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Activation of the neutrophil respiratory burst by plasma from periodontitis patients is mediated by pro-inflammatory cytokines

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Abstract

Aim: To determine the effect of periodontitis patients' plasma on the neutrophil oxidative burst and the role of albumin, immunoglobulins (Igs) and cytokines.

Materials and Methods: Plasma was collected from chronic periodontitis patients ($n = 11$) and periodontally healthy controls ($n = 11$) and used with/without depletion of albumin and Ig or antibody neutralization of IL-8, GM-CSF or IFN-α to prime/stimulate peripheral blood neutrophils, isolated from healthy volunteers. The respiratory burst was measured by lucigenin-dependent chemiluminescence. Plasma cytokine levels were determined by ELISA.

Results: Plasmas from patients were significantly more effective in both directly stimulating neutrophil superoxide production and priming for subsequent formyl-met-leu-phe (fMLP)-stimulated superoxide production than plasmas from healthy controls ($p < 0.05$). This difference was maintained after depletion of albumin and Ig. Plasma from patients contained higher mean levels of IL-8, GM-CSF and IFN-α. Individual neutralizing antibodies against IL-8, GM-CSF or IFN-α inhibited the direct stimulatory effect of patients' plasma, whereas the ability to prime for fMLP-stimulated superoxide production was only inhibited by neutralization of IFN-α. The stimulating and priming effects of control plasma were unaffected by antibody neutralization.

Conclusions: This study demonstrates that plasma cytokines may have a role in inducing the hyperactive (IL-8, GM-CSF, IFN-α) and hyper-reactive (IFN-α) neutrophil phenotype seen in periodontitis patients.

Key words: IL-8; inflammation; neutrophil priming; periodontitis; ROS

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Periodontitis is one of the most prevalent chronic inflammatory diseases of humans, which is initiated by a plaque biofilm and propagated by the host's inflammatory-immune response. It results in a non-resolving inflammation, which leads to periodontal tissue destruction (Hajishengalis 2009). The pathogenic role of neutrophils in mediating tissue destruction in periodontitis has been suggested in several previous studies (Asman et al. 1985, Chapple 1997, Fredriksson et al. 1998, 1999, Chapple & Matthews 2007, Restaíno et al. 2007, Wright et al. 2008). Neutrophils isolated from periodontitis patients are hyperactive with respect to the generation of reactive oxygen species (ROS) in the absence of any overt stimulus (Matthews et al. 2007a), as well as being hyper-reactive to Fcγ- and Toll-like receptor stimulation with increased release of elastase (Figueredo et al. 1999) and ROS (Gustafsson & Asman 1996, Fredriksson et al. 1999, Matthews et al. 2007b). The hyperactivity and reactivity of peripheral blood neutrophils from periodontitis patients may be a constitutive feature of the cells

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themselves, or a constitutive characteristic of the host in relation to the elaboration of priming agents into plasma. Rates of ROS production are important determinants of oxidative stress, a phenomenon that is associated with periodontitis (Chapple & Matthews 2007). Mechanisms promoting increased neutrophil life span, their extended duration within the periodontal tissues and those factors increasing neutrophil numbers may be important contributors to the reported, neutrophil-mediated periodontal tissue damage. Neutrophils may be further primed either by bacterial products such as lipopolysaccharide (LPS) and formyl-met-leu-phe (fMLP) or by plaque-induced host immune modulators (Matthews et al. 2007a); for example, IL-8, IFN-α and GM-CSF prime for an increased respiratory burst from the peripheral blood neutrophils of patients with periodontitis relative to matched controls following a subsequent second stimulus.

Neutrophil priming has been implicated in the development of various inflammatory diseases (Elbim et al. 1994, Partrick et al. 2000, Brown et al. 2004). It is possible that neutrophil hyper-responsiveness in periodontitis patients is due to increased levels of priming substances within plasma or an increased susceptibility of neutrophils to priming substances in a patient's plasma.

Plasma factors such as pro-inflammatory cytokines (Gainet et al. 1999), acute phase proteins (Ebersole et al. 1997), soluble immune complexes (Wilton et al. 1992, Lu et al. 1994), activated complement (Abid et al. 2007), soluble adhesion molecules (Hayashi et al. 1996) or bacterial components (Ishikawa et al. 1997) are at elevated concentrations in periodontally diseased individuals. Moreover, patients with chronic periodontitis, aggressive periodontitis and gingivitis have increased levels of immunoglobulin (Ig) and IgG subclass production with reactivity towards *gingivalis* fraction IV compared with healthy controls (Trindade et al. 2008). In addition, a prolonged IgG response is indicative of persistent immune activation and periodontal destruction. Higher serum *P. gingivalis*-specific IgG1 levels were also observed with treated and maintained periodontitis patients compared with healthy controls (Sakai et al. 2001).

We have previously demonstrated a potential role for the periodontal pathogen *P. gingivalis* in modifying IL-8 neutrophil chemoattraction and priming (Dias et al. 2008), and for peripheral blood IFN-α in neutrophil priming in periodontitis patients (Wright et al. 2008). By priming the neutrophil for an enhanced respiratory burst when exposed to an activating second stimulus, these cytokines have the potential to increase neutrophil-derived ROS release both locally within the periodontal tissues and also within the systemic circulation.

The aim of the reported studies was, therefore, to elucidate the nature of activating and priming proteins/peptides within the peripheral blood of periodontitis patients that may, at least in part, be responsible for the reported exaggerated ROS release from peripheral blood neutrophils that is observed in periodontitis patients.

Material and Methods

Materials

Recombinant IL-8, mouse anti-human IL-8 monoclonal antibody (mAb), anti-IFN-α mAb and anti-GM-CSF mAb were purchased from R&D Systems (Abingdon, UK). All other reagents were obtained from Sigma Chemical Company (Poole, UK).

Patients

Plasma samples from chronic periodontitis patients ($n = 11$; age range = 45–60 years; five males, six females) were kindly provided by Dr. E. M. Allen (National University of Ireland Cork, Ireland) who, at the time of the study, was an external PhD student with the Periodontal Research Group in Birmingham. She was trained and calibrated within the group before patient recruitment and sample collection. Age- and gender-matched periodontally healthy control individuals ($n = 11$; age range = 37–62 years) were recruited from staff of the Birmingham Dental Hospital. Chronic periodontitis was defined as described previously (Brock et al. 2004, Chapple et al. 2007), the mildest case had 12 teeth with clinical attachment loss of $\geqslant 5$ mm, in excess of the consensus criteria of the European Federation of Periodontology (Tonetti & Claffey 2005). All study participants were systemically healthy (by medical history questionnaire) never-smokers, were not pregnant, did not take medications that may affect the course of periodontal inflammation and had no special dietary requirements. Ethical approval was granted by the South Birmingham Local Research Ethics Committee (LREC 5643) and donors gave their informed consent after the risks and benefits of partaking in the study were explained. Ethical approval for collection of blood from healthy volunteers was provided by Aston University and donors gave their informed consent. All plasma samples were snap frozen in liquid nitrogen following separation and maintained at a minimum temperature of -80°C before assay.

Collection and isolation of peripheral blood neutrophils

Venous blood was collected from systemically and periodontally healthy donors into 4% sodium citrate (weight/volume) in phosphate-buffered saline, with a citrate/blood ratio of 1:9. Neutrophils were isolated as described by Matthews et al. (2007a) using Percoll® density centrifugation (Sigma-Aldrich, Poole, UK). Isolated cell viability was determined immediately before assay by trypan blue exclusion and was typically $>98\%$.

Chemiluminescence ROS assay

Assays were performed (37°C) using an Orion II Berthold microplate-luminometer UK Ltd (Harpenden, UK). Lucigenin (1 mM in PBS) and fMLP (5 mM in DMSO) stock solutions were prepared and stored at -20°C in small aliquots. Fresh aliquots were used for each experiment to avoid freeze-thaw cycles. Lucigenin was diluted to a final assay concentration of 100 μM in PBS and fMLP was diluted to 1 μM in PBS. PBS-washed primary neutrophils (5×10^5 cells) in 100 μL of PBS buffer were incubated with 100 μM lucigenin in white microplate wells previously blocked with 1% BSA overnight. After incubation for 30 min. at 37°C, light emission in relative light units (RLUs) was recorded in order to study baseline ROS release for 30 min. before addition of plasmas or fMLP (1 μM) to stimulate the respiratory burst and further measurements were recorded for another 30 min.

Depletion of plasma albumin and Ig

The major plasma components albumin and Igs are modulated independently

during inflammatory events and have the potential to activate the neutrophil respiratory burst. In order to eliminate potential confounding effects of a reduced albumin–globulin ratio in periodontitis compared with healthy patient plasmas, albumin and Ig were removed by the Aurum serum protein mini kit (BioRad, Hemel Hempstead, Hertfordshire, UK) according to the manufacturer's instructions. To assess the efficacy of depletion, plasma was separated by two-dimensional electrophoresis as previously described (Aldred et al. 2006). Briefly, lyophilized plasma proteins (50 μg) pre- or post-albumin and Ig depletion were re-suspended in rehydration buffer (40 mM Tris, 6 M urea, 2 M thiourea, 2% CHAPS, 2% SB3-10, bromophenol blue, 0.5% biolytes (BioRad), 2 mM Destreak (GE Healthcare) and added to the rehydration tray, with IPG strips (three to ten) overlaid to rehydrate passively at 20°C overnight. IEF focusing was achieved before separation by gradient SDS-PAGE, staining with Flamingo (Biorad) and visualization by fluorescence scanning densitometry (Pharos, Biorad). Effective depletion was typically observed for both albumin and Ig (Fig. 1) and previously reported (Björhall et al. 2005). Depleted plasmas were subsequently incubated with healthy control neutrophils and the respiratory burst activity measured in the presence and absence of 1 μM fMLP.

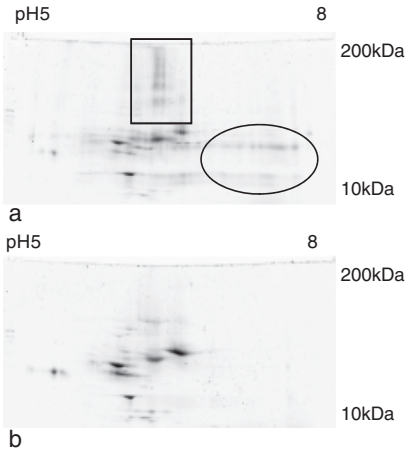

Fig. 1. Two-dimensional electrophoresis of plasma pre- and post-albumin and immunoglobulin depletion. Plasma proteins were resolved by isoelectric focusing in the first dimension and SDS-PAGE (4–20%) in the second dimension. Spots were imaged after labelling with Flamingo (Biorad). (a) Pre-depletion of albumin and immunoglobulin with rectangle drawn to show albumin and oval shape to show globulins; and (b) post-depletion.

Neutralisztion of plasma IL-8, GM-CSF and IFN-α and effect of plasmas on lucigenin-dependent chemiluminescence

Heat-inactivated (57°C for 15 min.) plasma samples from periodontitis patients ($n = 11$) and matched controls ($n = 11$) were treated with 5 μg/mL anti-IL-8 (1 μg/mL for 10^5 cells), 5 μg/mL anti-GM-CSF, or 5 μg/mL anti-IFN-α antibody for 15 min. at room temperature in three independent experiments. Chemiluminescence (CL) was recorded over the last 10 min. pre-stimulation period to study baseline superoxide production from isolated neutrophils from three independent systemically and periodontally healthy donors (Fig. 2). Albumin/Ig-depleted, antibody neutralized or untreated plasma (10 μL) was added into microwells containing neutrophils from the three volunteers in independent experiments. Pre-stimulation radical release was measured for 30 min. Cells were then stimulated with 1 μM fMLP and RLUs measurements were taken for a further 30 min. The peak RLU values pre- and post-stimulation are reported as mean RLU ± standard error of the mean (SEM). All samples were analysed in triplicate, with paired patient and control samples analysed at the same time. The analyst (I. H. K. D.) was blinded to the origins of each sample pair.

Measurement of plasma IL-8, GM-CSF and IFN-α

Plasma samples were diluted 1 in 2 in sample diluent and levels of IL-8, GM-CSF and IFN-α were measured in triplicate using ELISA [IL-8 (Peprotech, London, UK), GM-CSF (Diaclone Research, Besancon, France) and IFN-α (Amersham Biosciences, Little Chalfont, UK)] according to the manufacturer's instructions.

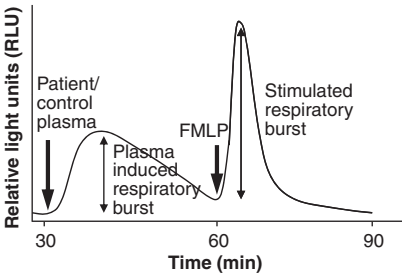

Fig. 2. Schematic representation of the neutrophil respiratory burst in the presence of plasma and a second stimulus, formyl-met-leu-phe (fMLP).

Data analysis

Data were analysed using Graphpad Prism software (version 3.00). Unless specified all data are presented as the mean ± SEM of at least three independent experiments, performed in triplicate. Statistical analysis was performed using a Wilcoxon signed rank test when comparing the differences between paired-sample medians. When comparing column means from more than two samples the one-way analysis of variance followed by Tukey's multiple comparison test was used.

Results

Periodontal plasma elicits an enhanced respiratory burst in neutrophils

Incubation of healthy, resting neutrophils (5×10^5) with plasma (1:20 vol:vol) from 11 age-, gender-matched periodontitis patient or control subjects induced a significant respiratory burst (Fig. 3a). As measured in the CL assay, patient plasma induced significantly higher ROS detected as lucigenin-dependent luminescence (median = 297 RLU) than age-/gender-matched, healthy control plasma (median = 165 RLU; $p < 0.001$). To investigate the effect of different plasma components on neutrophil hyperactivity and hyperreactivity, depletion and inhibition approaches were undertaken. Albumin- and IgG-depletion of control and patient plasma resulted in a significant reduction of the directly stimulated, lucigenin-dependent luminescence peak value compared with non-depleted, paired samples ($p < 0.05$). However, depleted patient plasma still stimulated a greater respiratory burst (median = 77.3 RLU) than depleted control plasma (mean = 41.6 RLU; $p < 0.001$; Fig. 3a).

fMLP stimulation of neutrophils (Fig. 3b) after incubation with periodontal plasma resulted in a significantly higher respiratory burst (median = 5995 RLU) compared with neutrophils pre-treated with control plasma (median = 3133 RLU; $p < 0.01$). This enhanced priming effect of patients' plasma for fMLP-stimulated ROS production was maintained after Ig/albumin depletion ($p < 0.001$; Fig. 3b).

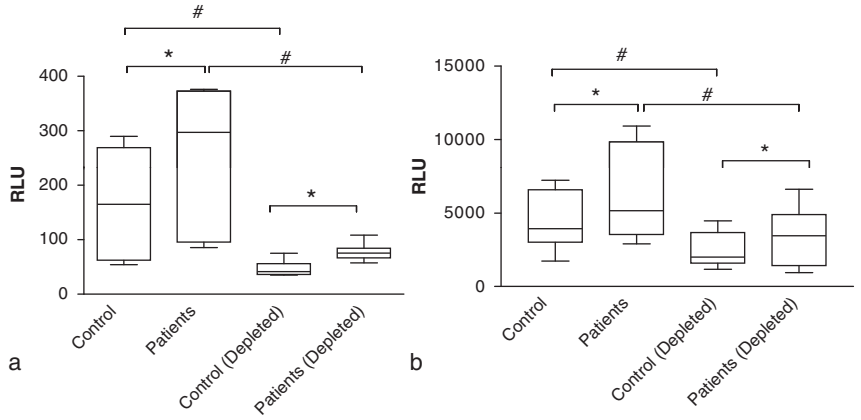

Fig. 3. The effect of periodontally healthy and diseased peripheral blood plasma on the neutrophil respiratory burst. Peak relative light unit (RLU) values for both (a) pre-formyl-met-leu-phe (fMLP) and (b) post-fMLP respiratory burst with or without albumin/Ig depletion were plotted and significant differences between control plasmas ($n = 11$) and patient plasmas ($n = 11$) were calculated using Wilcoxon signed rank test, where $^{*}p < 0.001$ and $^{\#}p < 0.05$. These data represent triplicate analysis of each plasma tested on neutrophil preparations from three healthy control donors. Box and whisker plot showing minimum value, lower quartile, median, upper quartile and maximum value.

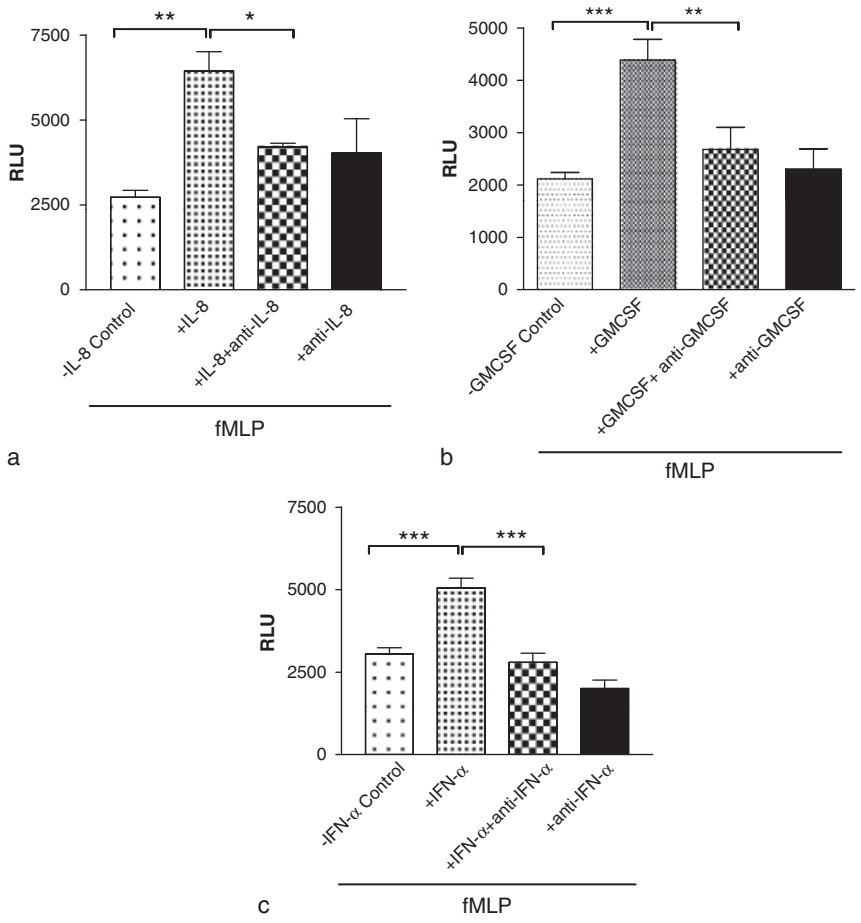

Fig. 4. The neutralizing ability of anti-IL-8, anti-GM-CSF and anti-IFN-α antibodies. The peak chemiluminescent relative light unit (RLU; mean ± SED) values for post-formyl-met-leu-phe (fMLP)-stimulated respiratory burst are presented for three independent experiments, where $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. Cells were pre-incubated ± cytokine ± anti-cytokine antibody before stimulation with fMLP.

IL-8, GM-CSF and IFN-α prime the fMLP-induced respiratory burst in neutrophils and priming is inhibited in the presence of neutralizing antibody

To determine whether specific cytokines were responsible for the observed effects of periodontal plasma on the respiratory burst of healthy neutrophils, experiments were undertaken using neutralizing antibodies. Initially, the neutralizing ability of anti-cytokine antibodies on the fMLP-stimulated respiratory burst after cytokine priming of neutrophils suspended in PBS was determined (Fig. 4). The fMLP-stimulated respiratory burst generated by neutrophils after priming with IL-8 (mean ± SEM; 6435 ± 235.8 RLU), GM-CSF (mean ± SEM; 4385 ± 684 RLU) and IFN-α (mean ± SEM; 5052 ± 506 RLU) was significantly higher than that obtained by non-primed, fMLP-stimulated control cells (range 1965–3257 RLU). The priming ability of IL-8, GM-CSF and IFN-α was significantly neutralized by the addition of 5 μg/mL monoclonal anti-IL-8, monoclonal anti-GM-CSF and monoclonal anti-IFN-α antibody ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).

Neutralization of IL-8, GM-CSF and IFN-α reduces periodontal plasma-mediated activation of neutrophil respiratory burst

Both control and periodontitis patient cytokine-neutralized plasmas stimulated a lower neutrophil respiratory burst compared with paired non-neutralized plasma samples (Fig. 5a and b). However, the decrease was significant only for periodontitis patient plasmas where the respiratory burst in the presence of anti-IL-8 antibody-neutralized plasma was 46.3% of control. In contrast, specific antibody-mediated neutralization of GM-CSF and IFN-α in plasma reduced respiratory burst to 67.8% and 68.9% of non-neutralized plasma, respectively (Fig. 5b). After the 30-min. stimulation/priming with plasma alone, cells were stimulated with 1 μM fMLP. Addition of anti-IL-8 or anti-GM-CSF antibody to both control and patient plasmas during the pre-stimulatory period had no effect on the subsequent fMLP-stimulated respiratory burst activity, suggesting that these cytokines did not prime for fMLP stimulation. Similarly, neutralization of IFN-α had no effect on the priming ability of control plasma for fMLP-stimulated CL (Fig. 5a). By contrast, a significant

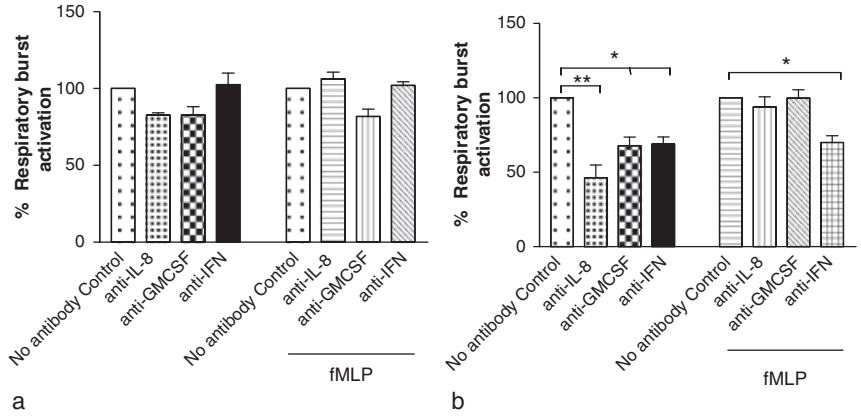

Fig. 5. The effect of anti-IL-8, anti-GM-CSF and anti-IFN-α neutralizing antibody on plasma-induced neutrophil respiratory burst. Peak relative light unit (RLU) values for (a) control plasma and (b) patient plasma were plotted as a percentage of no antibody control (100%). Peak RLU values were used to evaluate statistical significance by analysis of variance followed by Tukey's comparison. $^{*}p<0.05$ and $^{**}p<0.01$. Cells were pre-incubated with plasma ± anti-cytokine antibody and directly stimulated chemiluminescence (CL) recorded for 30 min. (left-hand columns). Subsequently, cells were stimulated with fMLP and peak CL determined (right-hand columns).

Table 1. Cytokine levels within plasma from controls and periodontitis patients determined by ELISA ($n = 11$; mean ± SEM)

Donor group	Concentration (pg/mL)		
	IL-8	GM-CSF	IFN-α
Control	38.76 ± 4.76	14.10 ± 0.48	0.487 ± 0.28
Patient	50.68 ± 6.18*	16.08 ± 1.10	1.002 ± 0.32*

*Significantly different from periodontally healthy control plasma levels: $p<0.05$.
SEM, standard error of the mean.

decrease in the fMLP-stimulated respiratory burst was found for IFN-α-neutralized periodontitis plasma (70%) compared with paired non-neutralized samples (100%; $p<0.05$).

Plasma cytokine levels

Plasma levels of IL-8, GM-CSF and IFN-α were measured by ELISA (Table 1). Mean plasma levels of IL-8 ($p<0.05$), GM-CSF (p = NS) and IFN-α ($p<0.05$) in patient samples were higher compared with control samples.

Discussion

Hyperactivity and reactivity of neutrophils may contribute to the destructive changes observed in inflammatory periodontitis (Chapple & Matthews 2007). The interactions between the microbial plaque biofilm and the host's inflammatory response create a complex environment resulting in increased levels of bacterial products and host inflammatory factors within both the gingival crevicular fluid as well as the systemic circulation (Matthews et al. 2007a). Levels of plasma factors are useful biomarkers for assessing the severity of periodontitis (Loos 2005). It is biologically plausible that these mediators prime or induce activity/reactivity of neutrophils in patients with periodontitis. In this regard, understanding the functional contributions of various plasma components that are elevated in periodontitis should provide valuable information about the neutrophil respiratory burst, regardless of whether the reported hyperactivity and reactivity is constitutional in nature or arises secondary to, hitherto unknown priming/stimulating agents.

The ability of plasma to activate the neutrophilic respiratory burst has been reported previously (Pascual et al. 1998). Confirming these observations, this paper has shown that plasma from periodontally healthy patients, activated neutrophils to generate a measurable respiratory burst. Interestingly, plasma from patients with periodontitis was significantly more effective in both directly stimulating the neutrophil respiratory burst and priming for subsequent fMLP-stimulated superoxide production than plasma from periodontally healthy subjects. This suggests the presence of additional neutrophil stimulatory factors in the plasma of patients with periodontitis that may explain the observed hyperactive and hyper-reactive neutrophil phenotype, with respect to ROS generation, reported in this disease (Gustafsson & Asman 1996, Fredriksson et al. 1999, Matthews et al. 2007a, b).

To investigate this further, plasma was independently depleted of factors known to stimulate neutrophils in in vitro and ex vivo studies. To our knowledge, this is the first report describing this approach to investigate putative factors that increase the neutrophil respiratory burst in periodontitis.

Although not demonstrated in chronic periodontitis, a lower plasma albumin–globulin ratio compared with periodontally healthy controls, which positively correlates with clinical parameters, has been reported in patients with aggressive periodontitis (Shi et al. 2008). Albumin is a major acute phase protein found to be inversely associated with inflammation and, together with Ig, represents 70–80% of the protein content of plasma and might have a role in plasma-induced activation of the neutrophil respiratory burst (Pascual et al. 1998). Depletion of albumin and IgG from plasma significantly decreased neutrophil ROS production by both control and patient plasma. However, plasma from patients with periodontitis retained the ability to directly stimulate and prime for a higher fMLP-stimulated respiratory burst than plasma from controls, suggesting that other factors in plasma were responsible for this enhanced activity.

We have previously reported that gingipains secreted by the periodontal pathogen *P. gingivalis* could manipulate the IL-8-primed neutrophil respiratory burst (Dias et al. 2008). In order to elucidate whether IL-8 present in the plasma of patients with periodontitis could contribute to an enhanced respiratory burst, IL-8 was neutralized with anti-IL-8 neutralizing antibody. This significantly reduced the respiratory burst elicited by neutrophils incubated with plasma from periodontitis patients. There was no significant decrease in the respiratory burst with IL-8-neutralization of plasma from control subjects compared with non-neutralized control plasma. This finding, together with the ELISA data, indicates the presence of elevated IL-8 activity in plasma from

periodontitis patients, which may contribute to the observed enhanced baseline extracellular ROS production in peripheral neutrophils from periodontitis patients (Matthews et al. 2007a). By contrast, neutralization of IL-8 had no effect on the ability of patient plasma to prime for fMLP-stimulated ROS generation indicating that elevated IL-8 levels alone are not responsible for the known hyper-reactivity of patient neutrophils to Fcγ- and Toll-like receptor stimulation (Gustafsson & Asman 1996, Fredriksson et al. 1999, Matthews et al. 2007b).

We have previously demonstrated that in vitro priming by GM-CSF enhanced baseline extracellular ROS production in peripheral neutrophils from periodontitis patients (Matthews et al. 2007a). This observation is consistent with previous findings of GM-CSF-dependent up-regulation of neutrophil-mediated pathology (Waterman & Shaafi 1995, Mansfield et al. 2002). In the current work, neutralization of GM-CSF in patient plasma reduced its ability to directly stimulate the neutrophil respiratory burst compared with non-neutralized plasma but, as found for IL-8, had no effect on priming for fMLP stimulation. Thus, GM-CSF levels in the plasma of periodontitis patients may play a role in baseline neutrophil hyperactivity, with respect to ROS production, without necessarily playing a significant priming role for subsequent stimulation.

The ability to enhance degranulation and the respiratory burst of neutrophils by interferons has been reported in previous studies (Atzeni et al. 2002). The data reported here support the concept that an elevated plasma IFN-α level in periodontitis patients is a contributor to both neutrophil hyperactivity and hyperreactivity. Previously, IFN-α has been shown to act as a priming agent, enhancing the fMLP, leukotriene B4 and influenza A virus-induced respiratory burst (Little et al. 1994). Our data show that the elevated IFN-α in the plasma of periodontitis patients is able to cause both enhanced baseline extracellular ROS production by, and prime for the fMLP-stimulated respiratory burst in, neutrophils isolated from healthy individuals. This suggests an important role for this cytokine in the reported hyperactive and reactive phenotype of neutrophils freshly isolated from periodontitis patients. Our group has reported previously that levels of IFN-α in the plasma of periodontitis patients are elevated relative to healthy controls and that IFN-α-stimulated gene expression is elevated in peripheral blood neutrophils from periodontitis patients (Wright et al. 2008). In comparison, LPS did not replicate the interferon-stimulated gene expression signature that was observed in vivo. According to this latter study, the most likely IFNs to stimulate these genes are type I IFNs, such as IFN-α that we now show in the present study to mediate both direct stimulation and priming of the neutrophil respiratory burst.

Neutrophil ROS release is a result of the enhanced activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. NADPH oxidase is activated through phosphorylation of the cytoplasmic subunits p47phox, p67phox and p40phox and their translocation to the membrane to associate with membrane components of gp91 and p22phox. Although IL-8, GM-CSF, IFN-α and fMLP stimulate neutrophils through different signalling pathways, they commonly upregulate the activity of protein kinase C to phosphorylate p47phox. The convergence of different signalling pathways to upregulate the assembly and activity of NADPH oxidase may be involved in increased baseline and stimulated ROS production by neutrophils demonstrated in peripheral neutrophils from periodontitis patients (Matthews et al. 2007a, b).

Collectively, the reported studies have investigated the stimulatory and priming activities of plasma from periodontitis patients on the neutrophil respiratory burst. The data suggest that IL-8, GM-CSF and IFN-α may be responsible for direct activation of the oxidative burst and have a role in hyperactivity of peripheral neutrophils in periodontitis. In addition, the role of plasma IFN-α as a pathophysiological priming agent may also be important for the observed hyper-reactivity of periodontitis neutrophils. The reported ability of IL-8, GM-CSF and IFN-α to elicit the oxidative burst of neutrophils combined with their elevated levels present in patients' plasma provides support for their involvement in the pathogenesis of chronic periodontitis.

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Clinical Relevance

Scientific rationale for the study: Periodontitis patients are characterized by a 'hyper-inflammatory' phenotype, a major component of which is exaggerated neutrophil ROS release. Here we examine mechanisms that contribute to this destructive response.

Principal findings: Blood plasma from periodontitis patients, but not from unaffected controls, contains sufficient pro-inflammatory cytokines to directly stimulate and to prime for neutrophil ROS production, which may explain in part, the hyper-responsive neutrophil phenotype.

Practical implications: Understanding the control of dysregulated periodontal inflammation may lead to the development of novel corrective immune-modulatory therapies.