RESEARCH REPORTS
Clinical

L. Shaddox[1,2], J. Wiedey[1], E. Bimstein[3], I. Magnuson[2], M. Clare-Salzler[4], I. Aukhil[1], and S.M. Wallet[1,2,*]

Departments of [1]Periodontology, PO Box 100434, [2]Oral Biology, [3]Pediatrics, College of Dentistry and [4]Pathology, College of Medicine, University of Florida, Gainesville, FL 32608, USA; *corresponding author, swallet@dental.ufl.edu

J Dent Res 89(2):143-148, 2010

Hyper-responsive Phenotype in Localized Aggressive Periodontitis

ABSTRACT

The 'hyper-responsive' trait is an increased inflammatory response upon stimulation of innate immune receptors. Our objective was to determine if a hyper-reactive trait is present in a cohort diagnosed with aggressive periodontitis (LAgP). Peripheral blood was collected from 30 LAgP, 10 healthy unrelated, and 10 healthy sibling participants and stimulated with lipopolysaccharide (LPS) from *E. coli* and *P. gingivalis*. Cyto/chemokine response profiles were evaluated and analyzed by ANOVA. Elevated levels of pro-inflammatory cyto/chemokines were detected in *E. coli* and *P. gingivalis* LPS-stimulated LAgP cultures when compared with those of healthy unrelated control individuals. Periodontally healthy siblings presented with attenuated hyper-inflammatory cyto/chemokine profiles. Regression analysis demonstrated the hyper-reactive trait to be concomitant expression of pro-inflammatory cyto/chemokines and an absence of anti-inflammatory mediator expression. Our findings demonstrate hyper-responsive trait in a LAgP cohort, along with an attenuated hyper-responsiveness in healthy siblings, which can be induced in response to multiple TLR ligations.

KEY WORDS: LAgP, immunity, cytokines, chemokines, inflammation, TLR.

DOI: 10.1177/0022034509353397

Received July 14, 2008; Last revision June 16, 2009; Accepted July 28, 2009

INTRODUCTION

Immunological mediators play a relevant role within the pathogenesis of disease, including periodontal diseases. Perspectives on the contribution of innate immunity to disease can lead to therapeutic considerations and goals for the treatment. Although bacteria must be present for periodontal disease to occur, a susceptible host is also required (Southerland *et al.*, 2006; Meng *et al.,* 2007). Host responses confer protection; however, an abnormal immune response can result in exacerbated tissue destruction. Such an abnormal inflammatory response, known as a 'hyper-responsive' phenotype, has been linked to multiple inflammatory processes, where the phenotype has been described as an increased inflammatory response upon stimulation by Toll-like receptors (TLR). For instance, increased responsiveness to LPS has been described in the gut during inflammatory bowel disease (Chadwick *et al.*, 2002), in the lung during Hermansky-Pudlak syndrome (Young *et al.,* 2006), and in circulating monocytes during type 1 and type 2 diabetes (Mohammad *et al.*, 2006; Shi *et al.*, 2006; Kim *et al.*, 2007; Devaraj *et al.*, 2008). The 'hyper-responsive' phenotype in periodontal disease, although considered an important feature, is not well-understood. Hyper-responsiveness to bacterial antigens results in increased production of pro-inflammatory mediators, which induce tissue and bone destruction (Salvi *et al.*, 1997a,b; Naguib *et al.*, 2004). Components of bacteria have been shown to be stimulators of a variety of cytokines, chemokines, and growth factors through their interaction with TLRs (Akira *et al.*, 2006). While TLRs play an important role in innate immunity, they can be double-edged swords, because abnormal activation of their signaling can be detrimental to the host, such as seen in the 'hyper-responsive' phenotype.

Aggressive periodontitis (AgP) is a severe and rapidly progressing form of periodontitis, characterized by early onset and familial aggregation; affected individuals are otherwise clinically healthy (Armitage, 1999; Meng *et al.*, 2007). When localized, this disease affects first molars and incisors. Although it is recognized that the host inflammatory response plays an important role in the pathogenesis of this disease, our understanding of immune regulation in LAgP is still limited.

It was the objective of this study to determine whether a hyper-responsive trait was present in a cohort of African-Americans diagnosed with LAgP compared with periodontally healthy related siblings and unrelated participants.

MATERIALS & METHODS

Participant Population and Clinical Measurements

All data and samples collected were obtained under a protocol approved by the Institutional Review Board (IRB) and participants' informed consent at the University of Florida (Gainesville, USA). Participants were recruited from the Leon County Health Department (Tallahassee, FL) from August, 2006, to November, 2007. Inclusion criteria were: being from 4 to 19 yrs old; being African-American; having been diagnosed with localized aggressive periodontal disease (Armitage, 1999); or being an age-, sex, and race-matched periodontally healthy individual. Exclusion criteria were: having been diagnosed with systemic diseases or conditions that influence the progression and/or clinical characteristics of periodontal disease; having taken antibiotics within the preceding 3 mos; currently taking medications that could influence the characteristics of the disease; being a smoker; or being pregnant/lactating. A 10-mL sample of peripheral blood was taken, along with complete medical and dental histories and periodontal clinical parameters, including: (1) pocket depth (PD), (2) gingival margin position (GM), (3) clinical attachment loss (CAL), (4) bleeding on probing (BoP), and (5) radiographic examination (periapical and bite-wing x-rays). All measurements were performed with the use of a UNC-15 periodontal probe at 6 sites *per* tooth.

In vitro Stimulation and Detection of Cyto/Chemokines

Heparinized blood was diluted 1:4 in RPMI1640 (Invitrogen, Carlsbad, CA, USA) and stimulated with 1 μg/mL of ultrapure TLR4 (cat# tlrl-pelps) or TLR2 (cat# tlrl-pglps) agonists (InvivoGen, San Diego, CA, USA), or left untreated. Culture supernatants were collected 24 hrs post-stimulation. We used Beadlyte® 22-plex cyto/chemokine detection kits (Millipore, St. Charles, MO, USA) to detect and quantitate 22 cyto/chemokines. Culture supernatants and cytokine capture-bead cocktails were incubated for 2 hrs, and then incubated with biotin-labeled anti-cytokine for 1.5 hrs, and with a 1:12.5 dilution of streptavidin-phycoerythrin (SAV-PE) for 30 min. All incubations were performed at RT in the dark while samples were gently shaken. Data were acquired with the use of a Luminex®100™ and analyzed with Beadview software (Millipore), with standard curves and five-parameter logistics. Unstimulated levels were subtracted from stimulated levels to normalize.

Statistics

Statistical analyses were performed with GraphPad Prism software. ANOVA analyses were used for comparisons among experimental groups for each cytokine evaluated. We used Spearman analysis to determine non-parametric correlations (r values) between cytokines evaluated. A p value < 0.05 was considered significant.

RESULTS

Participant Cohort

In total, 30 persons with LAgP, 10 periodontally healthy siblings, and 10 unrelated healthy control participants were recruited for this study (Fig. 1). Demographic and clinical descriptions of the participant pools are summarized in the Table.

TLR4 Ligation Results in Hyper-responsiveness

To determine the potential for aberrant immunological responses in LAgP participants, we measured cyto/chemokine responses to *in vitro* TLR-stimulated peripheral blood leukocytes. No differences in percentage or absolute counts of neutrophils, lymphocytes, or monocytes were seen among the participant groups (data not shown). TLR4 ligation resulted in elevated levels of multiple pro-inflammatory cytokines and chemokines in LAgP cultures when compared with control cultures (Fig. 2). Elevated levels of IL1α, IL1β, IL2, IL12p40, IFNγ, TNFα GM-CSF, and IL6 were detected in cultures from LAgP participants (Fig. 2a) when compared with cultures from unrelated periodontally healthy control individuals. In addition, the chemokines IL8, CXCL10, CCL2, and CCL3 were also found to be elevated in TLR4-stimulated LAgP cultures (Figs. 2b, 2c). While the anti-inflammatory cytokines IL4 and IL5 in TLR4-stimulated cultures from LAgP participants were similar to those seen in periodontally healthy unrelated control cultures (Fig. 2d), levels of IL10 were significantly higher in cultures from LAgP participants (Fig. 2d).

P. gingivalis LPS Induces an Altered Pattern of Hyper-responsiveness

To determine if a 'hyper-responsive' phenotype could be elicited in response to additional PAMPs, we performed the same stimulation assays using a TLR2 agonist. Similar to what was seen with the TLR4 agonist, TLR2 ligation elicited elevated levels of pro-inflammatory cytokines in LAgP cultures when compared with cultures from healthy unrelated control individuals (Fig. 3). The same cyto/chemokine profile was not observed under these stimulatory conditions (Figs. 2, 3). While levels of IL1α, IL1β, and IL12p40 were significantly higher in TLR2-stimulated cultures from LAgP participants than in those from control individuals, IL2 and CXCL10 levels were relatively similar (Fig. 3a). Hyper-secretion of IL12p40 again correlated with elevated levels of TNFα and IFNγ, along with increases of GM-CSF, IL6, IL8, CCL3, and CCL2 (Fig. 3). Similar to what was seen with TLR4 ligation, the anti-inflammatory cytokine IL10 was also elevated in the TLR2-stimulated LAgP cultures, while IL4 and IL5 levels were similar to those in unrelated healthy control individuals (Fig. 3c).

Periodontally Healthy Siblings of LAgP Participants Have Attenuated Hyper-reactivity

In addition to periodontally healthy unrelated control individuals, our cohort consisted of periodontally healthy siblings of those diagnosed with LAgP. A robust cytokine response from the healthy siblings was detected as well, although the hyper-responsiveness was less than in their counterparts with disease (Figs. 2, 3). Specifically, IL2, IL12p40, TNFα, IFNγ, IL6, and CXCL10 were all elevated in cultures stimulated with TLR4 agonists, as compared with those cultures from unrelated healthy control individuals. However, some of these cytokine levels were statistically lower than those observed in cultures from participants with LAgP, indicating an attenuated hyper-reactive phenotype

(Fig. 2). Again, while a different pattern of cyto/chemokine induction was seen in response to TLR2 ligation, IL1β, IL2, IL12p40, CCL2, CCL3, and IL6 levels were elevated compared with those in cultures from unrelated healthy control individuals, although statistically lower than those observed in cultures from participants with LAgP. Unlike responses of participants with LAgP, IL10 levels in cultures from periodontally healthy siblings were not elevated in response to either LPS stimulation (Fig. 3).

Hyper-reactive Phenotype is a Concomitant Expression of Pro-inflammatory Cytokines

To determine if a given participant was 'hyper'-secreting all cytokines analyzed, or if the response was selective for individual cytokines, we performed Pearson's correlation analysis for all cytokines described above. Indeed, this analysis demonstrated that when one cytokine was induced by *E. coli* LPS to be expressed at a high level, so were all others (r value $\geq$ 0.8) (p value $\leq$ 0.001). While there was correlation in the 'hyper-expression' of these same cytokines in response to *P. gingivalis* LPS, the correlation was less significant (r value $\geq$ 0.7; p value $\leq$ 0.01). For instance, in a comparison of the correlation between IL1α and IFNγ induced by *E. coli* LPS, the correlation r value was 0.8158. but the *P. gingivalis* correlation r value was 0.7888. Similarly, correlation r values for IFNγ and IL2 were 0.9364 and 0.7105 for *E. coli* and *P. gingivalis* responses, respectively. Analysis of these data indicates that while both stimulations induce a hyper-inflammatory phenotype, the nature of this phenotype is dependent on the stimuli.

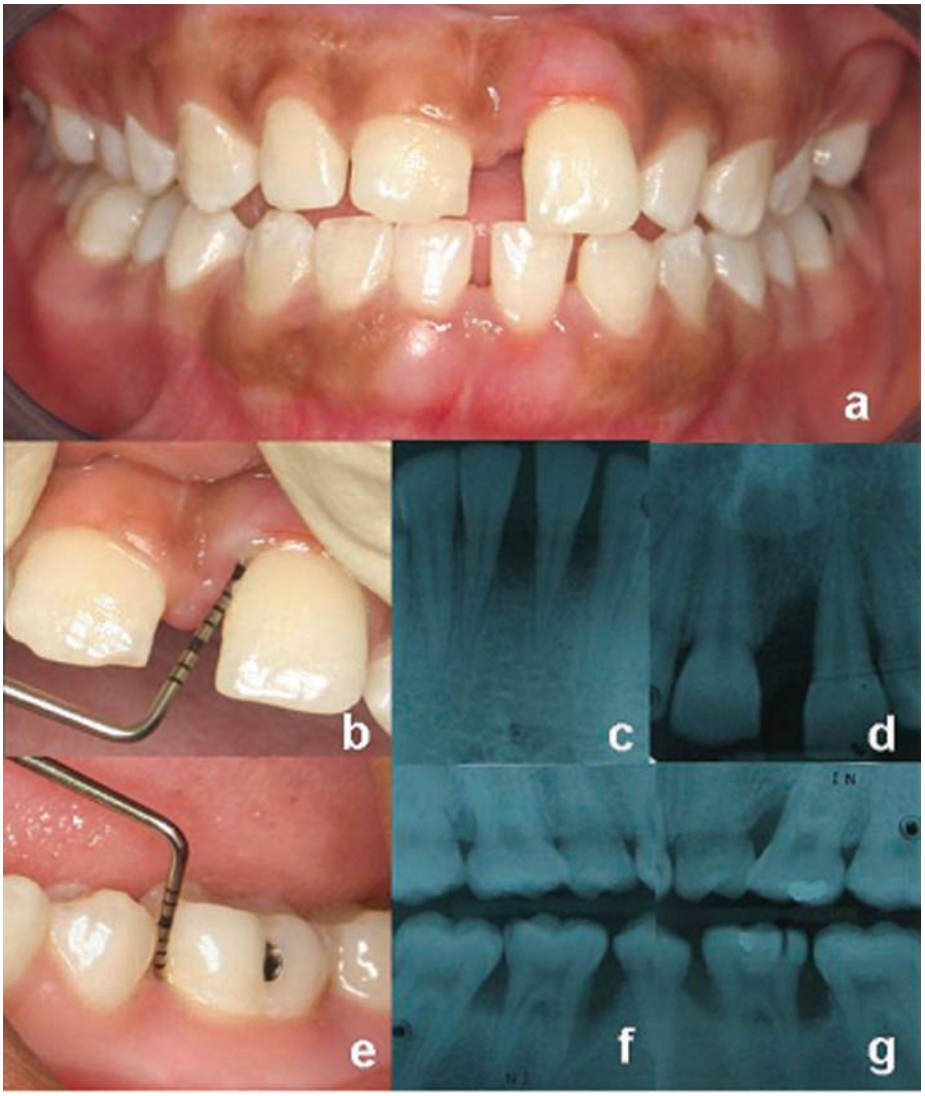

Figure 1. Representative clinical images of a localized aggressive periodontitis cohort. **(a)** Frontal smile photo. **(b)** Slight sign of marginal inflammation around teeth 9 and 25. Areas of enamel demineralization on canines, pre-molars, and molars. Probing depth of 4 mm on mesial-buccal aspect of tooth 9. Radiographic images of lower **(c)** and upper **(d)** anterior teeth show severe bone loss around tooth 25 **(c)** and 9 **(d)**. Probing depth of 6 mm on mesial-buccal aspect of tooth 19, with no evident clinical signs of inflammation **(e)**. Bite-wing radiographic images of right **(f)** and left **(g)** posterior teeth demonstrate severe vertical bone loss around all first molars and furcation involvement on lower first molars.

DISCUSSION

To handle the array of continuous microbial challenge present in the subgingival biofilm, our immune system confers protective immune responses, yet, destructive immune responses can occur, especially when the challenge overwhelms the host, or becomes dysregulated during the course of disease. Here we describe an unregulated immune response to TLR ligation in a cohort diagnosed with LAgP.

To mediate an effective response, immune cells must find their way to sites of infection or inflammation, which can be induced by several substances, including IL1, TNFα, and LPS, by inducing the production of chemoattractants, causing migration *via* a receptor-mediated gradient (Silva *et al.*, 2007). The first cytokine identified to have chemotactic activity was IL8, a selective neutrophil chemoattractant (Yoshimura *et al.*, 1987). Neutrophils represent the first line of host defense against microbial infection and are important in maintaining periodontal health, although hyperactivity of this cell type has been associated with periodontal tissue destruction (Silva *et al.*, 2007). IL8 has also been shown to induce differentiation of osteoclasts, where over-expression, like that seen here, can lead to excessive osteoclastogenesis and bone destruction (Bendre

Table. Demographic and Clinical Description Participant Cohort

Participants	Age (yrs)	Gender	PD (mm)	CAL (mm)	BoP (%)	# Sites ≥ 4 mm	# Sites ≥ 7 mm
LAgP	13.53 ± 4.38	5 M 25 F	5.28 ± 0.7	3.47 ± 1.23	10.11 ± 8.3	12.40 (8.61%)	3.37 (2.55%)
SIB	12.4 ± 4.97	6 M 4 F	2.12 ± 0.36	0	1.46 ± 1.05	–	–
CON	13 ± 4.65	3 M 8 F	2.29 ± 0.5	0	1.21 ± 0.97	–	–

Means followed by standard deviations of clinical parameters. PD and CAL means in persons with LAgP were calculated from affected teeth only. All other parameters were based on whole-mouth calculations. Persons with localized aggressive periodontitis (LAgP) (n = 30); healthy siblings (SIB) (n = 10); healthy unrelated control individuals (CON) (n = 10). PD = pocket depth; CAL = clinical attachment loss; BoP = bleeding on probing.

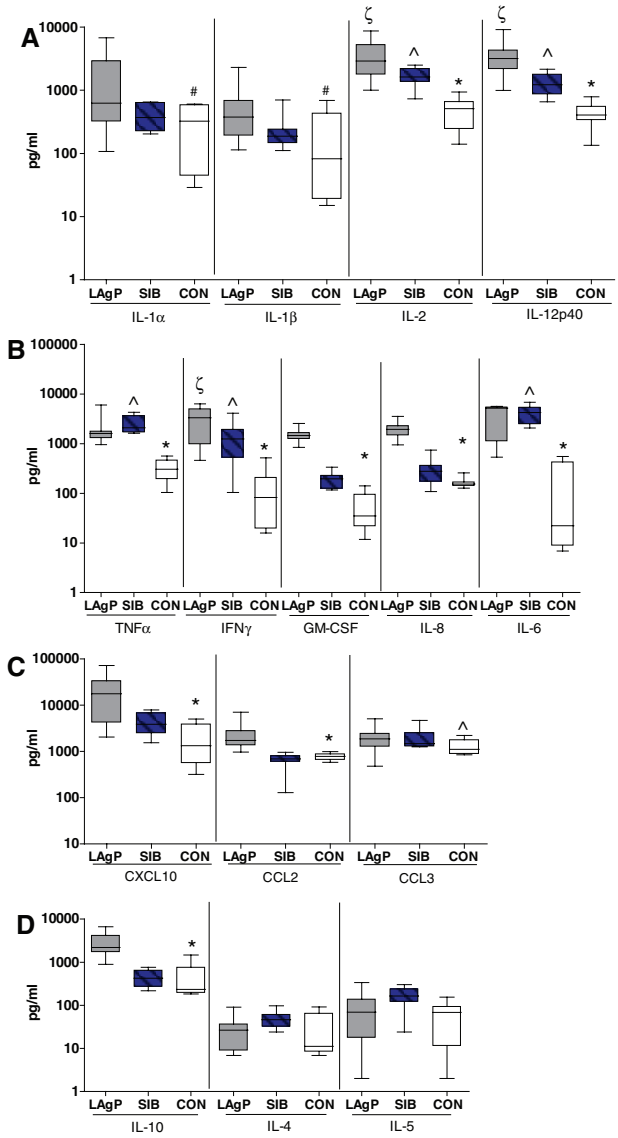

Figure 2. Hyper-reactivity of peripheral blood cells to TLR4 ligation. Supernatants from TLR4-stimulated peripheral blood cells were used for cytokine detection. Persons with localized aggressive periodontitis (LAgP) (n = 30), healthy siblings (SIB), and healthy unrelated control individuals (CON) (n = 10). Box-plots show the median (horizontal line), inter-quartile range (box), 10th-90th percentiles (vertical lines). *p value < 0.001. #p value ≤ 0.008, LAgP *vs.* CON. ^p value ≤ 0.05, SIB *vs.* CON. ξp value ≤ 0.006, LAgP *vs.* SIB for the indicated cyto/chemokines.

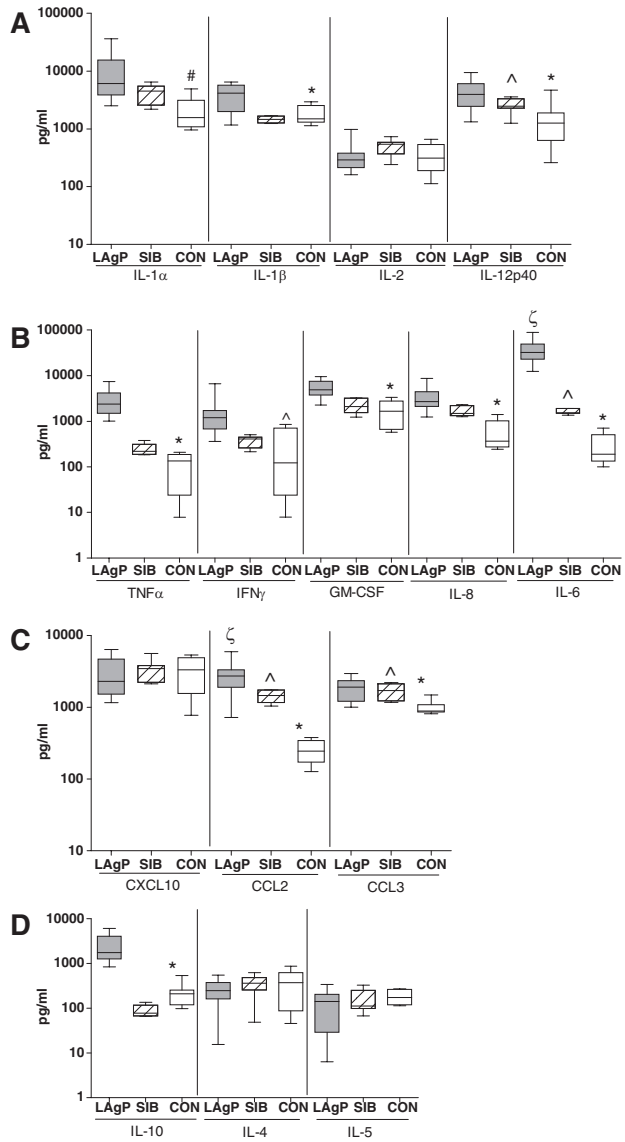

Figure 3. Hyper-reactivity of peripheral blood cells to TLR2 ligation. Supernatants from TLR2-stimulated peripheral blood cells were used for cytokine detection. Persons with localized aggressive periodontitis (LAgP) (n = 16), healthy unrelated control individuals (CON) (n = 10). Box-plots show the median (horizontal line), inter-quartile range (box), and 10th-90th percentiles (vertical lines).*p value < 0.0001. #p value < 0.005 LAgP *vs.* CON; ^p value ≤ 0.05 SIB *vs.* CON; ξp value ≤ 0.006 LAgP *vs.* SIB for indicated cyto/chemokines.

et al., 2003). CCL2 and CCL3 are major chemoattractants of macrophages, playing a significant role in the killing of pathogens and in the release of pro-inflammatory mediators such as TNFα and IL1 (Hanazawa *et al*., 1993; Yamamoto *et al*., 1996; Baker, 2000; Gemmell *et al.*, 2001; Kinane and Lappin, 2001). While these mediators enhance the cellular immune response, in excess, as demonstrated in this study, they induce bone resorption (Choi *et al*., 2000; Han *et al.*, 2001). CXCL10 is also expressed in diseased periodontal tissues and is thought to increase the migration of IFNγ-producing Th1 cells, which are involved in the activation of macrophages and osteoclasts, again contributing to the progression of tissue destruction (Kabashima *et al.,* 2002; Garlet *et al.*, 2003). Analysis of these data demonstrates that the fine balance between cytokines and chemokines under various inflammatory conditions may directly or indirectly contribute to the outcome of osteoclastogenesis. In the context of our data, over-production of these chemokines, and the consequential chemoattraction of inflammatory cells and hyper-secretion of their cytokines in the bone environment, contributes to the disruption of bone homeostasis and results in unchecked tissue destruction.

Cyto/chemokine biology is intertwined, resulting in positive and negative feedback mechanisms used to control infection and the inflammatory processes these molecules induce. Here, we have elucidated how dysregulation of this process can result in exacerbated inflammation and tissue destruction. We have demonstrated a hyper-responsiveness of several cytokines which then have direct and indirect effects on other soluble mediators. For instance, we saw elevated levels of IL1α and ILβ in our LAgP TLR4-stimulated cultures. IL1 assists the humoral and adaptive immune response by stimulating B-cell proliferation and T-cell production of IL2 and IL2R. Indeed, these cultures also contained elevated levels of IL2. In addition, higher levels of IL12p40, another pro-inflammatory cytokine, were also detected. IL12p40 is also responsible for increased B- and T-cell proliferation, as well as for inducing secretion of IL2, IFNγ, and TNFα by the adaptive immune system. To this end, TLR4-stimulated LAgP cultures had significantly higher levels of both TNFα and IFNγ, which, in turn, are known to induce the expression of multiple cyto/chemokines such as GM-CSF, IL6, IL8, CXCL10, CCL2, and CCL3, also seen up-regulated in these cultures. In addition, IL10, an anti-inflammatory cytokine which was induced in our LAgP cultures, is produced by monocytes after stimulation and is known to inhibit a multitude of events associated with activation of the adaptive immune response, although these actions can be overcome by high levels of IL2, similar to those levels detected in our LAgP cultures. Although the majority of the cyto/chemokines induced in the TLR4-stimulated cultures were also induced in the TLR2-stimulated cultures, some differences were seen. Here, CXCL10 was not induced beyond that seen in cultures from periodontally healthy unrelated control individuals. CXCL10 is induced by IFNγ, TNFα, and TLR4 ligands, but its induction can be inhibited by IL10 and IL4. Here, in the absence of elevated levels of IL2, elevated levels of IL10 may be having some effect on the 'hyper-responsive' phenotype through controlling CXCL10 levels. Because CXCL10 recruits many cells to the site of infection, the initiation and perpetuation of host tissue destruction through this hyper-responsive mechanism may also be controlled, resulting in a net decreased 'hyper-responsiveness' upon TLR2 ligation. Analysis of these data indicates that stimulation by TLR2 agonists can induce a 'hyper-responsive' phenotype, although one different from that induced by TLR4 agonists. Analysis of these data, taken together, indicates a clear 'hyper-responsive' phenotype which, upon activation, results in robust inflammatory responses and eventual host tissue destruction. The positive feedback mechanism, in which this phenotype perpetuates itself, makes it difficult for natural immune-dampening mechanisms (such as the production of IL10) to be effective, which could indicate that successful treatment of periodontal disease in these populations may need to incorporate an anti-inflammatory component.

Surprisingly, we observed a robust cytokine response from the healthy siblings of participants with LAgP, although to a lesser extent than that in their counterparts with disease. To us, this would suggest the possibility of a genetic linkage of this 'hyper-responsive' phenotype. In addition, as we follow these now-healthy siblings for oral maintenance, we would anticipate that the siblings with this attenuated hyper-inflammatory state would develop LAgP. We would also anticipate that at the point of disease induction, the threshold level of this response should be elevated similar to what is currently seen in their counterparts with disease. These results would enable us to define a specific threshold of inflammatory response that will dictate disease initiation in healthy but susceptible individuals.

In summary, analysis of our data demonstrates a 'hyper-responsive' phenotype in individuals with LAgP that, upon activation by common periodontal pathogens, could contribute to exacerbated inflammatory responses and eventual rapid host tissue destruction. These data lay the groundwork for the development of screening tools to identify individuals prone to periodontal destruction, allowing for better monitoring, earlier clinical intervention, and the prevention of irreversible damage to host tissues. Findings presented here support the notion that therapy for persons with LAgP may need to be directed at over-active inflammatory processes.

ACKNOWLEDGMENTS

This research was supported by UFCD Student Summer Research Fellowship and Research Funds, and by NIH U24DE 016509-01 and NIH/NIDCR R21DE019196. Special thanks to the Leon County Dental Clinic staff, Tiffany Davis, and Drs. Edward Zapert and John Bidwell.

REFERENCES

Akira S, Uematsu S, Takeuchi O (2006). Pathogen recognition and innate immunity. *Cell* 124:783-801.

Armitage GC (1999). Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 4:1-6.

Baker PJ (2000). The role of immune responses in bone loss during periodontal disease. *Microbes Infect* 2:1181-1192.

Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ (2003). Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. *Bone* 33:28-37.

Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, *et al*. (2002). Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 122:1778-1783.

Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, *et al*. (2000). Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. *Blood* 96:671-675.

Devaraj S, Dasu MR, Rockwood J, Winter W, Griffen SC, Jialal I (2008). Increased Toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. *J Clin Endocrinol Metab* 93:578-583.

Garlet GP, Martins W Jr, Ferreira BR, Milanezi CM, Silva JS (2003). Patterns of chemokines and chemokine receptors expression in different forms of human periodontal disease. *J Periodontal Res* 38:210-217.

Gemmell E, Carter CL, Seymour GJ (2001). Chemokines in human periodontal disease tissues. *Clin Exp Immunol* 125:134-141.

Han JH, Choi SJ, Kurihara N, Koide M, Oba Y, Roodman GD (2001). Macrophage inflammatory protein-1alpha is an osteoclastogenic factor in myeloma that is independent of receptor activator of nuclear factor kappaB ligand. *Blood* 97:3349-3353.

Hanazawa S, Kawata Y, Takeshita A, Kumada H, Okithu M, Tanaka S, *et al*. (1993). Expression of monocyte chemoattractant protein 1 (MCP-1) in adult periodontal disease: increased monocyte chemotactic activity in crevicular fluids and induction of MCP-1 expression in gingival tissues. *Infect Immun* 61:5219-5224.

Kabashima H, Yoneda M, Nagata K, Hirofuji T, Maeda K (2002). The presence of chemokine (MCP-1, MIP-1alpha, MIP-1beta, IP-10, RANTES)-positive cells and chemokine receptor (CCR5, CXCR3)-positive cells in inflamed human gingival tissues. *Cytokine* 20:70-77.

Kim F, Pham M, Luttrell I, Bannerman DD, Tupper J, Thaler J, *et al*. (2007). Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. *Circ Res* 100:1589-1596.

Kinane DF, Lappin DF (2001). Clinical, pathological and immunological aspects of periodontal disease. *Acta Odontol Scand* 59:154-160.

Meng H, Xu L, Li Q, Han J, Zhao Y (2007). Determinants of host susceptibility in aggressive periodontitis. *Periodontol 2000* 43:133-159.

Mohammad MK, Morran M, Slotterbeck B, Leaman DW, Sun Y, Grafenstein H, *et al*. (2006). Dysregulated Toll-like receptor expression and signaling in bone marrow-derived macrophages at the onset of diabetes in the non-obese diabetic mouse. *Int Immunol* 18:1101-1113; *erratum* in *Int Immunol* 19:581, 2007.

Naguib G, Al-Mashat H, Desta T, Graves DT (2004). Diabetes prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation. *J Invest Dermatol* 123:87-92.

Salvi GE, Collins JG, Yalda B, Arnold RR, Lang NP, Offenbacher S (1997a). Monocytic TNF alpha secretion patterns in IDDM patients with periodontal diseases. *J Clin Periodontol* 24:8-16.

Salvi GE, Yalda B, Collins JG, Jones BH, Smith FW, Arnold RR, *et al*. (1997b). Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. *J Periodontol* 68:127-135.

Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 116:3015-3025.

Silva TA, Garlet GP, Fukada SY, Silva JS, Cunha FQ (2007). Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease. *J Dent Res* 86:306-319.

Southerland JH, Taylor GW, Moss K, Beck JD, Offenbacher S (2006). Commonality in chronic inflammatory diseases: periodontitis, diabetes, and coronary artery disease. *Periodontol 2000* 40:130-143.

Yamamoto M, Kawabata K, Fujihashi K, McGhee JR, Van Dyke TE, Bamberg TV, *et al*. (1996). Absence of exogenous interleukin-4-induced apoptosis of gingival macrophages may contribute to chronic inflammation in periodontal diseases. *Am J Pathol* 148:331-339.

Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, *et al*. (1987). Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA* 84:9233-9237.

Young LR, Borchers MT, Allen HL, Gibbons RS, McCormack FX (2006). Lung-restricted macrophage activation in the pearl mouse model of Hermansky-Pudlak syndrome. *J Immunol* 176: 4361-4368.