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Gender differences in HLA phenotype frequencies found in German patients with generalized aggressive periodontitis and chronic periodontitis

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HLA antigens have been considered as risk factors for periodontitis. Differences in prevalence and in the extent of attachment loss between males and females have suggested that gender-dependent HLA deviations could play a role in individual predisposition to periodontitis. The aim of the present study was therefore to investigate the incidence of gender-dependent HLA associations in 50 patients with generalized aggressive periodontitis (AP) and 102 patients with chronic periodontitis (CP) in comparison to 102 probands without any attachment loss caused by periodontitis. HLA typing was carried out using a microlymphocytotoxic test and a polymerase chain reaction with sequence-specific primers (PCR-SSP). Female AP patients showed an increase in the frequency of HLA-A*68/69 and a decrease in the frequency of DRBblank* (non-DRB3/4/5*) and DQB1*05-positive probands. Only in female CP patients was HLA-DQB1*0303 absent, whereas HLA-DQB1*06 homozygosity increased significantly. With regard to the (AP + CP) periodontitis group as a whole, the increased frequency of HLA-DQB1*06 homozygosity in females was similar to the findings obtained in the AP group. Evidently, gender is a confounding variable, which should be considered in further studies of HLA and periodontitis.

Key words: aggressive periodontitis; chronic periodontitis; gender differences; HLA

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There is evidence that bacteria play a primary role in the etiology of all types of periodontitis. For instance, interventions such as scaling, root planning and surgical elimination of pathologically deepened pockets could help to halt advanced cases of periodontitis (30). The use of antibiotics in treating necrotizing ulcerative gingivitis (51), localized juvenile periodontitis and refractory periodontitis has improved the therapeutic outcome (57). Microbiota obtained from lesion sites in patients with adult periodontitis were markedly different from microbiota taken from healthy sites in the same patients and also from sites in patients suffering from localized juvenile periodontitis (52, 55). However, a recent study (12) has identified various herpes viruses in patients with periodontal disease, which could cause transient local immunosuppression and thereby subgingival overgrowth of periodontopathic bacteria.

Genetic immunological mechanisms may determine individual host susceptibility, and thus the onset of periodontal diseases, or individual predisposition to mild or aggressive severe forms of periodontitis, but have not been elucidated sufficiently. In particular, aggressive forms of periodontitis suggest the existence of such genetic susceptibility fac-

tors. For example, several studies have demonstrated a disproportionately high prevalence within one family in which 40–50% of siblings were affected (4, 6, 32, 34, 49). Furthermore, the prevalence and sex ratio, in particular for localized juvenile periodontitis, vary geographically and/or racially in different populations. In Blacks, this disease is more frequent, with a prevalence of >1%, than in Caucasians, and males are more often affected than females. In Caucasians, the prevalence is lower, at 0.1%, and females are more often affected (10, 31, 36). Suzuki (53) reported that the prevalence of rapidly progressive periodontitis was 2 to 3 times higher in females than in males for rapidly progressive periodontitis type A (lower immune defense function and minimal plaque accumulation), but not for type B (normal immune defense and significant plaque accumulation). Several authors have also reported gender differences in prevalence and in the extent of attachment loss in adult populations. Summarizing this result, chronic periodontitis among adults was more frequent in males than females and more prevalent in Blacks than Caucasians (1, 23, 48). These gender- and race-dependent differences in aggressive and chronic forms of periodontitis could represent further evidence of the existence of underlying genetic susceptibility and resistance factors. With particular regard to possible genetic factors, the significance of various HLA markers has been investigated in several studies determining individual susceptibility factors for localized juvenile periodontitis, rapidly progressive periodontitis and adult periodontitis. From bacterial mimicry with HLA (18, 25) and HLA-dependent immunoreactivity to bacterial antigens (9, 22), an association with periodontopathic bacteria can be assumed. The most striking positive associations with periodontitis were found for HLA-A*24 (19, 28, 35, 46, 50) and HLA-DR4 (2, 5, 16, 27, 41). Negative associations with periodontitis were found for HLA-A10 (A*25/26/34/66) (3).

Gender may thus play a role in individual predisposition to periodontitis. To date, investigations on gender-dependent HLA associations with periodontitis have not been carried out, but studies on patients with rheumatic diseases (Reiters's disease, ankylosing spondylitis, acute anterior uveitis and psoriatic arthritis) (26), psoriasis vulgaris (33), acute lymphoblastic leukaemia (13, 15) and chronic lymphoid leukaemia (14) have revealed such gender-dependent HLA deviations. The primary aim of this study was therefore to evaluate males and females separately for positive and negative HLA associations in patients with generalized aggressive periodontitis (AP) (formerly called rapidly progressive periodontitis or generalized early-onset periodontitis) and chronic periodontitis (CP) (previously called adult periodontitis) and to compare the findings with those obtained in a control group of healthy individuals who were periodontitis-free.

Materials and methods

Study population

Fifty German Caucasian patients with AP and 102 German Caucasian patients with CP were investigated. The two groups were subdivided into males and females and separately evaluated and compared with a group of 102 German Caucasian individuals who were free of periodontitis. The numbers of males and females and important demographic variables such as age range and median and smoking status are presented in Table 1. The clinical assessment included determination of the following parameters: anamnesis, smoking status, approximal plaque index (API) (29) and modified sulcular bleeding index (SBI). Both the maximum clinical probing depth (PD) and maximum clinical attachment loss (AL) for each tooth were derived by measuring six sites around each tooth and recording the maximum values. Alveolar bone loss on interproximal tooth surfaces was estimated by panoramic radiograph saided by single radiographs when necessary. Furthermore, a group of 157 German Caucasian blood donors without periodontal assessment were also included, representing the normal HLA marker distribution found in the population.

Out of a group of 4131 patients with different forms of periodontal disease, who received treatment at our Department of Operative Dentistry and Periodontology over a 4-year period, we selected 50 cases of AP, according to the following minimal published clinical criteria (56) for AP: age of onset of periodontitis under 35 years, at least eight teeth with an attachment loss of 4 mm or more, at least three affected teeth other than molars or incisors, more vertical than horizontal approximal bone loss in the affected sites, minimal accumulation of mineralized plaque in comparison to chronic periodontitis, bleeding on probing, increased mobility of certain teeth, rapid course, and no systemic diseases. In a few cases our investigations also included patients with AP who were older than 35 years at the time the HLA typing was carried out, but it was proved from case histories and previous X-ray protocols that they developed the disease before they turned 35.

A total of 102 cases with CP were selected from a group of 6800 individuals receiving dental treatment at two of our dental departments over a period of 4 years fulfilling the following criteria: age of onset >38, at least 10 teeth present, at least five teeth with an attachment loss of 4 mm or more, more horizontal than vertical approximal bone loss in the affected sites, bleeding on

Table 1. Demographic values and smoking status in relation to gender-specific differences in all investigated cohorts

Variable	Aggressive periodontitis ($n = 50$)	Chronic periodontitis ($n = 102$)	No periodontitis ($n = 102$)	Blood donors ($n = 157$)
Prevalence	1.21	1.5	1.5	
Median age (range)	33.0 (19–43)	52.5 (38–73)	61.0 (38–95)	29.2 (19–39)
Number (males/females)	22/28	39/63	40/62	63/94
Male:female ratio	1 : 1.27	1 : 1.62	1 : 1.55	1 : 1.49
Percentage smokers	35.6	44	32.2	
Percentage smokers (male/female)	40/32	62.5/34.8	48.0/38.0	

probing, often extensive accumulation of mineralized plaque, increased mobility of certain teeth, slow course, and no systemic diseases.

The 102 periodontitis-free probands were selected from the same 6800 individuals who fulfilled the following inclusion criteria: older than 38 years, probing depth <3.5 mm, recession of maximum 4 mm at facial or oral surfaces not caused by periodontitis, and no approximal alveolar bone loss in X-rays. We selected controls displaying a lack of adequate oral hygiene with an API of more than 30%.

The 157 blood donors were not examined for periodontal diseases since they represented the normal distribution of HLA antigens in our geographic region. All blood donors were systemically checked according to guidelines for blood donation in Germany. In particular, probands who were infected with the human immunodeficiency virus 1 or 2 or any variety of hepatitis virus were excluded. All patients and controls were of Caucasian descent and were unrelated and free from general diseases known to be associated with certain HLA markers.

Serologic HLA typing

Anticoagulated blood samples (20 ml) were taken from all patients and control probands. Lymphocytes as indicator cells were separated from peripheral blood by density gradient centrifugation (7). All probands were typed for HLA-A, -B, and -Cw antigens (Lymphotype 144, Biotest, Dreieich, Germany) using the standard National Institute of Health (NIH) microlymphocytotoxicity test following the manufacturer's instructions.

Genomic HLA typing

DNA was prepared from blood leukocytes using a salting-out procedure to extract DNA from human nucleated cells (38), i.e. after lysis of erythrocytes in red cell lysis buffer (RCLB) and protein digestion in proteinase K solution. DNA was extracted by precipitation of proteins using a saturating salt solution. The final cleaning of DNA was effected by ethanol precipitation resulting from the withdrawal of water.

All patients and controls were DNA typed by standard polymerase chain reaction–sequence-specific priming (PCR-SSP) (PE 9600, Perkin Elmer, Weiterstadt, Germany) for HLA-A, -B and -Cw (Deutsche Dynal AG, Hamburg, Germany) and HLA-DRB1, -DRB3/4/5, and -DQB1 markers using a low-resolution technique (Histotype-DR, Histotype-DQ, BAG, Lich, Germany) according to the protocol provided by the manufacturer.

Quality control

Quality was verified by regular HLA typing of control samples from the Institute for Standardization and Demonstration in Medical Laboratories (INSTAND e.V., Düsseldorf, Germany) and from the International DNA Exchange, UCLA Tissue Typing DNA Laboratory (Los Angeles, CA).

Degree of differentiation in HLA typing

Using a serologic technique (the microlymphocytotoxic test), we determined MHC class I markers (HLA-A, -B, and -Cw) and their splits. By using an additional PCR-SSP procedure these serologic findings were confirmed and extended. Because the serologic typing of HLA class II markers (DRB1, DRB3/4/5 and DQB1) is difficult and unsafe, it was only carried out with PCR-SSP. All the HLA markers investigated are listed in Table 2.

Statistical analysis

Each of the three patient groups (AP, CP and AP + CP) were compared with periodontitis-free controls and were separated into male and female prob-

Table 2. List of the investigated class I and II HLA markers in all patient and control groups

HLA-A	HLA-B			HLA-Cw	HLA-DR	HLA-DQ
A*01	B*51 (5)	B*50 (21)	B*73	Cw*01	DRB1*01	DQB1*05 (1)
A*02	B*52 (5)	B*54 (22)	B*78	Cw*02	DRB1*15 (2)	DQB1*06 (2)
A*03	B*07	B*55 (22)		Cw*03	DRB1*16 (2)	DQB1*02
A*23 (9)	B*08	B*56 (22)	Bw*4	Cw*04	DRB1*03	DQB1*0301 (7)
						DQB1*0304 (7)
A*24 (9)	B*44 (12)	B*27	Bw*6	Cw*05	DRB1*04	DQB1*0302 (8)
						DQB1*0303 (9)
A*25 (10)	B*45 (12)	B*35		Cw*06	DRB1*11 (5)	DQB1*0308 (9)
A*26 (10)	B*13	B*37		Cw*07	DRB1*12 (5)	DQB1*04
A*34 (10)	B*14	B*40 (60)		Cw*08	DRB1*13 (6)	
A*66 (10)	B*15 (62)	B*40 (61)		Cw*blank	DRB1*14 (6)	
A*11	B*15 (63)	B*41			DRB1*07	
A*29 (19)	B*15 (75)	B*42			DRB1*08	
A*30 (19)	B*15 (76)	B*46			DRB1*09	
A*31 (19)	B*15 (77)	B*47			DRB1*10	
A*32 (19)	B*38 (16)	B*48				
A*33 (19)	B*39 (16)	B*53			DRB3*(DR52)	
A*74 (19)	B*57 (17)	B*59			DRB4*(DR53)	
A*68 (28)						
A*69 (28)	B*58 (17)	B*67			DRB5*(DR51)	
A*36	B*18	B*15 (71)			DRBblank*	
					(non-DRB3/4/5)	
A*80	B*49 (21)	B*15 (72)				

Class I markers were detected by microlymphocytotoxic test and PCR-SSP, whereas class II markers were detected only by PCR-SSP. The main markers are shown in parentheses. HLA-DRB*blank indicates that none of the supertypes DRB3*/DRB4*/DRB5* was detectable.

ands. Statistical analysis was performed according to the published (17) instructions on the design and interpretation of studies on a major histocompatibility complex in a disease, i.e. HLA phenotype frequencies in patients and controls were determined by directly counting the probands as being positive for a certain HLA antigen and were represented as a percentage (pf %) of the total number of probands in the group (*N*). Statistical comparison between patients and healthy individuals was based on a 2×2 contingency table and performed by χ^2 testing with Yates' continuity correction. If there were less than five patients in a group who tested positive for a certain HLA allele, Fisher's exact test was used. A P value < 0.05 was considered significant. The relative disease risk (RR) of an observed association was calculated using a 2×2 contingency table. If less than five patients tested positive or negative for a certain HLA allele a modification according to Haldane (24) was used. $RR > 1$ indicated a positive disease association and $RR < 1$ indicated a negative disease association.

Quantitative clinical variables such as API, SBI, PD and AL were described in terms of the mean. *t*-tests were performed to compare differences in means between males and females within a cohort. P values < 0.05 were accepted as statistically significant.

Databank searching for MHC ligands

Using two different MHC databases, MHCPEP (8) and SYFPEITHI (44), we looked for HLA class II markers that were positively or negatively associated with the AP, CP or AP + CP group with regard to their anchor amino acid residues. Whereas the entries in the database SYFPEITHI were compiled only from published reports, the database MHCPEP contains additional direct submissions of experimental data. A 53-kDa outer membrane protein from *Porphyromonas gingivalis* (NKVPIIVKRAAIRASMTITQQ: Ag53 p141–161), which was found to stimulate T cells from patients with early-onset periodontitis very strongly (40), was used to search for possible motifs binding to the HLA class II markers revealed in the present study. Finally, we looked for published motifs of other periodontopathic antigenic peptides which could bind to our striking HLA class II markers.

Results

Clinical findings

In all cohorts, there were more females than males. Smokers were more frequent among male patients and the male periodontitis-free controls (Table 1). Since clinical measurements for the CP group were taken after initial therapy, including scaling and root planning, we found the lowest values for API and SBI in this group. Values for API were not significantly different between females and males amongst all groups, while values for SBI were significantly higher in the female patient groups and the female control group than in the male groups. With regard to PD and AL, no significant differences between males and females were found (Table 3).

HLA deviations in the AP group

The significantly decreased frequency of HLA-DRBblank* (non-DRB3/4/5*) and the significantly increased frequency of HLA-DRB1*13 in the AP group as a whole compared with the controls was mainly caused by deviations found in AP females in comparison with female periodontitis-free probands. Furthermore, HLA-A*68/69 (A28) occurred at a significantly higher frequency and HLA-DQB1*05 at a significantly lower frequency in AP females than in female controls, whereas AP males and the AP group as a whole showed no significant differences for these alleles relative to controls.

No significant differences were revealed between male patients and male controls. However, the significantly higher occurrence of HLA*29 in the AP group as a whole was attributed to the increased frequency of this marker in AP males, whereas in female patients HLA-A*29 was in the normal range. HLA-A*31 and HLA*30/31 were absent in both genders (Table 4).

HLA deviations in the CP group

HLA-DQB1*0303 (DQ09) was absent in female CP patients and in the normal range in male CP patients. Moreover, homozygosity for HLA-DQB1*06 was significantly more frequent only in female patients.

In the CP group as a whole, the deviations of HLA-A*03, -A*11, -B*14, and -Cw*08 were caused by similar deviations in both genders. The positive associations of HLA-B*14 and -Cw*08, as well as the negative association of HLA-B*27, were caused by a deviation in the controls from the normal distribution (there was no deviation within the AP group) (Table 5).

HLA deviations in the combined (AP + CP) periodontitis group

In addition to analyzing patients with CP and AP separately, the two patient groups were combined (CP + AP) and compared with the controls. Similarly to the CP group, homozygosity for HLA-DQB1*06 was significantly more frequent only in female periodontitis patients as opposed to male patients. Additionally, the significantly decreased frequency of the marker HLA-A*31 in the combined periodontitis group was mainly caused by devi-

Table 3. Approximal plaque index (API), sulcular bleeding index (SBI), pocket depth (PD) and attachment loss (AL) in relation to gender-specific differences

Variable	Aggressive periodontitis ($n = 50$)	Chronic periodontitis ($n = 102$)	No periodontitis ($n = 102$)
API (%)			
Total	42.7	18.8	56.9
Males/females	36.4/47.3	17.6/19.4	55.5/57.8
SBI (%)			
Total	55.36	19.1	60.8
Males/females	44.6/64.0*	14.7/21.3*	54.9/64.6*
PD (mm)			
Total	5.7	4.9	1.9
Males/females	5.6/5.8	5.0/4.9	1.9/2.0
AL (mm)			
Total	6.7	5.7	3.4
Males/females	6.7/6.7	5.8/5.6	3.2/3.5

*$P < 0.05$.

Table 4. Striking HLA phenotype frequencies in patients with aggressive periodontitis (AP) and the periodontitis-free control group (NP) in relation to gender.

	Total			Female			Male		
HLA	AP ($n = 50$) pf (%)	NP ($n = 102$) pf (%)	P_c/RR	AP ($n = 28$) pf (%)	NP ($n = 62$) pf (%)	P_c/RR	AP ($n = 22$) pf (%)	NP ($n = 40$) pf (%)	P_c/RR
A*68/69	22.00↑	9.80	n.s.	28.57↑	6.45↓	0.007/5.39	13.64	15.00	n.s.
A*29	8.00↑	0.98↓	0.040/6.55	3.57	0.00↓	n.s.	13.64↑	2.50	n.s.
A*31	0.00↓	8.82↑	0.024/0.10	0.00↓	9.86↑	n.s.	0.00↓	7.50↑	n.s.
A*30/31	0.00↓	11.76↑	0.007/0.07	0.00↓	11.29↑	n.s.	0.00↓	12.50↑	n.s.
DRBblank*	18.00↓	34.31↑	0.036/0.42	17.86↓	41.94↑	0.026/0.30	18.18↓	22.50↓	n.s.
DQB1*05	22.00↓	34.31↑	n.s.	17.86↓	43.55↑	0.019/0.28	27.27	20.00↓	n.s.
DRB1*13	36.00↑	20.59↓	0.046/2.17	42.86↑	24.19	n.s.	27.27	15.00↓	n.s.

The arrows indicate a higher (↑) or lower (↓) frequency within a group compared with the distribution in blood donors. pf = frequency of HLA phenotype; $P_C = P$ corrected (Yates or Fisher); RR = relative risk; n.s. = not significant.

Table 5. Striking HLA phenotype frequencies in patients with chronic periodontitis (CP) and in the periodontitis-free control group (NP) in relation to gender

	Total			Female			Male		
HLA	CP ($n = 102$) pf (%)	NP ($n = 102$) pf (%)	P_c/RR	CP ($n = 63$) pf (%)	NP ($n = 62$) pf (%)	P_c/RR	CP ($n = 39$) pf (%)	NP ($n = 40$) pf (%)	P_c/RR
A*03	19.61↓	32.35↑	0.039/0.51	20.63↓	33.87↑	n.s.	17.95↓	30.00	n.s.
A*11	14.71↑	5.88↓	0.041/2.76	15.87↑	4.84↓	0.040/3.34	12.82↑	7.50	n.s.
B*14	5.88	0.00↓	0.014/13.8	6.35	0.00↓	n.s.	5.13	0.00↓	n.s.
B*27	8.82	14.71↑	n.s.	6.35	17.74↑	0.045/0.34	12.82↑	10.00	n.s.
Cw*08	5.88	0.00↓	0.041/13.8	6.35	0.00↓	n.s.	5.13	0.00↓	n.s.
DQB1*0303	2.94↓	6.86	n.s.	0.00↓	8.06	0.028/0.08	7.69	5.00↓	n.s.
DQB1*06,06	9.80↑	4.90	n.s.	11.11↑	1.61↓	0.032/5.54	7.69	10↑	n.s.

The arrows indicate a higher (↑) or lower (↓) frequency within a group compared with the distribution in blood donors. pf = frequency of HLA phenotype; $P_C = P$ corrected (Yates or Fisher); RR = relative risk; n.s. = not significant.

Table 6. Striking HLA phenotype frequencies in patients with periodontitis (AP + CP) and in periodontitis-free controls (NP) in relation to gender

	Total			Female			Male		
HLA	AP + CP ($n = 152$) pf (%)	NP ($n = 102$) pf (%)	P_c/RR	AP + CP ($n = 91$) pf (%)	NP ($n = 62$) pf (%)	P_c/RR	AP + CP ($n = 61$) pf (%)	NP ($n = 40$) pf (%)	P_c/RR
A*11	13.82↑	5.88	0.044/2.56	14.29↑	4.84↓	n.s.	13.11↑	7.50	n.s.
A*24	15.79	21.57	n.s.	19.78	19.35	n.s.	9.84↓	25.00	0.049/0.33
A*29	7.24↑	0.98↓	0.017/5.50	5.49	0.00↓	n.s.	9.84↑	2.50↓	n.s.
A*31	2.63↓	8.82↑	0.029/0.30	2.20↓	9.68↑	0.048/0.24	3.28	7.50↑	n.s.
A*30+31	3.95↓	11.76↑	0.022/0.31	3.30↓	11.29↑	n.s.	4.92↓	12.50↑	n.s.
B*60 (B40)	15.13↑	10.78	n.s.	17.58↑	17.74↑	n.s.	11.48	0.00↓	0.025/11.15
DQB1*06,06	10.35↑	4.90	n.s.	10.99↑	1.61↓	0.024/5.28	9.84	10.00	n.s.

The arrows indicate a higher (↑) or lower (↓) frequency within a group compared with the distribution in blood donors. pf = frequency of HLA phenotype; $P_C = P$ corrected (Yates or Fisher); RR = relative risk; n.s. = not significant.

ations found in female patients and controls.

In male periodontitis patients, HLA-A*24 was significantly decreased, whereas this marker was in the normal range in female patients and in the combined periodontitis group. The significant positive association of HLA-B*60 (as a split of HLA-B40) in male periodontitis patients was attributed to an absence of this marker in the male controls, as the frequency of this marker in male patients corresponded to the normal distribution. HLA-A*11, which was positively associated with CP + AP (combined group), showed the same tendency of deviations in both genders, but without statistical significance. Similarly to the AP group, the significantly increased frequency of HLA-A*29 was caused by an absence of this marker in female controls and an increased frequency in male patients (Table 6).

Searching MHC databases for results for binding motifs

In neither database were there any entries for known periodontal pathogens for ligands or T-cell epitopes to match the striking MHC class II markers revealed in our investigations. For Ag53

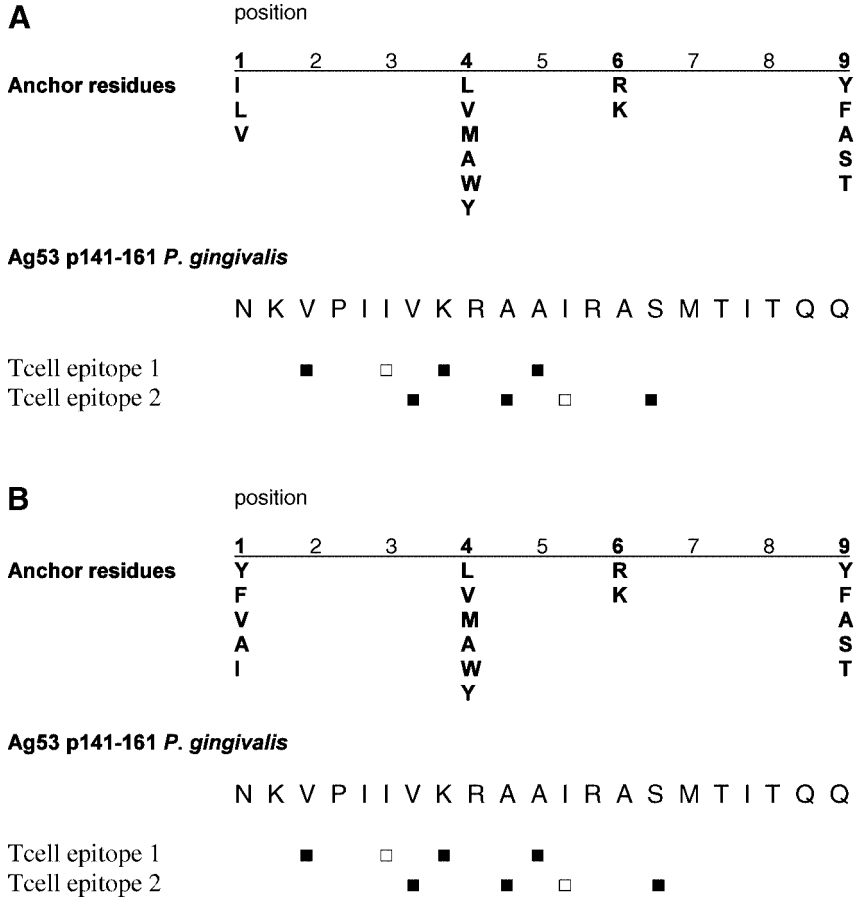

Fig. 1. (A) Possible motifs binding to HLA-DRB1*1301. Anchor amino acid residues for the HLA-DRB1*1301 molecule (44) are shown. Possible binding motifs found in the Ag53 p141–161 peptide are shown by ?. ? indicates insufficient match to the anchor. *(B)* Possible motifs binding to HLA-DRB1*1302. Anchor amino acid residues for the HLA-DRB1*1302 molecule (44) are shown. The boxes have the same meanings as those in (A).

p141–161, we found possible motifs binding to HLA-DRB1*1301 and HLA-DRB1*1302 (both high-resolution alleles of HLA-DRB1*13) as well as HLA-DQB1*0602 (split of HLA-DQB1*06). The anchor amino acid residues of DRB1*1301 and DRB1*1302 were located at the first, fourth, sixth and ninth positions (44). For Ag53 p141–161, we found in both alleles two possible T-cell epitopes matching three of the four anchor residues. Epitope 1 matched the first, sixth and ninth, and epitope 2 the first, fourth and ninth residues (Figs 1A and B). The anchor residues of HLA-DQB1*0602 were located at the first, third, fourth, sixth and ninth positions (44). At this point, three possible T-cell epitopes were detected. Epitope 1 matched four of five anchor residues and epitopes 2 and 3 each matched three of five residues (Fig. 2).

Discussion

Our patients and periodontitis-free probands were selected on the basis of the inclusion and exclusion criteria for studies on HLA-associated diseases. All patients with AP who were treated in our department over a period of 4 years were HLA typed. The prevalence of our AP was 1.2%. This was lower than the values found in another study (45) on rapidly progressive periodontitis which revealed a prevalence ranging from 2 to 5%. In the AP cohort of the present study there were more females, but the male:female ratio of 1 : 1.27 was lower than that found by Suzuki (53). Comparison of our epidemiologic data for CP patients and periodontitis-free controls with that of other epidemiolgic studies is not meaningful, because only a proportion of the total number of CP patients treated at two different departments could be examined for striking HLA markers. Among the blood donors, we found a higher proportion of females. This suggested that females in general are more willing to donate blood than their male counterparts, a social effect that should also be taken into consideration. Female patients and periodontitis-free probands had higher values for API and correspondingly significantly increased values for SBI. Although smokers were more frequent in male periodontitis patients and male periodontitis-free probands than in females, we did not find any significant gender differences for PD or AL.

Because there were more female than male patients with AP in this and in other studies, and because gender-dependent HLA deviations have been demonstrated in several types of rheumatic disease with similarities in their pathophysiology to periodontitis (37), gender-dependent genetic susceptibility or resistant factors could exist and therefore separate HLA typing for females and males was required.

In contrast to most other studies on associations between HLA and periodontitis, our patient groups were compared with 102 periodontitis-free probands as a control group. We therefore experienced a much greater contrast between 'affected' and 'healthy'. The minimum age of the controls was restricted to 38 years, excluding the possibility that AP could develop at a later age. However, CP could appear later than the age of 38 years and so a strict differentiation between periodontal healthy controls and CP is impossible. All patient groups and the control group were examined both as a whole and for females and males separately, in order to investigate gender-determined HLA deviations. The data for blood donors represented the normal distribution and corresponded to values already published (20). This group was checked for infections and diseases according to the guidelines for blood donation in Germany, but was not examined for periodontal diseases and therefore represents a heterogeneous group in terms of periodontal diseases. Therefore, a direct comparison between blood donors and patient groups was not in any way meaningful. Rather, the blood donor group was used to estimate whether any HLA deviation affected the patients, the controls or both groups. Significantly increased or

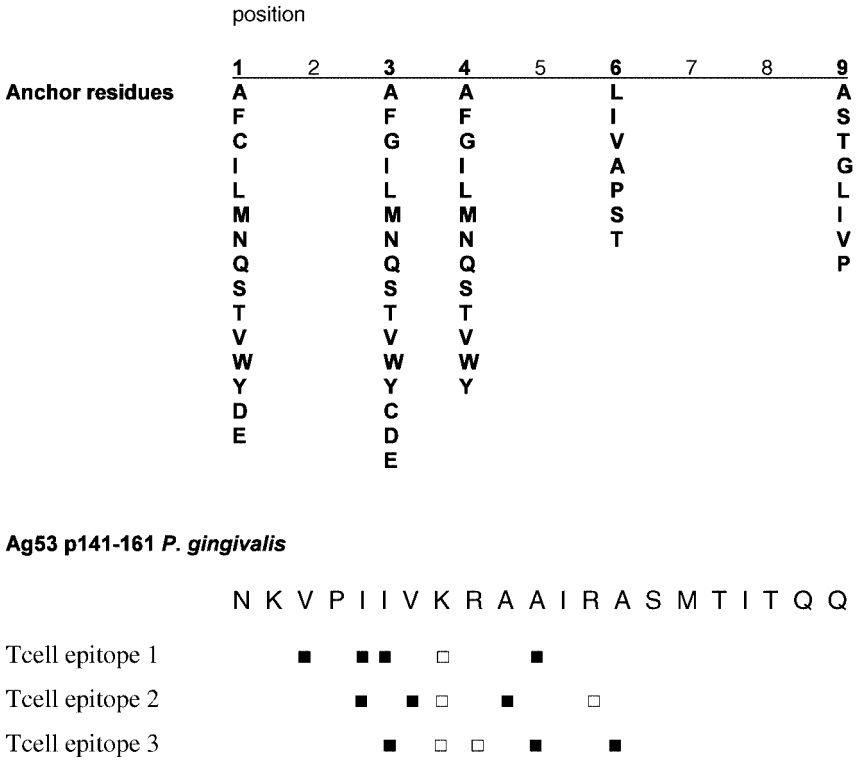

Fig. 2. Possible motifs binding to HLA-DQB1*0602. Anchor amino acid residues for the HLA-DQB1*0602 molecule are shown (44). Possible binding motifs found in the Ag53 p141–161 peptide are shown. The boxes have the same meanings as those shown in Fig. 1.

decreased HLA frequencies in patients in comparison to controls and the normal distribution could be associated with higher or lower susceptibility to periodontitis, respectively.

Separate HLA typing for males and females revealed some striking HLA markers, which to date are unidentified, as well as some discrepancies in the results of other studies on the association of class I and II HLA markers in periodontitis. The significant negative association of HLA-DQB1*0303 with CP in females has not previously been described. The increased frequency of HLA-A*68/69 (A28)-positive AP females has not been confirmed in other groups with rapidly progressive periodontitis, but is supported by findings in a Danish Caucasian juvenile periodontitis group (46). In contrast to our findings, in two previous studies of adult and juvenile periodontitis, respectively (39, 54), the marker A28 (A*68/89) and its split A68 (A*68) were found to be decreased. This may be a hint that HLA-A28 plays a role in periodontitis. In contrast to previous studies on juvenile periodontitis (19, 46, 50) and rapidly progressive periodontitis (3, 19, 28), we revealed a significantly lower frequency of HLA-A*24 in our AP + CP females. Moreover, in contrast to the increased frequency of HLA-DQB1*0503 (high-resolution allele of DQB1*05) in Japanese early onset periodontitis (EOP) patients (40, 41), we obtained a lower frequency of HLA-DQB1*05, predominantly in female AP patients. However, gender dependent HLA phenotype frequences of HLA-A*24 and HLA-DQB1*05 have not been considered in these former studies. HLA-B*14, -B*27 and -Cw*08 cannot be considered as susceptibility or resistance factors for CP females or for the CP group as a whole, because these HLA deviations were caused by differences in the periodontitis-free control group.

A Japanese study on EOP patients (41) revealed a higher frequency of HLA-DQB1*0602 and supported the finding of the present study of a higher frequency of HLA-DQB1*06 homozygosity in CP and AP + CP females. HLA-DQB1*0602 could be linked to HLA-DRB1*1501, and this HLA-DRB1 molecule was found to be responsible for T-cell proliferation in Japanese EOP patients (40).

Takashiba et al. (54) found two possible motifs binding to HLA-DRB1*1501 in an outer membrane protein of *P. ginigivalis* (Ag53 p141–161). To search for motifs binding to the striking MHC class II markers found in the present study, two different databases were used in which possible anchor amino acid residues were characterized. At least three anchor amino acid residues had to be matched by the antigenic peptide for binding to a MHC class II molecule. When Ag53 was applied to HLA-DRB1*1301 and -DRB1*1302 (alleles of the marker HLA-DRB1*13 increased in the AP group as a whole) as well as HLA-DQB1*0602 (allele of HLA-DQB1*06 with a significantly increased frequency of homozygosity in CP and AP + CP females), two or three possible T-cell epitopes, respectively, emerged (Figs 1A,B and 2). HLA-DQB1*0501 (allele of HLA-DQB1*05 decreased in our AP females) prefers leucine as an anchor at relative position 1 and tyrosine/phenylalanine/tryptophan at relative position 5. When the Ag53 p141–161 peptide was applied, in contrast to HLA-DRB1*13 and HLA-DQB1*06, no binding motifs could be defined. Anchor residues for HLA-DRBblank* (negatively associated with AP) and HLA-DQB1*0303 (not present in CP females) have not yet been characterized (44). However, this theoretical 'epitope prediction' allowed only a preselection of antigenic peptides. The ability to bind certain HLA molecules and to activate T cells should be investigated in further studies. Nevertheless, this result could be a possible indication that certain HLA class II alleles could directly affect the capability to bind certain bacterial antigens and in this manner influence susceptibility or resistance to periodontitis.

In comparison to MHC class II molecules, the HLA-A, -B, and -C markers present peptides from proteins compounded within a cell (virus peptides and cancer peptides), and therefore binding of peptides from periodontitis bacteria is improbable. Nevertheless, previous studies and our investigations have revealed positive as well as negative associations of several HLA class I markers in periodontitis. Possible reasons for these associations are molecular mimicry of HLA for certain periodontopathic bacteria (18) with impaired immune response or tissue destruction by autoimmune reactions. Furthermore, striking HLA class I

markers could be linked to (as yet unknown) susceptibility or resistance factors to periodontitis. The ability of class I markers to bind viruses could play an adjuvant role in the pathogenesis of periodontitis. In a recent study, Contreras and Slots (12) detected herpes viruses (Epstein-Barr virus and cytomegalovirus) in gingival biopsies from patients with periodontitis. It was inferred that virus infection impairs periodontal defense, thereby permitting overgrowth of periodontopathic bacteria. It has been reported that several MHC class I molecules are able to bind to virus peptides. For example, HLA-A*29, which was significantly increased in our AP and CP + AP groups, and HLA-A*11, which was significantly increased in our CP and CP + AP groups, are able to bind peptides from Epstein-Barr virus (EBV) (42, 47, 58). However, HLA-A*3002 (an allele of HLA-A*30 not present in the AP group and significantly decreased in the AP + CP group) is able to bind EBV-EBNA-3 176–184 (47).

The present study has revealed some gender-determined differences in HLA deviations in relationship to AP and CP. Thus, there is not only a direct relationship between gender and the disease (especially its aggressive forms), but also an indirect relationship in terms of certain HLA factors. In addition to other factors, such as analysis of infrequent HLA alleles in groups of insufficient size, different geographical/ethnological origins of groups, use of only a serologic typing technique, investigation of either class I or class II HLA markers, and control groups without periodontal examination, differences in the sex ratio of patients or control groups could explain discrepancies in the results of some previous HLA studies. Only by evaluating HLA deviations separately for females and males was it possible to define HLA markers positively or negatively associated with periodontitis which were inconspicuous in groups that were not separated. Moreover, it was possible to determine whether significant HLA deviations in patient groups as a whole were attributable to males, females or both genders. In the AP + CP group, females but not males showed significant HLA deviations. Therefore, it would be useful to investigate the possible associations between certain HLA markers and sex hormones. For instance, HLA-B*27 is linked to high testosterone levels in men suffering from HLA-related rheumatic diseases (26). Regarding the different striking HLA markers in AP and CP, the results suggest that the two periodontal diseases are under different genetic control. In the present paper and in other studies, however, no striking associations between HLA markers and periodontitis have been revealed. Therefore, our findings suggest that certain HLA markers or other genetic factors linked to them could affect, but not completely determine, susceptibility or resistance to periodontitis. Evidently, gender is an additional confounding variable which should be considered in further studies on HLA and periodontitis. Because of the large number of potential periodontal pathogens (bacteria and viruses) and the apparent commensal nature of many of them (43), searching HLA databases for possible ligands or T-cell epitopes could be useful for the preselection of antigenic peptides before carrying out further binding studies. It is possible that HLA-DRB1*13 and HLA-DQB1*0601 are able to bind an outer membrane protein of *P. gingivalis* (Ag53), and this could lead to an accelerated T-cell response and hyperimmune reactions to *P. gingivalis*, and thus to higher susceptibility to AP and CP, respectively. Further studies on other antigenic peptides and their ability to bind to several striking HLA markers, as well as investigations into other genetic risk factors that could be linked to HLA, are required.

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References

1. Albandar JM, Brunelle JA, Kingman A. Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. J Periodontol 1999: **70**: 13–29.
2. Alley CS, Reinhardt RA, Maze CA et al. HLA-D and T lymphocyte reactivity to specific periodontal pathogens in type 1 diabetic periodontitis. J Periodontol 1993: **64**: 974–979.
3. Amer A, Singh G, Darke C, Dolby AE. Association between HLA antigens and periodontal disease. Tissue Antigens 1988: **31**: 53–58.
4. Beaty TH, Boughman JA, Yang P, Astemborski JA, Suzuki JB. Genetic analysis of juvenile periodontitis in families ascertained through an affected proband. Am J Hum Genet 1987: **40**: 443–452.
5. Bonfil JJ, Dillier FL, Mercier P et al. A 'case control' study on the role of HLA DR4 in severe periodontitis and rapidly progressive periodontitis. J Clin Periodontol 1999: **26**: 77–84.
6. Boughman JA, Astemborski JA, Suzuki JB. Phenotypic assessment of early onset periodontitis in sibships. J Clin Periodontol 1992: **19**: 233–239.
7. Böyum A. A one-stage procedure for isolation of granulocytes and lymphocytes from human blood. Scand J Clin Lab Invest 1986: **21**: 77–89.
8. Brusic V, Rudy G, Kyne AP, Harrison LC. MHCPEP, a database of MHC-binding peptides: update 1997. Nucl Acids Res 1998: **26**: 368–371.
9. Buckley CE, Dorsey FC, Corley RR, Ralph WB, Woodbury MA, Amos DB. HL-A-linked human immune-response genes. Proc Natl Acad Sci USA 1973: **70**: 2157–2161.
10. Cogen RB, Wright JT, Tate AL. Destructive periodontal disease in healthy children. J Periodontol 1992: **63**: 761–765.
11. Colin BW, Putnins EE. The Periodontal Disease Classification System of the American Academy of Periodontology – an update. J Assoc Dent Can 2000: **66**: 594–597.
12. Contreras A, Slots J. Herpesviruses in human periodontal disease. Short review. J Periodont Res 2000: **35**: 3–16.
13. De Moor P, Boogaerts M, Louwagie A. More familial leukaemia in ALL patients with both unexplained high transcortin levels and an HLA antigen Cw3. Br J Haematol 1988: **69**: 225–227.
14. Dorak MT, Machulla HK, Hentschel M, Mills KI, Langner J, Burnett AK. Influence of the major histocompatibility complex on age at onset of chronic lymphoid leukaemia. Int J Cancer 1996: **17**: 134–139.
15. Dorak MT, Owen G, Galbraith I et al. Nature of HLA-associated predisposition to childhood acute lymphoblastic leukemia. Leukemia 1995: **9**: 875–878.
16. Dyer JK, Peck MA, Reinhardt RA et al. HLA-D types and serum IgG responses to Capnocytophaga in diabetes and periodontitis. J Dent Res 1997: **76**: 1825–1832.
17. Dyer P, Warrens A. Design and interpretation of studies of the major histocompatibility complex in disease. In: Lechler R, ed. HLA and disease. San Diego: Academic Press, 1994: 100–120.
18. Ebringer A. Ankylosing spondylarthritis, HLA B27 and the theory of

crossed tolerance. Rev Rhum Mal Osteoartic 1983: **50**: 763–769.
19. Firatli E, Kantarci A, Cebeci I et al. Association between HLA antigens and early onset periodontitis. J Clin Periodontol 1996: **23**: 563–566.
20. Gjertson DW, Geer L, Lee SH et al. Population studies. In: Gjertson DW, Terasaki PI, ed. HLA 1998. Lenexa, Kansas: American Society for Histocompatibility and Immunogenetics, 1998: 103–326.
21. Goteiner D, Goldman MJ. Human lymphocyte antigen haplotype and resistance to periodontitis. J Periodontol 1984: **55**: 155–158.
22. Greenberg LJ, Gray ED, Yunis EJ. Association of HL-A5 and immune responsiveness in vitro to streptococcal antigens. J Exp Med 1975: **141**: 935–943.
23. Grossi SG, Genco RJ, Machtei EE et al. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. J Periodontol 1995: **66**: 23–29.
24. Haldane JBS. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet 1956: **20**: 309–311.
25. Hirata AA, Terasaki PI. Cross-reactions between streptococcal M proteins and human transplantation antigens. Science 1970: **168**: 1095–1096.
26. James WH. Sex ratios and hormones in HLA related rheumatic diseases. Ann Rheum Dis 1991: **50**: 401–444.
27. Katz J, Goultschin J, Benoliel R, Brautbar C. Human leukocyte antigen (HLA) DR4. Positive association with rapidly progressing periodontitis. J Periodontol 1987: **58**: 607–610.
28. Klouda PT, Porter SR, Scully C et al. Association between HLA-A9 and rapidly progressive periodontitis. Tissue Antigens 1986: **28**: 146–149.
29. Lange DE, Plagmann HC, Eenboom A, Promesberger A. Clinical methods for the objective evaluation of oral hygiene. Dtsch Zahnarztl Z 1977: **32**: 44–47.
30. Lindhe J, Nyman S. The effect of plaque control and surgical pocket elimination on the establishment and maintenance of periodontal health. A longitudinal study of periodontal therapy in cases of advanced disease. J Clin Periodontol 1975: **2**: 67–79.
31. Loe H, Brown LJ. Early onset periodontitis in the United States of America. J Periodontol 1991: **62**: 608–616.
32. Long JC, Nance WE, Waring P, Burmeister JA, Ranney RR. Early onset periodontitis: a comparison and evaluation of two proposed modes of inheritance. Genet Epidemiol 1987: **4**: 13–24.
33. Mallon E, Bunce M, Wojnarowska F, Welsh K. HLA-CW*0602 is a susceptibility factor in type I psoriasis, and evidence Ala-73 is increased in male type I psoriatics. J Invest Dermatol 1997: **109**: 183–186.
34. Marazita ML, Burmeister JA, Gunsolley JC, Koertge TE, Lake K, Schenkein HA. Evidence for autosomal dominant inheritance and race-specific heterogenity in early-onset periodontitis. J Periodontol 1994: **65**: 623–630.
35. Marggraf E, Von Keyserlingk-Eberius HJ, Komischke B, Wöllert N. Die Assoziation von Histokompatibilitätsantigenen (HLA-Antigene) mit profunden Parodontopathien. Dtsch Zahnärztl Z 1983: **38**: 585–589.
36. Melvin WL, Sandifer JB, Gray JL. The prevalence and sex ratio of juvenile periodontitis in a young racially mixed population. J Periodontol 1991: **62**: 330–334.
37. Mercado F, Marshall RI, Klestov AC, Bartold PM. Is there a relationship between rheumatoid arthritis and periodontal disease? J Clin Periodontol 2000: **27**: 267–272.
38. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 1988: **16**: 1215–1218.
39. Moses JH, Tsichti H, Donaldson P, Smith PB, Johnson NW, Bodmer JG. HLA and susceptibility to juvenile periodontitis in Afro-Caribbeans. Tissue Antigens 1994: **43**: 316–319.
40. Ohyama H, Matsushita S, Kato N et al. T cell responses to 53-kDa outer membrane protein of *Porphyromonas gingivalis* in humans with early-onset periodontitis. Hum Immunol 1998: **59**: 635–643.
41. Ohyama H, Takashiba S, Oyaizu K et al. HLA Class II genotypes associated with early-onset periodontitis: DQB1 molecule primarily confers susceptibility to the disease. J Periodontol 1996: **9**: 888–894.
42. Pepperl S, Benninger-Doring G, Modrow S, Wolf H, Jilg W. Immediate-early transactivator Rta of Epstein-Barr virus (EBV) shows multiple epitopes recognized by EBV-specific cytotoxic T lymphcytes. J Virol 1998: **11**: 8644–8649.
43. Podmore M, Ebersole JL, Kinane DF. Immunodominant antigens in periodontal disease: a real or illusive concept? Crit Rev Oral Biol Med 2001: **12**: 179–185.
44. Rammensee HG, Bachmann J, Emmerich NPN, Bachor OA, Stevanovic S. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 1999: **50**: 213–219.
45. Rateitschak KH, Rateitschak EM, Wolf HF. Periodontology (4). Rapidly progressing periodontitis. Schweiz Monatsschr Zahnmed 1988: **98**: 51–56 [French, German].
46. Reinholdt J, Bay I, Svejgaard A. Association between HLA-antigens and periodontal disease. J Dent Res 1977: **56**: 1261–1263.
47. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte response to Epstein-Barr virus infection. Annu Rev Immunol 1997: **15**: 405–431.
48. Salonen LW, Frithiof L, Wouters FR, Hellden LB. Marginal alveolar bone height in an adult Swedish population. A radiographic cross-sectional epidemiologic study. J Clin Periodontol 1991: **18**: 223–232.
49. Saxen L, Nevanlinna HR. Autosomal recessive inheritance of juvenile periodontitis: test of hypothesis. Clin Genet 1984: **25**: 332–335.
50. Shapira L, Eizenberg S, Sela MN, Soskolne A, Brautbar H. HLA A9 and B15 are associated with the generalized form, but not the localized form, of early-onset periodontal diseases. J Periodontol 1994: **65**: 219–223.
51. Shinn DLS. Metronidazole in acute ulcerative gingivitis. Lancet 1962: **1**: 1191.
52. Slots J. The predominant cultivable microflora of advanced periodontitis. Scand J Dent Res 1977: **85**: 114–121.
53. Suzuki JB. Diagnosis and classification of the periodontal diseases. Dent Clin North Am 1988: **32**: 195–216.
54. Takashiba S, Ohyama H, Oyaizu K, Kogoe-Kato N, Murayama Y. HLA genetics for diagnosis of susceptibility to early-onset periodontitis. J Periodontal Res 1999: **34**: 374–378.
55. Tanner AC, Haffer C, Bratthall GT, Visconti RA, Socransky SS. A study of the bacteria associated with advancing periodontitis in man. J Clin Periodontol 1979: **5**: 278–307.
56. Tonetti MS, Mombelli A. Early onset periodontitis. In: Lindhe J, Karring T, Lang NP, ed. Clinical periodontology and implant dentistry. Copenhagen: Munksgaard, 1998: 226–257.
57. van Winkelhoff AJ, Rams TE, Slots J. Systemic antibiotic therapy in periodontics. Periodontol 2000 1996: **10**: 45–78.
58. Zhang QJ, Gavioli R, Klein G, Masucci MG. An HLA-A11-specific motif in nonamer peptides derived from viral and cellular proteins. Proc Natl Acad Sci USA 1993: **15**: 2217–2221.