International Poster Journal IPJ of Dentistry and Oral Medicine

Int Poster J Dent Oral Med 2005, Vol 7 No 04, Poster 293

HLA associations to periodontopathic bacteria

Language: English

Authors:
OA Dr. Stefan Reichert
Universitätspoliklinik für Zahnerhaltungskunde und Parodontologie, Martin-Luther-Universität Halle-Wittenberg

Dr. med. dent. Jamal M. Stein
Klinik für Zahnerhaltung, Parodontologie und Präventive Zahnheilkunde, RWTH Aachen

Univ.-Prof. Dr. Hans-Günter Schaller
Universitätspoliklinik für Zahnerhaltungskunde und Parodontologie, Martin-Luther-Universität Halle-Wittenberg

Dr. rer. nat. Helmut Machulla
Interdisziplinäres HLA Labor, Martin-Luther-Universität Halle-Wittenberg

Date/Event/Venue:
25.-28. August 2004
Joint Meeting of the CED, NOF of the IADR
Istanbul

Introduction

Our previous studies revealed HLA class I- and II-associations to aggressive (AP) and chronic (CP) periodontitis (Machulla et al. 2002, Reichert et al. 2003, Stein et al. 2003). Antigenic peptides were bound by HLA molecules over specific pockets of the hypervariable peptid binding region in order to present them T lymphocytes. In patients with advanced periodontitis, HLA class II-molecules were expressed by Langerhans cells in both oral gingival epithelium and pocket epithelium (Nunes et al. 1994). Colonization of periodontopathic bacteria and antigen specific immune response could be controlled by polymorphism of HLA alleles. That's why an association from HLA to periodontopathic bacteria could be exist.

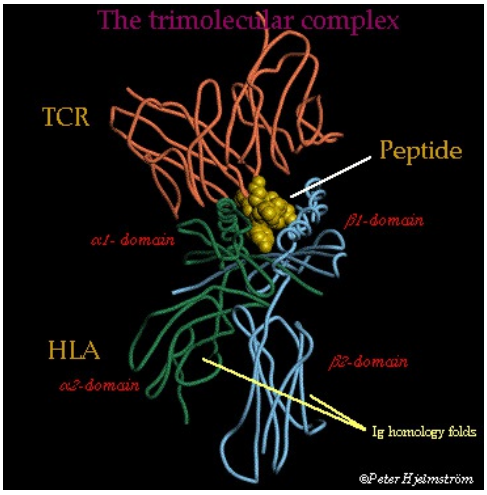

Fig. 1 (Hjelmström et al. 1996) Presentation of an antigenic peptide through the hypervariable binding groove of a HLA class II molecule (HLA-DQ). The T cell receptor recognises as well as structures of the HLA molecule and bound peptide. The length of the peptides vary from 13-25 amino acids.

Objectives

The goal of the present study was to evaluate HLA associations to A. actinomycetemcomitans (A.a.), P. gingivalis (P.g.), P. intermedia (P.i.), T. forsythensis (T.f.) and T. denticola (T.d.) in patients with both aggressive and chronic periodontitis.

Material and Methods

In both patient groups the smoking status, the approximal plaque index (API), bleeding on probing (BOP), pocket depth (PD), clinical attachment loss in general (CAL) and on the microbial test-sites (CALBact.) were determined. For the microbial assessment subgingival plaque samples were taken from the deepest four pockets (CALBact. > 5 mm) by means of sterile paper points. Bacterial infection was analyzed employing a PCR-rSSO microDent DNA-Strip technique (Hain Lifescience GmbH, Nehren, Germany). HLA-A, -B, -Cw, -DR, -DQ typing was performed by both CDC (Complement Depending Cytotoxicity assay, BAG, Lich, Germany) and polymerase chain reaction with sequence-specific primers (PCR-SSP, GenoVision VertriebsmbH Schwechat, Austria). Statistical calculations were carried out by Chi² testing, with Yates correction or Fishers Exact test, if appropriate. In order to determine the adjusted odds ratio (OR) of certain HLA markers for an infection with one of the periodontopathic bacteria the confounding variables age, gender, nicotine consumption and CALBact. were additional included in a logistic regression model. All patients and controls were of Caucasian descent and were unrelated and free from other general diseases known to be associated with certain HLA markers.

Results

AP patients were in comparison to CP patients significantly younger, more frequently smoker and A.a. positive. CP patients were significantly more infected with T.d. (Table 1).

Variable	**Chronic periodontitis (CP)**	**Aggressive periodontitis (AP)**	**P values**	**AP + CP**
	N = 35	**N = 33**		**N = 68**
Median age (range)	47 (27-68)	37 (15-55)	**0,0001**	42,5 (15-68)
females %	62,9	57,6	0,656	60,0
smoker %	14,3	48,5	0,002	30,9
API % (SD)	62,1 (10,5)	53,5 (34,8)	0,286	58,0 (29,9)
BOP % (SD)	64,4 (28,7)	71,6 (28,7)	0,317	67,8 (28,7)
PD mm (SD)	5,4 (1,2)	5,8 (1,6)	0,212	5,6 (1,4)
CAL mm (SD)	6,1 (1,5)	6,5 (1,9)	0,338	6,3 (1,7)
CAL Bact. (SD)	7,4 (1,8)	7,6 (2,0)	0,613	7,5 (1,9)
A.actinomycetem-comitans (%)	9 (25,7)	16 (48,5)	**0,052**	25 (36,8)
P. gingivalis (%)	33 (94,3)	26 (78,8)	0,079	59 (86,8)
P. intermedia (%)	22 (62,9)	21 (63,6)	0,947	43 (63,2)
T. forsythensis (%)	34 (97,1)	29 (87,9)	0,191	63 (92,6)
T. denticola (%)	35 (100)	29 (87,9)	**0,050**	64 (92,1)

Table 1: Demografic and clinical parameters of all investigated cohorts

The proof of P.g. was significantly positively correlated with the occurrence of P.i. and T.f.. Both, P.i. and T.f. were positively correlated with the detection of T.d. (Table 2).

	A.a.	**P.g.**	**P.i.**	**T.f.**	**T.d.**
A.a. r	1,000	-,152	,012	-,136	,061
P		,215	,922	,270	,621
P.g. r	-,125	1,000	,242*	,389**	,087
P	,215		,047	,001	,482
P.i. r	,012	,242*	1,000	,136	,328**
P	,922	,047		,270	,006
T.f. r	-,136	,389**	,136	1,000	,408**
P	,270	,001	,270		,001
T.d. r	,016	,087	,328**	,408**	1,000
P	,621	,482	,006	,001	

Table 2: Correlations between the five proved bacteria. *P <0.05, **P < 0.01

The occurrence of an A.a. Infection was found to be positively associated with the haplotypes HLA-B*08:Cw*07:DRB1*03:DRB3*(DR52):DQB1*02 and HLA-DRB1*13:DRB3*(DR52):DQB1*06 as well as the HLA-B super-type Bw6. On the other hand a homozygosity for HLA-Bw4 and HLA-DRB1*04:DRB4*(DR53):DQB1*03(DQ7/DQ8) were connected with a reduced risk for an A.a. infection. The HLA markers A*02 and A*23/*24(A9) increased the infection risk for P.g. and the complex P.g., T.f., T.d., respectively. An infection with P.i. occurred less frequent in patients expressing HLA-B*51/*52(B5), HLA-DRB3*(DR52) homozygous and two combinations with HLA-DRB1*13. T.f. was negatively associated with HLA-A*33 (Table 3).

HLA-	**Number HLA positive patients**	**Microorganisms**	**infected patients %**		**OR**	**P values**
			HLA +	**HLA-**		

Bw6	55	A.a.	43,6	7,7	8,2	**0,052**
B*08:Cw*07:DRB1*03:DRB3*:DQB1*02	8	A.a.	75,0	31,7	6,2	**0,039**
DRB1*13:DRB3*:DQB1*06	13	A.a.	61,5	30,9	4,5	**0,029**
Bw4 homozygous	13	A.a.	7,7	43,6	0,1	**0,052**
DRB1*04:DRB4*:DQB1*03(DQ7/DQ8)	11	A.a.	9,1	42,1	0,2	0,077
A*02	30	P.g.	100,0	76,3	*	*
		P.g., T.f., T.d.	93,3	71,7	5,5	**0,040**
A*23/ A*24 (A9)	14	P.g., T.f., T.d.	100,0	75,9	*	*
B*51 /B*52 (B5)	8	P.i.	25,0	68,3	0,2	**0,030**
DRB3*(DR52) homozygous	8	P.i.	25,0	68,3	0,1	**0,015**
B*44:DRB1*13	4	P.i.	0,0	67,2	0,001	0,746
DRB1*13:(DRB3*:DQB1*06) only AP group	6	P.i.	16,7	74,1	0,07	**0,024**
A*33	6	T.f.	66,7	95,2	0,03	**0,025**

Table 3: HLA associations to five periodontopathic bacteria. *OR could not be calculated

Conclusions

Single HLA markers, HLA homocygosities and HLA combinations could influence the infection with A.a., P.g., P.i., T.f. and T.d.. However, the mechanisms of the HLA - bacteria association are unknown. As well as bacterial mimicry with HLA (Ebringer 1983) and HLA dependent immunoreactivity to bacterial antigens (Buckley et al. 1973, Greenberg et al. 1975) could be assumed. In addition another study revealed that HLA-B8:DR3 positive subjects showed the significant lowest levels of serum IgA (Modica et al. 1989). This finding could be related to the high incidence of an A.a. infection among HLA-B*08:Cw*07:DRB1*03:DRB3*:DQB1*02 positive patients. Further studies about the binding of periodontopathic and protective peptides at striking HLA molecules are required.

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Abbreviations

HLA	human leucozyte antigen
AP	aggressive periodontitis
CP	chronic periodontitis
PCR-SSP	polymerase chain reaction with sequence-specific primers
A.a.	Actinobacillus actinomycetemcomotans
P.g.	Porphyromonas gingivalis
P.i.	Prevotella intermedia
T.f.	Tannerella forsytensis
T.d.	Treponema denticola

This Poster was submitted by OA Dr. Stefan Reichert.

Correspondence address:
OA Dr. Stefan Reichert
Universitätspoliklinik für Zahnerhaltungskunde und Parodontologie
Martin-LutherUniversität Halle-Wittenberg
Große Steinstrasse 19
06108 Halle (Saale)

Poster Faksimile:

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HLA ASSOCIATIONS TO PERIODONTOPATHIC BACTERIA

*S. Reichert[1], J. Stein[2], H.-G. Schaller[1], B. Seliger[3] & H.K.G. Machulla[3]

[1] Dept. of Operative Dentistry and Periodontology, Martin-Luther-University Halle-Wittenberg,
[2] Dept. of Periodontology RWTH Aachen, [3] Interbranch HLA Laboratory, Dept. GHATT
Martin-Luther-University Halle-Wittenberg, Germany

Introduction

Our previous studies revealed HLA class I- and II-associations to aggressive (AP) and chronic (CP) periodontitis (*Machulla* et al. 2002, *Reichert* et al. 2003, *Stein* et al. 2003). Antigenic peptides were bound by HLA molecules over specific pockets of the hypervariable peptid binding region in order to present them T lymphocytes (Fig. 1). In patients with advanced periodontitis, HLA class II-molecules were expressed by Langerhans cells in both oral gingival epithelium and pocket epithelium (*Nunes* et al. 1994). Colonization of periodontopathic bacteria and antigen specific immune response could be controlled by polymorphism of HLA alleles. That´s why an association from HLA to periodontopathic bacteria could be exist.

Fig. 1 (*Hjelmström* et al. 1996)
Presentation of an antigenic peptid through the hypervariable binding groove of a HLA class II molecule (HLA-DQ). The T cell receptor recognises as well as structures of the HLA molecule and bound peptide. The length of the peptides vary from 13-25 amino acids.

Aim

The goal of the present study was to evaluate HLA associations to A. actinomycetemcomitans (A.a.), P. gingivalis (P.g.), P. intermedia (P.i.), T. forsythensis (T.f.) and T. denticola (T.d.) in patients with both aggressive and chronic periodontitis.

Material and Methods

In both patient groups the smoking status, the approximal plaque index (API), bleeding on probing (BOP), pocket depth (PD), clinical attachment loss in general (CAL) and on the microbial test-sites (CAL_{test}) were determined. For the microbial assessment subgingival plaque samples were taken from the deepest four pockets (CAL_{test} > 5 mm) by means of sterile paper points. Bacterial infection was analyzed employing a PCR-rSSO microDent DNA-Strip technique (Hain Lifescience GmbH, Nehren, Germany). HLA-A, -B, -Cw, -DR, -DQ typing was performed by both CDC (Complement Depending Cytotoxicity assay, BAG, Lich, Germany) and polymerase chain reaction with sequence-specific primers (PCR-SSP, GenoVision Vertriebsmbh Schwechat, Austria). Statistical calculations were carried out by Chi² testing, with Yates correction or Fishers Exact test, if appropriate. In order to determine the adjusted odds ratio (OR) of certain HLA markers for an infection with one of the periodontopathic bacteria the confounding variables age, gender, nicotine consumption and CAL_{test} were additional included in a logistic regression model. All patients and controls were of Caucasian descent and were unreleated and free from other general diseases known to be associated with certain HLA markers.

Results

AP patients were in comparison to CP patients significantly younger, more frequently smoker and A.a. positive. CP patients were significantly more infected with T.d. (Table 1).

Table 1 Demografic and clinic parameters of all investigated cohorts.

Variable	Chronic periodontitis (CP) N = 38	Aggressive periodontitis (AP) N = 33	Significance	AP + CP N = 68
Median age (range)	47 (27-68)	37 (15-55)	0.0001	42.5 (15-68)
females %	52.6	57.6	0.558	60.0
smoker %	14.3	48.5	0.002	30.9
API % (SD)	52.1 (10.5)	53.3 (14.8)	0.396	58.0 (26.9)
BOP % (SD)	64.4 (28.7)	71.6 (28.7)	0.317	67.9 (28.7)
PD mm (SD)	5.4 (1.2)	5.8 (1.6)	0.212	5.5 (1.4)
CAL mm (SD)	6.1 (1.5)	5.7 (1.9)	0.338	6.3 (1.7)
CAL_{test} (SD)	7.4 (1.8)	7.6 (2.0)	0.613	7.5 (1.9)
A.actinomycetem-comitans (%)	9 (25.7)	16 (48.5)	0.362	25 (36.8)
P. gingivalis (%)	33 (94.3)	26 (78.8)	0.379	59 (86.8)
P. intermedia (%)	22 (62.9)	21 (63.6)	0.947	43 (63.2)
T. forsythensis (%)	34 (97.1)	29 (87.9)	0.191	63 (92.6)
T. denticola (%)	35 (100)	29 (87.9)	0.060	64 (92.1)

The proof of P.g. was significantly positively correlated with the occurrence of P.i. and T.f.. Both, P.i. and T.f. were positively correlated with the detection of T.d. (Table 2).

Table 2 Correlations between the five proved bacteria. *P<0,05, **P < 0,01

	A.a.	P.g.	P.i.	T.f.	T.d.
A.a. r	1.000	-.132	.012	-.136	.061
P		.215	.922	.270	.621
P.g. r	-.125	1.000	.242*	.389**	.087
P	.215		.047	.001	.482
P.i. r	.012	.242*	1.000	.136	.328**
P	.922	.047		.270	.006
T.f. r	-.136	.389**	.136	1.000	.408**
P	.270	.001	.270		.001
T.d. r	.016	.087	.329**	.408**	1.000
P	.621	.482	.006	.001	

The occurrence of an A.a. infection was found to be positively associated with the haplotypes HLA-B*08:Cw*07:DRB1*03:DRB3*(DR52):DQB1*02 and HLA-DRB1*13:DRB3*(DR52):DQB1*06 as well as the HLA-B supertype Bw6. On the other hand a homozygosity for HLA-Bw4 and HLA-DRB1*04:DRB4*(DR53):DQB1*03(DQ7/DQ8) were connected with a reduced risk for an A.a. infection. The HLA markers A*02 and A*23/*24(A9) increased the infection risk for P.g. and the complex P.g., T.f., T.d., respectively. An infection with P.i. occurred less frequent in patients expressing HLA-B*51/*52(B5), HLA-DRB3*(DR52) homozygous and two combinations with HLA-DRB1*13. T.f. was negatively associated with HLA-A*33 (Table 3).

Table 3 HLA associations to five periodontopathic bacteria. *OR could not be calculated

HLA-	Number HLA positive patients	Micro-organisms	Infected patients % HLA +	HLA-	OR	p
Bw6	66	A.a.	49.8	7.7	8.3	0.000
B*08:Cw*07:DRB1*03:DRB3*:DQB1*02	6	A.a.	75.0	31.7	6.2	0.094
DRB1*13:DRB3*:DQB1*06	13	A.a.	61.5	30.8	4.5	0.029
Bw4 homozygous	13	A.a.	7.7	43.6	0.1	0.002
DRB1*04:DRB4*(DR53):DQB1*03(DQ7/DQ8)	11	A.a.	9.1	42.1	0.2	0.077
A*02	30	P.g.	100.0	76.3	*	*
		P.g. T.f. T.d.	93.3	71.7	8.6	0.040
A*23/*24(A9)	14	P.g. T.f. T.d.	100.0	75.8	*	*
B51/B*52 (B5)	8	P.i.	25.0	66.3	0.2	0.030
DRB3*(DR52) homozygous	8	P.i.	25.0	66.3	0.1	0.018
Bw4:DRB1*13	4	P.i.	0.0	67.2	0.001	0.748
DRB1*13:DRB3*:DQB1*06 only AP group	6	P.i.	16.7	74.1	0.07	0.024
A*33	6	T.f.	66.7	95.3	0.05	0.006

Discussion and Conclusion

Single HLA markers, HLA homocygosities and HLA combinations could influence the infection with A.a., P.g., P.i., T.f. and T.d.. However, the mechanisms of the HLA - bactera association are unknown. As well as bacterial memory with HLA (*Ebringer* 1983) and HLA dependent immunoreactivity to bacterial antigens (*Buckley* et al. 1973, *Greenberg* et al. 1975) could be assumed. In addition another study revealed that HLA-B8 DR3 positive subjects showed the significant lowest levels of serum IgA (*Modica* et al. 1989). This finding could be related to the high incidence of an A.a. infection among HLA-B*08 Cw*07:DRB1*03:DRB3*:DQB1*02 positive patients. Further studies about the binding of periodontopathic and protective peptides at striking HLA molecules are required.

IADR 25-28 AUGUST, ISTANBUL 2004

stefan.reichert@medizin.uni-halle.de