR4-positive and -negative individuals

	DR4-negative	DR4-positive
n	15	13
Sex (female / male)	10:5	8:5
Age*	34 ± 10	33 ± 8
DMFS*	26 ± 22	23 ± 20
CFU*†	0.41 ± 0.69	0.58 ± 1.2
CFU (median)	0.006	0.13

* Mean ± standard deviation.
† Colony-forming units × 10^6.

Table 2. Distribution (*n*) of HLA-DR4 in relation to levels of mutans streptococci

	Low level	High level	Total
DR4-positive	5	8	13
DR4-negative	8	7	15
Total	13	15	28

with the NIH technique (15). The study design was approved by the Ethics Committee of Lund University.

The study data were complemented with clinical recordings of caries and fillings, since retention sites and open caries lesions can influence the number of bacteria found in saliva. Four bitewing radiographs, two on each side of the dentition, were taken using the parallel technique. The bitewing radiographs were read and diagnosed for caries by the same member of the team. Only carious surfaces with a radiolucency involving the dentin were included in the DMFS index.

Results

Of the 28 subjects, 13 were DR4-positive and 15 DR4-negative. The DR4-positive and DR4-negative subjects were similar with regard to mean age, DMFS, and salivary levels of mutans streptococci, although the median number of mutans streptococci in saliva tended to be higher for the DR4-positive group (Table 1).

Thirteen subjects with less than 10,000 mutans streptococci per milliliter saliva were assigned to the low-mutans group, and 15 subjects with more than 120,000 mutans streptococci per milliliter saliva to the high-mutans group. In the low-mutans group 38% of the subjects were DR4-positive, whereas in the high group 53% were positive for DR4 (Table 2).

Discussion

Our working hypothesis was that the level of mutans streptococci would be higher among HLA-DR4-positive subjects than among subjects with lymphocytes bearing other HLA-DR antigens. In an earlier study (10) we found that in a group of 76 immunosuppressed renal-transplanted patients, none of the HLA-DR4-positive patients had low levels of mutans streptococci. In the second part of the same study a similar trend was found among 94 healthy blood donors. The results obtained in the present study tend to support our previous findings, although some DR4-positive subjects had low levels of mutans streptococci (Table 2). The median level of mutans streptococci in saliva showed higher levels (not significantly) for DR4-positive subjects (Table 1). A noteworthy observation was that one woman who was homozygous for DR4 also manifested the highest level of mutans streptococci and the second highest DMFS index.

The subjects in this study were all students, staff, or faculty at a dental school, a fact that might have affected the results. It is reasonable to suppose that subjects at a dental school are better informed than a normal population concerning oral hygiene, fluoride, and diet, which can influence mutans streptococci to lower levels and explain why the DR4-positive subjects in this study also might be low-colonized. This is also supported by low DMFS indices in the whole group as compared with an age-matched adult Swedish population (15).

We consider it important to present our findings in the light of the results from the study of immunosuppressed patients (10), showing significant correlation between mutans streptococci and DR4. The differences might be explained as follows: As a consequence of the immunosuppression, the renal transplant patients produce lesser amounts of antibodies in general than do healthy subjects. The results of Lehner et al. (9) suggest that HLA-DR4 antigens may be associated with helper cells requiring high doses of mutans streptococcus antigens for the release of significant amounts of helper factor activity. As helper factors are required for the production of antibodies to the streptococcal antigen, immunosuppressed DR4-positive patients might harbor high levels of mutans streptococci without producing adequate antibody activity. For the healthy DR4-positive population, on the other hand, the T-cell activity might be sufficient to keep the mutans streptococci levels 'under control'.

In our previous studies we did not separately identify the species *S. mutans* and *S. sobrinus*. Nor did we in this study. The reason for this is that even though the PAc antigen is specific for *S. mutans* (17), it can cross-react with the PAg antigen from *S. sobrinus* (18). The antigen may also be an adhesin on *S. mutans* cells (19) and thus an important factor for the acquisition of this bacterium.

The present results, however, do not exclude the possible involvement of HLA-DR4 as a possible determinant of colonization by mutans streptococci in humans. Our earlier study of immunosuppressed individuals probably provided us with a unique

experimental situation, in which the involvement of the DR4 gene products could be seen without so many confounding factors. Thus, settling the question of whether the presence of the DR4 gene is a crucial determinant of streptococcal antibody response will probably entail investigation of antibody response levels in individuals carrying different DR antigens. Such research is currently under way at our department.

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References

1. Krangel MS, Orr HT, Strominger JL. Structure, function and biosynthesis of the major human histocompatibility antigens (HLA-A and HLA-B). Scand J Immunol 1980;11:561–71.
2. Dausset J, Svejgaard A, editors. HLA and disease. Copenhagen: Munksgaard; 1977.
3. Bratthall D. The global epidemiology of mutans streptococci. In: Johnson NW, editor. Risk markers for oral diseases. Vol. 1. Dental caries. Markers of high and low risk groups and individuals. Cambridge: Cambridge University Press; 1991. p. 313–26.
4. Kristoffersson K, Gröndahl H-G, Bratthall D. The more streptococcus mutans, the more caries on approximal surfaces. J Dent Res 1985;64:58–61.
5. Loesche WJ. Role of streptococcus mutans in human dental decay. Microbiol Rev 1986;50:353–80.
6. Salonen L, Allander L, Bratthall D, Helldén L. Mutans streptococci, oral hygiene and caries in an adult Swedish population. J Dent Res 1990;69:1469–75.
7. Togelius J, Bratthall D. Frequency of the bacterium streptococcus mutans in the saliva of selected human populations. Arch Oral Biol 1982;27:113–6.
8. Kristoffersson K, Birkhed D. Effects of partial sugar restriction for six weeks on numbers of Streptococcus mutans in saliva and dental plaque in man. Caries Res 1987;21:79–86.
9. Lehner T, Lamb JR, Welsh KL, Batchelor RJ. Association between HLA-DR antigens and helper cell activity in the control of dental caries. Nature 1981;292:770–2.
10. Wallengren ML, Ericson D, Forsberg B, Johnson U. Human leukocyte antigens in relation to colonization by mutans streptococci in the oral cavity. Oral Microbiol Immunol 1991; 6:292–4.
11. Köhler B, Bratthall D. Practical method to facilitate estimation of Streptococcus mutans levels in saliva. J Clin Microbiol 1979;9: 584–8.
12. Möller ÅJR. Microbiological examination of rootcanals and periapical tissues of human teeth. Methodological studies [thesis]. Odontol Tidskr 1966;74:no5–6.
13. Gould OG, Jordan HV, van Houte J. A selective medium for streptococcus mutans. Arch Oral Biol 1973;18:1357–64.
14. Kissmeyer-Nielsen F, Kjerbye KE. In: Curtoni ES, Mattiuz PL, Tosi RM, editors. Histocompatibility testing. Copenhagen: Munksgaard; 1967.
15. Vartdal F, Gaudernack G, Funderud S, Bratlie A, Lea T, Ugelstad J, et al. HLA class I and II typing using cells positively selected from blood immunomagnetic isolation—a fast and reliable technique. Tissue Antigens 1986;28:301–12.
16. Salonen L, Allander L, Bratthall D, Togelius J, Helldén L. Oral health status in an adult Swedish population. Prevalence of caries. Swed Dent J 1989;13:111–23.
17. Russell MW, Bergmeier L, Zanders ED, Lehner T. Protein antigens of Streptococcus mutans: purification and properties of a double antigen and its protease-resistant component. Infect Immun 1980;28:486–93.
18. La Polla RJ, Haron JA, Kelly CG, Taylor WR, Bohart C, Hendricks M, et al. Sequence and structural analysis of surface protein antigen I/II (SpaA) of Streptococcus sobrinus. Infect Immun 1991;59:2677–85.
19. Ma JK-C, Smith R, Lehner T. Use of monoclonal antibodies in local passive immunization to prevent colonization of human teeth by streptococcus mutans. Infect Immun 1987;55:1274–78.

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