2209 **LJP Neutrophil Population Characterized by Significant Numbers of Immature Cells.** M.A. DANIEL* and T.E. VAN DYKE (Boston University School of Dental Medicine, Boston, MA,USA)

Flow cytometric techniques were used to evaluate bacterial chemotactic factor receptor levels (FMLP) for both normal subjects and patients with localized juvenile periodontitis. Human neutrophils were incubated with a fluorescent (FITC) labeled synthetic bacterial peptide, FMLPL, to evaluate receptor number at the single cell level. Flow cytometry was performed with a Becton-Dickinson FACScan flow cytometer, and data analysis was performed using LYSIS (BD) and WINLIST (Verity Software) software. Multiple, correlated measures of both intrinsic and extrinsic parameters were obtained for heterogeneous cell samples, and provided a basis for statistical analysis. We were able to perform receptor analysis for large populations of neutrophils (greater than 10,000 cells examined in 5 seconds) with resolution at the single cell level. Furthermore, the use of this software provided not only single cell analysis, but also multiparametric analysis used to identify and analyze heterogeneous sub-populations of a seemingly homogeneous cell type. Our results confirmed previous reports of decreased numbers of bacterial peptide receptors on LJP neutrophils. The analysis also revealed significant proportions of very low binding neutrophils in the LJP patients studied(N=11). For LJP patients studied, this sub-population accounted for nearly 20% of the neutrophil population compared to 5% in control ($p<.01$). Flow cytometric analysis of the light scattering properties of these cells suggest they are an immature cell type. The data suggest that the chemotactic defect in LJP is due to an increase in the proportion of immature, non-responsive cells. This study was supported by NIDR Grants DE05656, DE00287, and DE06436.

2210 Histopathological characterization of Rapidly Progressive Periodontitis S. DOGAN*, G. HILLMANN and W. GEURTSEN (Department of Conservative Dentistry and Periodontology, Dental School, Hannover, Germany)

Purpose of this study was the histopathological characterization of diseased gingiva from patients with Rapidly Progressive Periodontitis (RPP) with light and electron microscopical techniques. Tissue samples from 20 patients with RPP and from 10 patients with adult periodontitis (AP) were fixed immediately after surgical procedures in a 3.5 % formaldehyde solution and embedded in Epon and LR White resin. The tissue at the sampling sites was highly inflamed. On the light microscopical level the pocket epithelium of patients with RPP revealed a thin non-keratinized lining of epithelial cells with an extensive proliferation of rete pegs. The epithelial cells were elongated and showed widened intercellular spaces. In the connective tissue plasma cells were dominant in dense inflammatory infiltrates. Frequently, the coronal parts of the pocket epithelium consisted of one or two strata only. On the electron microscopical level the epithelial cells were elongated and revealed wide intercellular spaces. Many epithelial cells were necrotic or revealed unusual electron densities. Polymorphonuclear leukocytes (PMNs) could be identified in the intercellular spaces. The continuity of the basal lamina was not disturbed. Adjacent to the basal lamina the connective tissue was characterized by a „homogeneous zone", which was free from infiltration and cellular remnants. The underlying connective tissue revealed a marked increase in vascularity, an extensive infiltration of plasma cells and a lot of cellular remnants. The extracellular matrix molecules, especially the collagens, were completely disturbed. On the other hand, the light microscopical investigation of biopsies of patients with AP revealed dense infiltrations of plasma cells in the connective tissue underlying the basal lamina. These infiltrates were localized in defined areas in kind of a „well-defined zone". Adjacent to this zone the gingiva was characterized by either a low degree of inflammation or a normal histology with collagen fibre bundles and fibroblasts. On the electron microscopical level the epithelial cells revealed only slight destructive processes. From our results we conclude that the histopathological characteristics of diseased gingival biopsies of RPP significantly differ from AP on the light and electron microscopical level.

2211 Experimental Gingivitis and Periodontitis Susceptibility. T.C. JOHNSON*, R.A. REINHARDT, J.B. PAYNE, J.K. DYER and K.D. PATIL. (UNMC College of Dentistry, Lincoln, Nebraska, USA).

Research on periodontitis susceptibility in the context of developing inflammation during bacterial accumulation is rare. The purpose of this study was to evaluate clinical, microbiological, and gingival crevicular fluid (GCF) bone-active inflammatory mediator profiles in age-similar periodontitis-resistant (gingivitis; n = 10) and periodontitis-susceptible (history of rapidly progressive periodontitis; n = 10) subjects during four weeks of experimental gingivitis. Prior to baseline, all subjects achieved good plaque control (Plaque Index [PII] ≈ 0) and gingival health (Gingival Index [GI] = 0), and had probing depths ≤ 4 mm on experimental teeth. For four weeks after baseline, oral hygiene around maxillary 2nd premolar-1st molar teeth was inhibited by a plaque guard. The plaque guard was removed weekly for GCF sampling, PII, GI, probing depth, and gingival recession measurements. Enzyme-linked immunosorbent assays (ELISAs) were performed to quantify the GCF interleukin (IL)-1β and prostaglandin (PGE_2) levels. Darkfield microscopy and DNA probe analysis were performed to correlate the microbiological profiles with clinical findings. Results indicated that clinical signs of inflammation progressed similarly in both groups. However, plaque accumulated more rapidly in the susceptible subjects with a PII at 4 weeks of 2.1 ± 0.1 compared to 1.5 ± 0.2 in the periodontitis-resistant group (logistic regression; $p < 0.0001$). Significant decreases in proportions of cocci; increased proportions of spirochetes, motile rods, non-motile rods, and fusiform species; and levels of putative periodontal pathogens were comparable in both groups. Increased production of GCF PGE_2 and IL-1β during experimental gingivitis was quantitatively similar in both patient groups. Hence, an increased rate of plaque mass development, possibly by transmission from other oral reservoirs, may play an important role in periodontitis susceptibility. Supported by UNMC College of Dentistry.

2212 **Periodontitis and Cytokine Expression in CD14 Deficient Patients.** K.M.MCNAMARA*, R.ARNOLD, R.WILDER, F.W.SMITH, Q.Z.WU, B.JONES, H.P.LAWRENCE, S.OFFENBACHER (The University of North Carolina, Chapel Hill, NC, USA).

CD14 is the monocyte receptor for lipopolysaccharide (LPS), which when activated triggers the release of proinflammatory mediators commonly associated with periodontal disease. Paroxysmal nocturnal hemoglobinuria (PNH) is a disorder in which the CD14 receptor is absent from both monocyte and neutrophil cell membranes. Since the LPS→monocyte→cytokine pathway is hypothesized to represent a critical pathway of periodontal pathogenesis, PNH patients and matched controls (age, gender, race) have been examined. Study participants have been assessed for periodontal status, levels of PGE_2 and IL-1β within the gingival crevicular fluid (GCF), and levels released from LPS stimulated peripheral blood monocytes in culture. Results from GCF-PGE_2 analysis indicate that PGE_2 levels are similar for PNH patients as compared to matched controls (13.0±4.9 ng/mL vs. 11.5±3.3 ng/mL). However, the GCF-IL-1β levels of PNH patients were significantly lower than those of matched controls (747.1±480 ng/mL vs. 1301.3±740 ng/mL; P=0.04 Wilcoxon). Monocyte responses to LPS at suboptimal concentration (0.3 μg/mL) demonstrated differences in PGE_2 and IL-1β secretion. PNH monocytes secreted significantly less PGE_2 in culture, as compared to matched controls; (3.8 vs. 27.6μg/mL) at $P<0.05$. A similar inhibition of monocytic IL-1β release was observed in PNH patients relative to matched controls (386.7 vs. 2540. ng/mL) at $P<0.05$. In this preliminary analysis of PNH patients there is a significant decrease in GCF- IL-1β, but not GCF-PGE_2, whereas there is a seven fold reduction in monocytic release to both IL-1β and PGE_2. Additional PNH patients will be needed to determine whether these impaired monocyte responses are associated with less severe periodontitis. This research is supported by NIH grant # DE-10519.

2213 ***P. gingivalis*-induced Rat Model of Periodontitis: Active/Inactive Phases.** M.E. RYAN*, N.S. RAMAMURTHY, E. GOTTESMAN, R.T. EVANS, T. SORSA and L.M. GOLUB (SUNY Stony Brook & Buffalo and U. Helsinki).

Previous studies in germfree rats established temporal relationships between *Porphyromonas gingivalis* (*P.g.*) infection and alveolar bone loss. The current study developed a pathogen-reduced *P. g*-infected model of periodontitis, based on microbial shifts, to replace cumbersome germfree experiments which are unrelated to the natural oral microenvironment. This time course study, using vinyl isolators (other studies used laminar airflow housing units), analyzed the response of 48 viral-free male Sprague Dawley rats, pathogen-reduced by gavaging for 3 days with 20mg each of Ampicillin/Kanamycin in 2% carboxymethylcellulose (CMC), followed by a 3 day washout period prior to 3 days of oral infection with *P. g.* 381, suspended in 5% CMC. Animals were sacrificed at baseline before infection, and at 7, 14, 21, 33 and 41 days after infection. Serum was analyzed for antibodies (Ab) to the fimbriae of *P. g.*, gingivae were assayed for matrix metalloproteinase (MMP) activity, and defleshed jaws were analyzed morphometrically. The Ab titre rose steadily to day 21 ($p<.003$) then rapidly declined. Active MMPs in gingival extracts also peaked at day 21 ($p<.001$) and then declined, as measured by functional assays and Western blot analysis which revealed elevated levels of MMPs 8, 9, and 13. Latent levels of the MMPs remained elevated from day 7 and beyond. Horizontal bone loss increased 50% by day 21 ($p<.05$) and plateaued thereafter. We conclude that a "window" of disease activity, as well as inactive phases, exists in this and our other models of *Porphyromonas gingivalis*-induced periodontitis. Supported by NIH # DE-00363, -03987, -04898 & Acad. of Finland.

2214 Adherence With Lymphocytes Increased Cytokine mRNA Expression in Gingival Fibroblasts. S. MURAKAMI*, H. HIRANO, E. HINO, Y. SHIMABUKURO and H. OKADA. (Dept. of Periodontol., Osaka Univ. Fac. of Dent., Osaka, Japan)

In inflammatory periodontal lesions, locally infiltrated lymphocytes should have an opportunity to directly interact with fibroblasts composing gingiva. In this study, we examined whether adherence of lymphoid cells to human gingival fibroblasts (HGF) could stimulate HGF to produce inflammatory cytokines. HGF were cultured in the presence or absence of human lymphoid cell lines for 3 hours. After the culture, messenger RNA (mRNA) were isolated from HGF and cytokine mRNA expression was examined by reverse transcription-polymerase chain reaction. We found that IL-1α, IL-1β and IL-6 mRNA expression was clearly increased when HGF were co-cultured with various human lymphoid cell lines. In addition, constitutive expression of mRNA of IL-1β converting enzyme by HGF suggests that HGF can secrete mature form of IL-1β. This increased mRNA expression of those cytokines in HGF was not induced by stimulation with culture supernatants of the lymphoid cells. In addition, HGF did not alter mRNA expression of those cytokines when HGF and lymphoid cells were separately cultured in the same well by membranes which were assembled in cylindrical cells and disturbed direct interactions between HGF and lymphoid cells. Noteworthy is a fact that IL-1β mRNA expression in HGF was synergistically increased when HGF were stimulated with exogenous IL-1β and direct interaction with the lymphoid cells simultaneouly. Present results demonstrated that HGF can be activated to increase mRNA expression of inflammatory cytokines through adhesive interactions with lymphoid cells and actively participate in inflammatory responses at diseased sites in periodontitis by supporting an effective cytokine network. This study was supported by Grant-in-Aid from Ministry of Education, Science and Culture in Japan (No. 08457504)

2215 **mRNA for Calcium Activated Protease System in Rat Gingiva** **T. R. SHEARER* and D. B. THRONEBURG (Oregon Health Sciences University, School of Dentistry, Portland, Oregon, USA).**

The purpose of this study was to demonstrate the presence of mRNAs for calpain protease and its natural inhibitor calpastatin in gingiva. Calpains (EC 3.4.22.17) are a family of calcium-activated, intracellular, neutral, cysteine proteases. Two nearly ubiquitous types are known: μ-calpain I requiring 5-50 μM Ca^{2+} and m-calpain requiring 150-1000 μM Ca^{2+} for half-maximal activation. Calpains have been found in almost all animal tissues assayed, where they seem to hydrolyze specific proteins to limited fragments. Gingival bacterial cysteine proteases are known; but to our knowledge, no data exists concerning the presence of calpains in gingiva.

The present study utilized gene specific primers in an oligo-dT primed RT-PCR assay for mRNAs for calpains and calpastatin. RNA was isolated from gingival samples from 12-14 day old Sprague-Dawley rats. Results on agarose gels showed bands at 536, 404, and 452 base-pairs. These were the expected PCR products corresponding to mRNAs for μ- and m-calpains, and calpastatin, respectively. Parallel controls using brain, kidney, lung, and lens samples were positive; while PCR with no RNA was negative. These are the first data demonstrating that the transcripts for the major components of the calpain system are present in gingival samples. We hypothesize that under healthy conditions, with only transient, localized spikes in intracellular calcium; proteolysis of specific substrates by gingival calpains may be important for cellular remodeling or differentiation. Under pathological conditions with larger increases in intracellular calcium, activation of intracellular calpains may contribute to proteolytic breakdown of gingiva. **Support: EY05786.**

2216 Activation of gingival collagenase gene expression by c-fos and c-jun MANORAMA TEWARI, P. J. REDDY, C.D. REDDY, R.C., NEWTON, R. TAUB, O.C. TUNCAY, AND DINESH S. TEWARI* (Department of Orthodontics, Temple University, School of Dentistry, Philadelphia, PA 19140)

Identification of the early signals that trigger gingival collagenase gene expression is of vital importance in understanding the pathogenesis of periodontal disease. Several growth factors and cytokines have been shown to activate members of the AP-1 family of transcription factors. In this study we investigated whether AP-1 participates in the regulation of collagenase gene expression by IL-1 in human gingival fibroblasts. Utilizing EMSA, we found that AP-1 DNA binding activity is increased in the gingival fibroblasts after IL-1 treatment and peaks at more than 30 fold at 1/2 h. Western blot analysis utilizing specific antibody, confirmed c-fos and c-jun protein induction. Northern blot analysis indicated that c-fos/c-jun gene expression is increased significantly by IL-1. Further, CAT transfection studies demonstrated the dose dependent transcriptional regulation of gingival collagenase gene expression by c-fos and c-jun and significant stimulation of AP-1-CAT activity by IL-1. Our results suggest that activation of AP-1 by IL-1 is an immediate-early response in gingival fibroblasts. The identification of AP-1 as an early factor in gingival fibroblasts provides clues as to the signal transduction pathways that are activated in diseased periodontal tissues within the first minutes of IL-1 stimulation. This study was supported by PHS grant (NIDR) 1R29 DE11706.