International Poster Journal IPJ of Dentistry and Oral Medicine

Int Poster J Dent Oral Med 2001, Vol 3 No 2, Poster 72

Interleukin-1 Polymorphism in patients with early Onset- and Adult Periodontitis

Language: English

Author(s): Julia Vonholdt, José Roberto Gonzáles, Jörg Michel, Jens Martin Herrmann, Jörg Meyle
Poliklinik für Parodontologie, Justus-Liebig-Universität, Germany

Date/Event/Venue:
June, 8th - 10th 2000
Europerio III
Geneva, Switzerland

Introduction

IL-1 is a multifunctional cytokine. Among others, it is involved in the mechanisms of autoimmunity and the pathogenesis of chronic inflammatory diseases (see Figure 1).

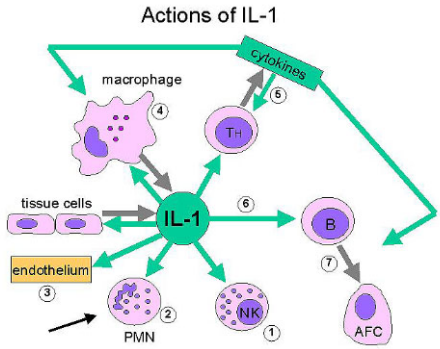

Fig. 1. IL-1 influences many cells and processes:
(1) NK cell cytocidal activity increases.
(2) Polymorphonuclear leukocytes (PMNs) are metabolically activated and move towards the site of IL-1 production by chemotaxis (black arrow).
(3) In the endothelium, adhesion molecules and procoagulants are induced, and permeability increases
(4) Prostaglandin and cytokine production and cytocidal activity increase in macrophages. Chemotaxis is also stimulated (black arrow).
(5) TH cell proliferation, IL-2 receptor expresion and cytokine production are enhanced.
(6) B-cell proliferation and differentiation into AFCs is stimulated and regulated
(7) by other cytokines.

There are three members of the IL-1 gene: IL-1A, IL-1B and IL-1 receptor antagonist (IL-1Ra).
This last one has been associated with several diseases including ulcerative colitis (Mansfield et al. 1994) systemic lupus erythematosus (Blakemore et al. 1994), alopecia areata (Tarlow et al. 1993) and lichen sclerosus (Clay et al. 1994).
Kornman et al. (1997) found a strong association between the severity of periodontitis and the composite genotype comprising allele 2 at position -889 of the IL-1A plus allele 2 at position +3953 of the IL-1B gene . However, this association was only detected in non-smokers, suggesting that smoking is a strong confounding factor for genetical analysis in adult periodontitis (Kornman et al. 1997).
Since the study of Kornman et al. several studies evaluating this association have been performed in different populations. In a recent publication, the IL-1 composite genotype was detected at a very low prevalence (2.3%) in a chinese population, in contrast to the 36% reported for Caucasians. This is a clear evidence for ethnic and racial differences in IL-1 gene polymorphisms.
There is substantial evidence that genetic and environmental factors contribute to the susceptibility of early onset periodontitis (EOP). A number of studies and case reports have demonstrated that EOP is found to aggregate within families (Page et al. 1984, Van Dyke et al. 1985). Heritable factors may be related to inflammatory immune mechanisms that, if rendered ineffective or hyperactive due to defective of inactive genes, could enhance susceptibility to the oral bacterial pathogens. Phagocyte recognition of bacteria opsonized with complement fragments (C3a and C3b) and IgG is a receptor-mediated event that involves membrane receptors for complement (CR1 and CR3) or Fc-gamma-R (Wilson et al. 1995). A defined polymorphism for Fc-gamma-RIIa has been associated with EOP and with phagocytic function of neutrophils in conjunction with more severe periodontitis in adults (Wilson et al. 1996).
Another genetic marker that has been frequently associated with EOP is the human leukocyte antigen (HLA), which plays an important role in regulating and mediating immune processes. Sofaer (1990) compiled and analyzed data from a number of these studies and concluded that the strongest negative associations with EOP are with HLA-A2 and that subjects with HLA-A9 of HLA-B15 may have an increased risk for LJP (Sofaer 1990).
In a recent study, Interleukin 1 gene polymorphisms were investigated in EOP caucasian patients and controls (Parkhill et al. 2000). The IL-1B genotype was found in increased frequency in EOP patients, however, a significant difference was only observed between EOP smokers and control smokers but not between nonsmokers from each group.
The aim of the present study was to investigate this genetic association in EOP and AP patients.

Material and Methods

18 AP (mean age ± SD: 46.8 ± 9.7) and 12 EOP (mean age ± SD: 26.3 ± 6.9) patients were included in the study. Periodontal diseases AP and EOP were defined as previously described by Salvi et al. (1998) (Salvi et al. 1998). EOP patients were distributed in two categories, the localised and the generalised form of the disease. For AP patients, 3 categories of bone loss were selected as described by Kornman et al. (1997). Additionally, a group of 15 healthy age-matched subjects were included as a control group (mean age ± SD: 25.6 ± 3.3). All patients and controls were selected according criteria for inclusion and excluded for a history of diabetes, requirement for antibiotic premedication, current pregnancy or lactation, chronic usage of anti-inflammatory drugs, a history of hepatitis or HIV infection. Subjects were evaluated clinically by one investigator and laboratory analyses were performed double blinded. Clinical parameters involved pocket depth (PD), clinical attachment level (CAL) and bleeding upon probing (BOP) at 6 sites/tooth, and modified plaque- and papillary bleeding index (PLI, PBI) at 4 sites/tooth. Genomic DNA was isolated from whole blood samples with the InstaGene(R) Whole Blood Kit (Bio-Rad Laboratories GmbH, Munich, Germany). This yields typically 5 ng DNA/micro liter and 5-10 micro liter were used in the amplification reactions. The IL-1 polymorphisms at positions +4845 for IL-1A and +3953 for IL-1B were amplified by polymerase chain reaction (PCR). Polymorphisms were detected by restriction-enzyme cleavage with slight modifications from the protocol described by Kornman et al (1997). The digested product was visualized after electrophoresis on a 3% MetaPhor(R)-agarose (Biozym, Oldendorf, Germany) gel stained with ethidium bromide.

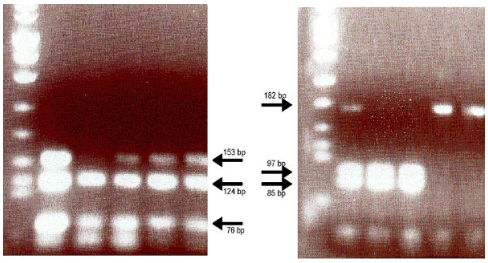

Fig. 2. Restriction pattern of IL-1A and IL-1B. Fnu 4HI (IL-1A) enzyme gave products of 76bp + 29bp + 124bp (allele 1) and 76bp and 153bp (allele 2). Taq I (IL-1B) enzyme gave products of 12bp + 85bp + 97bp (allele 1) and 12bp + 182bp (allele 2).

Results

13 patients with adult periodontitis had the severe form of the disease and 5 patients presented the moderate stage. Overall, 4 (30.7%) of the 13 patients with advanced AP (AAP) and 1 (20%) with moderate AP (MAP) carried the composite genotype. None of the patients with the generalised form of EOP (GEOP) carried the genotype and 33.3% of the EOP localised form (LEOP) carried the genotype. When these categories were combined, a higher ocurrence of the genotype was observed in the patients with AP (38.4%) than in patients with EOP. The occurrence of the genotype in association with the disease categories is shown in Figure 3.

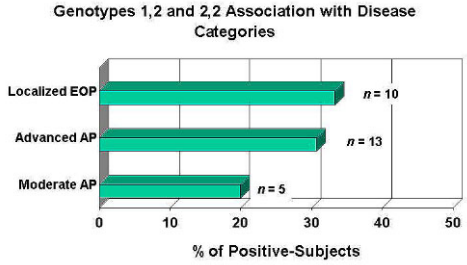

Fig. 3. The occurrence of the composite genotype (IL-1A +4845 allele 2 plus IL-1B +3953 allele 2) in the different disease groups. Localized EOP n = 10, advanced AP n = 13, moderate AP n = 5. Generalized EOP patients carrying the genotype were not observed

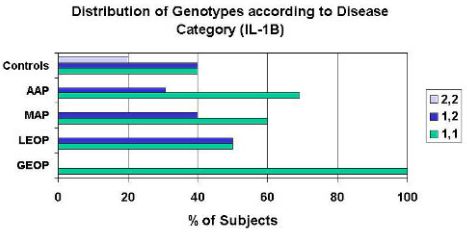

Fig. 4. The frequency of genotypes containing IL-1B allele 2 in the different disease groups. Localized EOP (LEOP) n = 10, generalized EOP (GEOP) n = 2, advanced AP (AAP) n = 13, moderate AP (MAP) n = 5. Generalized EOP patients carrying the genotypes 1,2 or 2,2 were not observed.

Figures 4 and 5 show the distribution of the genotypes positive and negative for both IL-1A and IL-1B genes according to disease severity and disease categories . Results 1,1 for both genes were considered negative, because no copy of the +4845 polymorphic allele 2 of the IL-1A and no copy of the +3953 allele 2 of the IL-1B were present. None of the patients with GEOP carried the genotypes 1,2 or 2,2 and only one patient with MAP carried the genotype. The 1,2 genotype was found increased in the LEOP group for both genes in comparison to the AAP group, even when a homozygously positive result was rare in both groups. A high prevalence of IL-1 polymorphism (53.3%) was found in the control group.
The clinical characteristics of AP, EOP patients and controls who were positive for the composite genotype and those who were negative are shown respectively in Figures 6, 7 and 8. Increased mean of PD and CAL were observed in the AAP patients and controls carrying the IL-1 composite genotype. However, the LEOP patients who were negative for the genotype showed increased levels of BOP, PD and CAL than the EOP patients who were positive.

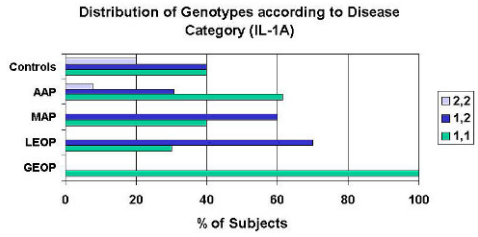

Fig. 5. The frequency of genotypes containing IL-1A allele 2 in the different disease groups. Localized EOP (LEOP) n = 10, generalized EOP (GEOP) n = 2, advanced AP (AAP) n = 13, moderate AP (MAP) n = 5. Generalized EOP patients carrying the genotypes 1,2 or 2,2 were not observed.

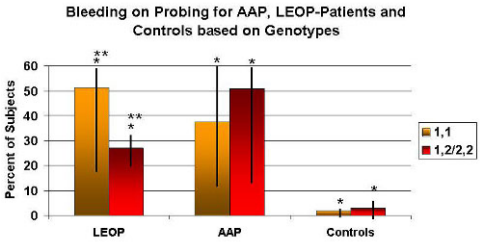

Fig. 6. BOP in the different disease groups (mean ± SD). Localized EOP (LEOP) n = 10, advanced AP (AAP) n = 13, controls n = 15. BOP was significantly increased in both disease categories compared to controls, and significantly increased between LEOP patients genotype 1,1 and genotypes 1,2/2,2.

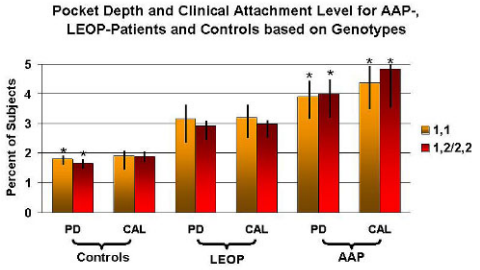

Fig. 7. PD and CAL in the different disease groups (mean ± SD). Localized EOP (LEOP) n = 10, advanced AP (AAP) n = 13, controls n = 15. PD and CAL were significantly increased in AAP disease compared to controls.

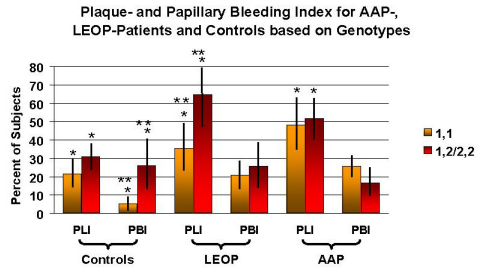

Fig. 8. PLI and PBI in the different disease groups (mean ± SD). Localized EOP (LEOP) n = 10, advanced AP (AAP) n = 13, controls n = 15. PLI and PBI were significantly increased in AAP an LEOP diseases compared to controls. Within groups, PLI and PBI were significantly increased in LEOP patients and controls with genotypes 1,2/2,2 compared to LEOP patients and controls with genotype 1,1.

Discussion and Conclusions

The present study revealed a similar frequency of the IL-1A (53%) and IL-1B (44%) genotypes containing the allele 2 in Caucasians as reported by Kornman et al. (1997) and Gore et al. (1998). However, the AP patient population in our study can not be entirely compared with that examined by Kornman et al. (1997) because of one aspect. Smoking has been established as a confounding factor in genetic analysis of IL-1 in patients with AP. In the present study, smokers were present in all patients groups, and it was not feasible to examine the effect of smoking because the sample size was small.
Ethnic differences in AP disease-associated polymorphisms within the IL-1 gene cluster have been recently reported (Armitage et al. 2000). Therefore, the patients examined in our study were exclusively Caucasians.
There is evidence that genetic factors are involved in the susceptibility to EOP but the role of the composite IL-1 genotype has not been extensively investigated in these patients and in different populations. One recent study reported a significant difference in the IL-1B genotype distribution between EOP smokers and control smokers. Again, the authors discussed the role of smoking and concluded that the interaction of smoking with the IL-1 genotype may increase susceptibility to EOP (Parkhill et al. 2000). These investigators detected a significantly increased distribution of allele 1 of the IL-1B gene in EOP patients versus controls. We obtained similar results in our study, although in the presence of a high percentage of positive controls. The highest prevalence of allele 2 in both genes occured in the EOP patient groups.
IL-1 genotype positive has been associated with BOP in patients during supportive therapy (Lang et al. 2000). We observed significant differences in BOP between AP patients who were genotype positive and genotype negative, as well as in controls. Conversely, BOP was found significantly increased in the patients with localised EOP who were genotype negative and those who were genotype positive (mean % ± SD: 51.40 ± 31.87 versus mean % ± SD: 27.00 ± 8.45). The reasons for this difference are not completely clear, but they raise an interesting question for further investigation, i.e. they may reflect population heterogeneity or a confounding factor such as smoking may be involved. Furthermore, it may reflect the limits of the IL-1 genotype in providing clinical information representing an individual risk factor in patients with EOP. In this case, it will be necessary to identify another candidate gene, or a combination of genes, that are convincing enough to explain genetic factors of the EOP phenotypes. However, we think that the sample population investigated was too small.
We conclude that at the moment the presence of this polymorphism alone is not sufficient to establish a relation with disease progression for AP and EOP patients. Further studies are necessary, i.e. multicenter studies in different populations would be necessary in order to develop a large database of EOP patients and their genotypes.

Bibliography

- Armitage, G. C., Wu, Y., Wang, H.Y., Sorrell, J., di Giovine, F.S. & Duff, G.W.: Low prevalence of a periodontitis-associated interleukin-1 composite genotype in individuals of chinese heritage. Journal of Periodontology 2000, 71, 164-171.
- Blakemore, A. I., Tarlowe, J.K., Cork, M.J., Gordon, C., Emery, P. & Duff, G.W.: Interleukin-1 receptor antagonist gene polymorphism as a severity factor in systemic lupus erythematous. Arthritis and Rheumatism 1994, 37, 1380-1385.
- Clay, F. E., Cork, M.J., Tarlow, J.K., Blakemore, A.I., Harrington, C.I., Lewis, F. & Duff, G.W.: Interleukin-1 receptor antagonist gene polymorphism association with lichen sclerosus. Human Genetics 1994, 94, 407-410.
- Gore, E. A., Sanders, J.J., Pandey, J.P., Palesch, Y. & Galbraith, G.M.: Interleukin-1beta +3953 allele 2: association with disease status in adult periodontitis. Journal of Clinical Periodontology 1998, 25, 781-785.
- Kornman, K. S., Crane, A., Wang, H.Y., di Giovine, F.S., Newman, M.G., Pirk, F.W., Wilson, T.G., Jr., Higginbottom, F.L. & Duff, G.W.: The interleukin-1 genotype as a severity factor in adult periodontal disease. Journal of Clinical Periodontology 1997, 24, 72-77.
- Lang N.P., Tonetti M.S., Suter J., Sorrell J., Duff G.W., Kornman K.S.: Effect of interleukin-1 gene polymorphisms on gingival inflammation assessed by bleeding on probing in a periodontal maintenance population. J Periodontal Research 2000, 35, 102-7.
- Mansfield, J. C., Holden, H., Tarlow, J.K., di Giovine, F.S., McDowell, T.L., Wilson, A.G., Holdsworth, C.D. & Duff, G.W.: Novel genetic association between ulcerative colitis and the antiinflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 1994, 106, 637-642.
- Page, R. C., Sims, T.J., Geissler, F., Altman, L.C. & Baab, D.A.: Abnormal leukocyte motility in patients with early - onset periodontitis. Journal of Periodontal Research 1984, 19, 591-594.
- Parkhill, J. M., Hennig, B.J.W., Chapple, I.L., Heasman, P.A. & Taylor, J.J.: Interleukin-1 gene polymorphisms and early onset periodontitis. Journal of Dental Research 2000 IADR Abstracts
- Salvi, G. E., Brown, D.L., Fujihashi, K., Kiyono, H., Smith, F.W., Beck, J.D. & Offenbacher, A.: Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. Journal of Periodontal Research 1998, 33, 212-225.
- Sofaer, J. A.: Genetic approaches in the study of periodontal diseases. Journal of Clinical Periodontology 1990, 17, 401-408.
- Tarlow, J. K., Blakemore, A.I., Lennard, A., Solari, R., Steinkasserer, A. & Duff, G.W.: Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Human Genetics 1993, 91, 403-404.
- Van Dyke, T. E., Schweinebraten, M., Cianciola, L.J., Offenbacher, S. & Genco, R.J.: Neutrophil chemotaxis in families with localized juvenile periodontitis. Journal of Periodontal Research 1985, 20, 503-541.
- Wilson, M. E. & Hamilton, R.G.: Immunoglobulin G subclass response of juvenile periodontitis subjects to principal outer membrane proteins of Actinobacillus actinomycetemcomitans. Infection and Immunity 1995, 63, 1062-1069.
- Wilson, M. E. & Kalmar, J.R.: Fc-gamma-RIIA (CD32) - a potential marker defining susceptibility to localized juvenile periodontitis. Journal of Periodontology 1996, 67, 323-331.

This Poster was submitted on 15.05.01 by Julia Vonholdt.

Correspondence address:
Julia Vonholdt
Poliklinik für Parodontologie
Justus-Liebig-Universität
Schlangenzahl 14
D-35392 Giessen

Poster Faksimile: