- to analyze genetically the polymorphism of apolipoprotein E and B in patients with EOP and to detect the levels of biochemical markers of cardiovascular disease in sera of patients;

Results

Twenty patients (7 males and 13 females, aged 15 - 26) generally healthy with EOP were enrolled in the study. The patients have been regularly clinically and immunologically examined at the Institute of Dental Research, Prague, Czech Republic for more then 10 years. They met the basic clinical diagnosis criterion – the presence of at least one periodontal pocket deeper than 3mm situated in the region of the molars or incisors, inflammation of gingiva and the presence of *A. a.* in the sulcular region. Persons with systemic disease or pregnant women were excluded from the group of the patients.

The patients were divided into two groups according to the presence of mild periodontal pockets after the forth year of the study. Group A contained 10 patients in whom periodontal pockets (PD≥3mm) were detected only in the 4th year of therapy, but no periodontal pocket and only mild inflammation of gingiva were detected in the subsequent years. Group B contained 10 patients in whom at least 1 periodontal pocket (PD≥3mm) and inflammation of gingiva persisted for more than 1 year. The state of the periodontium was improved in 15 patients later during the studied period, but in 5 other patients the disease strongly progressed (Krátká et al. in press).

We detected the risk IL-1 composite genome in 12 patients (60 %). We found a higher occurrence of risk IL-1 composite genome in the group of patients with progressive form of periodontitis than in patients with slow progression (Krátká et al in press).

We also analyzed the presence of bacteria in the sulcular fluid of 16 patients using the method of reverse hybridization of DNA. *Treponema denticola* was detected in 13 patients (81.3 %), *Porphyromonas gingivalis* in 10 patients (62.5 %), *Prevotella intermedia* in 7 patients (43.8 %), *A. a.* in 8 patients (50 %) and *Tannerella forsythia* in 5 patients (31.3 %). Two patients (12.5 %) did not harbor any of the monitored bacteria.

Patients without the IL-1 composite genome harbored 0 - 2 species of pathogenic bacteria in the sulcular area, whereas on average 3.2 species of pathogenic bacteria were detected in patients with the IL-1 composite genome. Although the progression of the disease was stopped during the treatment in most of the patients, the persistence of pathogenic bacteria in the sulcular region of patients presents the potential danger for the next progression of the disease if the change of the condition occurs (Krátká et al in press).

We analyzed the function of the polymorphonuclear cells by testing the metabolic outburst called INT test. Lower INT values than its physiology range were detected in 90 % of patients at early therapy. The same was detected in 53 % of patients after 5 years of therapy. We confirmed the detection of lower function of polymorfonuclear cells in patients with early-onset periodontitis as reported in literature (Procházková et al. 1996).

We compared the formation of cytokines in short - term (24 hours) cultivation of peripheral blood mononuclear cells (PBMC) with pokeweed mitogen (PWM), *E. coli* and *A. a.* between patients with EOP and their siblings with healthy periodontium but the presence of *A. a.* in sulcular region. We have found significantly higher production of IL-4 and significantly lower production of IFN-γ after *E. coli* and *A .a.* stimulation in patients than in their healthy siblings (Bártová et al. 2000).

We took up this study and we stimulated PBMC for 3, 5 and 7 days with standard strains of *E. coli* and *A . a.* and also with the strains of *A. a.* isolated from the sulcular region of each patients ("own *A. a.*") and healthy controls. The ELISPOT assays were used for analysis of number of cell producing cytokines (IL-4, IL-6, IL-10 and IFN-γ) and the ELISA assays were applied for estimation of concentrations of cytokines in supernatants.

The number of cells (spots) and the kinetics of reaction in healthy controls were independent on the strain of bacteria which was used for stimulation. However, in patients we detected strong suppression of immune reaction after the stimulation with standard strain of *A. a.* but not after the stimulation with own strain of *A. a.* or *E. coli* strain. We found significantly lower number of IL-4+ and IL-6+ spots when using the

standard strain of *A. a.* than when using own strain of *A. a.* after the 5th day of cultivation (Krátká et al. 2001).

Whereas the number of spots was similar between patients and healthy controls, the concentration of cytokines were higher in healthy controls than in patients. The significantly higher levels of IL-6 were detected in healthy donors than in patients after the stimulation with standard *A. a.* strain. The higher concentration of IL-4, IL-10 a IFN-γ and lower concentration of IL-6 were detected in patients after the stimulation of own *A. a.* strain of then after the stimulation of standard *A. a.* strain.

We found significantly higher IgM levels in healthy donors than in patients after the 7th day of cultivation of PBMC with standard *A. a.* and *E. coli*. We also found significantly higher concentration of IgA, but lower levels of IgM and IgG in healthy donors than in patients after the stimulation with own *A. a.* strain.

Putting these results together we assumed that PBMC of patients have not such a strong capability to accelerate the adequate immune response after the stimulation with pathogenic bacteria such as PBMC of healthy person.

It is supposed that polyclonal activation of B lymphocytes lead to the progression of disease. We detected the production of immunoglobulin after the 7th day of stimulation of PBMC with PWM. We measured the inhibition of immunoglobulin production after the stimulation with pokeweed mitogen and concanavalin A (PWM + ConA).

The significant inhibition of IgA and IgM was detected after PWM+ConA stimulation (when compared with PWM stimulation) in healthy donors but not in patients at the early study. The same results previously described Bártová et al. (1989) in the patients with adult periodontitis in the terminal state. After the therapy we found the inhibition of polyclonally produced immunoglobulins in most of patients.

Although we assumed the linkage between the low inhibition of polyclonally produced immunoglobulins and the progression of the early-onset periodontitis, we did not establish it during the longitudinal study.

We testified the in vitro production of immunoglobulins after the PWM stimulation and the inhibition of polyclonal immunoglobulin production also in the

patients with selective IgG immunodeficiency (IgGSD) and common variable immunodeficiency (CVID) (Krátká et al. 2002). We have studied also the effect of intravenous immunoglobulin (IVIG) administration applied to patients on *in vitro* immunoglobulin production. Polyclonally stimulated IgA and IgM production was suppressed by IVIG infusion in both groups of patients and was significantly higher than in healthy donors. Co-stimulation of PWM –stimulated cells with ConA caused an inhibition of immunoglobulin release in healthy donors and CVID patients before the infusion. The infusion supported the capability of ConA to inhibit IgG production in vitro in IgGSD patients but no in CVID patients.

We analyzed the cytokine production after the PWM and PWM + ConA stimulations of PBMC of 6 patients with EOP to find out the factors which could influence the inhibition or stimulation of polyclonal immunoglobulin production. We used the RayBio method in order to find out the presence of cytokines in supernatants from the of PBMC stimulation with the PWM and PWM + ConA. In 3 patients with and the 3 patients without the inhibition of immunoglobulins we detected higher amounts of IL-1α, IL-7, IL-15, IL-17, s TNF-RI and IL-6sR after PWM than PWM + ConA stimulation. On the other hand we detected higher levels of IL-6, IL-8, IL-13, IL-16, TGF-β a TNF-β after the PWM+ConA than PWM stimulation in both group of patients. We measured higher amounts of IL-1β, IL-10, IL-12p40, IL-12 p70, TNF-α a IFN-γ in PWM stimulation than PWM + ConA stimulation in patients with the inhibition of polyclonally produced immunoglobulins, but on the other hand the higher concentration of these cytokines we detected in PWM + ConA stimulation than PWM stimulation in patients without inhibition of polyclonally produced immunoglobulins. Then we used multiplexed analysis to estimate the concentrations of IL-4, IL-5, IL-10 and TNF-α in the samples. Higher concentrations of IL-10 and TNF-α were detected in samples of patients with the inhibition then in patients without it.

Periodontitis is one of the risk factors in the pathogenesis of atherosclerosis. That was the reason why we measured the immunological and biochemical markers of this disease in the sera of patients with EOP. We did not found any serious differences between the group of patients with slow and progressive disease.

We analyzed the genetic polymorphism of apolipoprotein E and B. We found the risk *apo ε2*, *apo ε4* or *apo B-100 Arg3500Gln* alleles in 9 patients with progressive periodontal disease, but only in one of patients with slow progression of the disease. These patients have higher genetically predisposition for the development of cardiovascular disease.

We found significantly lower concentrations of low-density lipoprotein (LDL) and cholesterol in *apo ε2+* patients than in *apo ε2-* patients. No significant differences were detected between the *apo ε4+* and *apo ε4-* patients. Two patients *apo ε4+* have been treated for hypertension for two years.

The higher presence of risk alleles for apolipoprotein found in the group of patients with progressive form of EOP was surprising and has not been pushed yet, but we have not found any biochemical signs of atherosclerosis development in most of the patients.