

International Poster Journal

Is the genetic background of the proinflammatory cytokine TNF-a a predictor for the development of aggressive and/or chronic periodontitis?

IP

TNF-a and periodontitis

Language: English

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Date/Event/Venue:

8.-11.03.2006 17. Tagung der Deutschen Gesellschaft für Humangenetik Heidelberg



Sensodyne-Poster-Studien-Award 2007 für das beste Poster in 2006

Introduction

Periodontitis is considered to be a chronic inflammatory disorder of the periodontal supporting tissue of teeth. That's why several factors of the immune response have been proposed as potential markers for the development of this disease. Cytokines, including the potent proinflammatory TNF-a, may be regarded as markers of the progression and severity of periodontitis as

well as indicators of an appropriate response to treatment. Genomic variants of TNF-a, including SNPs in the promoter c.-308G>A and c.-238G>A, were shown to trigger the expression of this cytokine. Therefore, the genetic background of TNF-a might play an important role in influencing the immune response to

periodontopathic bacteria via regulating the TNF-a expression.

Objectives

Objectives: The aim of the present clinical study was to evaluate the importance of genomic variants (c.-308G>A and c.-238G>A) as well as the corresponding haplotypes of TNF-a for the aetiology of chronic and aggressive periodontitis.

Material and Methods

1. Genomic investigations

1.1. DNA-isolation

Preparation of genomic DNA from human venous EDTA-blood was carried out using the blood extraction kit (Quiagen). 200µl EDTAblood and 20 µl protease were mixed in a 1,5 ml tube.

After adding of 200 µl denaturation buffer AL and pulse-vortexing for 15 sec the samples were incubated at 56°C for 10 min. 200 µl of ethanol was added to the samples, vortexed and the samples were applied to a QIAamp Spin Column were the DNA is bound. After two washing steps (buffer AW1 and AW2) the DNA bound to the column is dried by centrifugation.

200 µl distilled water is added to the samples, incubated at room temperature for 5 min and then centrifuged. The solved DNA is now in the filtrate. Long-term storage of DNA is possible at -20°C.

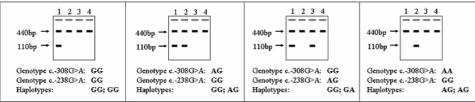
1.2. PCR

The detection of genotypes and haplotypes of TNF-a SNPs (c.-308G>A and c.-238G>A) was carried out using the CYTOKINE Genotyping array CTS-PCR-SSP Tray kit of the Collaborative Transplant Study, Department of Transplantation Immunology of the University Clinic of Heidelberg. For every PCR a fragment of 440bp of the human C-reactive protein was coamplified as a positive control.

The PCRs were performed using sequence specific primers for detection of possible haplotypes prepipetted and lyophilized in thinwalled plastic 96-well PCR trays.

For every PCR 10µl of a Mastermix containing 1U Taq-Polymerase (Invitek), 100ng genomic DNA, 5% glycerol, and PCR reaction buffer was added. PCR-program (2 min 94°C; 10 cycles: 15 sec 94°C, 1 min 64°C; 20 cycles: 15 sec 94°C, 50 sec 61°C, 30 sec 72°C) After cycling was completed, the PCR products were loaded onto a 2% agarosegel for electrophoresis. After electrophoresis, the ethidium bromide stained gel is photographed and interpreted.

Lane 1: sequence specific fragment at 110bp: G at pos. -308; G at pos. -238 Lane 2: sequence specific fragment at 110bp: A at pos. -308; G at pos. -238 Lane 3: sequence specific fragment at 110bp: G at pos. -308; A at pos. -238 Lane 4: sequence specific fragment at 110 bp: A at pos. -308; A at pos. -238



2. Investigation of periodontopathic bacteria in subgingival pockets

2.1. Sampling

Paper points for collection of subgingival samples were used to bind periodontopathogens of the deepest pocket of each quadrant.

2.2. DNA-isolation

Preparation of bacterial DNA was carried out using the QIAamp DNA Mini Kit (Quiagen).

The paper points were incubated with 180 µl ATL-buffer and 20 µl proteinase K and incubated at 70°C for 10 min.

200 µl buffer Al was added and the mixture was incubated at 96°C for 5 min.

The mixture (without paper points) was applied to a QIAamp Spin Column and washed twice with buffer AW1 and AW2. The DNA was solved in 400 µl AE-buffer and stored at -20°C.

2.3. PCR

For specific amplification of Haemophilus actinomycetemcomitans (Ha), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythensis (Tf), Treponema denticola (Td) the micro-Ident® test of HAIN-Diagnostik based on alkaline phosphatase mediated staining reaction was used.

Mastermix provided in the micro-Ident \mathbb{R} test (containing buffer, biotynilated primer, DNA for positive control'), 2U Taq-polymerase (Eppendorf), and 5 μ l of isolated bacgterial DNA were mixed.

PCR was performed (5 min 95°C; 10 cycles: 30 sec 95°C, 2 min 58°C; 20 cycles: 25 sec 95°C, 40 sec 53°C, 40 sec 70°C; 8 min 70°C) The quality of PCR product was checked by agarosegelelectrophoresis.

2.4. Hybridization

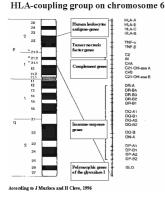
 $20 \ \mu$ l of the PCR product were mixed with $20 \ \mu$ l of the denaturation solution in the well of the tray and incubated at room temperature for 5 min.

1 ml prewarmed (45°C) hybridization buffer was added to the sample and a strip (hybridized with DNA sequences of each bacteria as well as a positive control) was placed in the well of the tray.

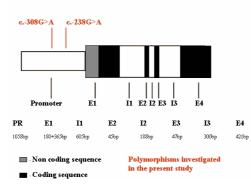
The tray was incubated at 45°C for 30 min in a shaking water bath.

After complete aspiration of hybridization buffer 1 ml of stringent wash solution was added and incubated at 45°C for 15 min. The strip was washed once with 1 ml rinse solution for 1 min and 1 ml of conjugate solution was added (room temperature for 30 min). After washing twice with 2ml rinse solution and once with 1 ml distilled water 1 ml of substrate solution was added. The substrate incubation time varied between 3 and 20 min and the occurrence of bacteria was evaluated visually by means of

The substrate incubation time varied between 3 and 20 min and the occurrence of bacteria was evaluated visually by means of colored bands. Two positive controls for amplification reaction and for conjugate were included in the test.



Genomic structure of TNFa



Results

1. Characterization of the patient groups

Clinical and demographical characterization

	Chronic periodontitis	Aggressive periodontitis	healthy controls	p values vs. controls	
	n =33	n=35	n=35	СР	AP
Mean age (years)	47.7 <u>+</u> 10.7	37.7 <u>+</u> 8.1	42.2 <u>+</u> 13.3	n.s.	n.s.
Gender (% male)	39.4	40	42.9	n.s.	n.s.
Smoking (%)	15.2	40	31.4	n.s.	n.s.
Approximal plaque index (%)	61.4±25.1	54.7 <u>+</u> 34	38.8±18.7	<0.001	0.020
Bleeding on probing (%)	62+28.4	73.7 <u>+</u> 28	40.2+29.4	0.001	<0.001
Pocket depth (mm)	5.3 <u>+</u> 1.3	5.9 <u>+</u> 1.5	2.8 <u>+</u> 1	<0.001	<0.001
Pocket depth on microbial test site (mm)	6.8 <u>+</u> 1.5	7.3 <u>+</u> 1.5	3.2 <u>+</u> 0.2	<0.001	<0.001
Clinical attachment loss in general (mm)	5.9 <u>+</u> 1.6	6.6+1.8	3.1 ± 1.1	<0.001	<0.001
Clinical attachment loss on microbial test site (mm)	7.4 <u>+</u> 1.8	8.2 <u>+</u> 1.7	3.3±0.4	<0.001	<0.001

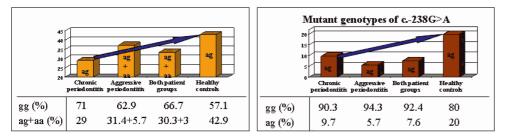
No significant association between the patient groups and the healthy control group could be proven investigating age, gender and smoking status. As expected, both patient groups showed significant more severe clinical symptoms compared to the control group.

1.2. Microbiological assessment

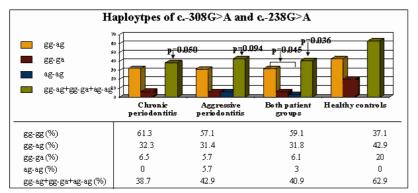
Haemophilus actinomycetemcomitans (%)	21.2	51.4	11.4	n.s.	<0.001
Porphyromonas gingivalis (%)	93.9	80	11.4	<0.001	<0.001
Prevotella intermedia (%)	60.6	65.7	25.7	0.004	<0.001
Tannerella forsythensis (%)	97	88.6	57.1	<0.001	0.003
Treponema denticola (%)	100	88.6	68.6	<0.001	0.041
Pg, Td, Tf (%)	90.9	71.4	11.4	<0.001	<0.001

As expected, both patient groups showed distinct and mostly significant increase in the occurrence of periodontopathic bacteria.

2. Genomic evaluation



For both polymorphisms of TNF-a a distinct but not significant increase in the frequency of the mutant genotypes could be detected in the control group compared with the two patient groups.



In the group of healthy controls a higher prevalence of carriers of the mutant haplotypes and combination of haplotype could be observed. Significances displayed reflect the increase of haplotype carriers of each patient group in comparison with carriers of the control group.

Conclusions

Investigating possible associations of the occurrence of chronic and/or aggressive periodontitis and the genetic background of the proinflammatory cytokine TNF-a (c.-308G>A, c.-238G>A) a distinct increase of probands carrying the mutant genotypes (c.-308G>A: ag + aa, c.-238G>A: ag) and haplotypes (ag, ga) could be shown in healthy controls compared with both patient groups. These results may be an indication for a genetically based altered, possibly more effective immune response to periodontopathic pathogens since these SNPs were considered to trigger the TNF-a production.

Abbreviations

API: Approximal plaque index BOP: Bleeding on probing CAL: Clinical attachment loss in general PD: Pocket depth SNP: small nuclear polymorphism TNF-a: Tumor necrosis factor alpha

This Poster was submitted by Dr. Susanne Schulz.

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Poster Faksimile:

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Materials and Methods

Genomic investigations

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Investigation of periodontopathic bacteria in subgingival pockets

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Results and Discussion

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Prevedella intermedia (%)	98.6					8.004	-8.0
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