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Reduced numbers of peripheral blood CD27$^+$ IgD$^-$ memory B cells in patients with aggressive periodontitis

Verringerte Anzahl von CD27$^+$ IgD$^-$ Memory-B-Zellen im peripheren Blut von Patienten mit aggressiver Periodontitis

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Abstract: Aggressive periodontitis (AgP) is a multifactorial disease with unknown association to the development and function of peripheral lymphocytes. The aim of this study was to elucidate a connection between the periodontal condition in 10 patients with AgP and their potential state of immunodeficiency. Based on full periodontal examination and radiographs, 10 females (ages 29.8±8.62 years) with established diagnosis of aggressive periodontitis were included in this study. Flow cytometric analysis revealed substantial reduction of switched memory B cells (IgM$^-$, IgD$^-$, CD27$^+$) in 9 of 10 patients, whereas numbers of naïve, IgM$^+$ memory, transitional, and activated B cells were normal. Serum levels of IgM, IgG, IgA, and subclasses were normal. In vitro differentiation of B cells showed normal amounts of secreted IgG and IgA at day 5 of culture. Our results indicate that lowered numbers of switched memory B cells – typically referred to the state of common variable immunodeficiency type I (Freiburg classification) – are unlikely to influence immunoglobulin serum levels or clinical anamnesis of our patients with AgP. Lipopolysaccharide-induced elevated levels of IL-1β and IL-8 and lowering of IL-4 are more likely to trigger a pro-inflammatory circle that attracts lymphocytes to local pockets of aggressive periodontitis.

Keywords: aggressive periodontitis; bone loss; memory B cells; primary immunodeficiency diseases.

Zusammenfassung: Die aggressive Parodontitis (agP) ist eine multifaktorielle Erkrankung mit unbekannter Assoziation zu Reifungsgrad und Funktion peripherer Lymphozyten. Das Ziel der durchgeführten Studie bestand in der Eruierung des Zusammenhangs zwischen dem parodontalen Gesundheitszustand von 10 Patienten mit agP und deren potentiellem Status einer Immundefizienz. Basierend auf einer vollständigen parodontologischen Untersuchung und Röntgenbefunden wurden 10 weibliche Patientinnen (Alter 29.8±8.62 Jahre) mit der gesicherten Diagnose einer agP in die Untersuchung eingeschlossen. Die durchflusszytometrische Untersuchung wies eine erhebliche Reduktion der Gedächtnis-B-Zellen mit Klassenwechsel (IgM$^-$, IgD$^-$, CD27$^+$) bei 9 von 10 Patientinnen nach, wohingegen die Zahlen der naiven, der IgM$^+$ Gedächtnis-B-Zellen, der transitionalen und der aktivierten B-Zellen den Normwerten entsprachen Die Serumspiegel von IgM, IgG, IgA und deren Subklassen lagen im Referenzbereich. Die in-vitro Differenzierung von B-Zellen zeigte normale Mengen sekretierten IgG und IgA nach 5 Tagen Zellkultivierung. Unsere Ergebnisse weisen darauf hin, dass eine reduzierte Anzahl von Gedächtnis-B-Zellen mit Klassenwechsel – die typischerweise zur Diagnosestellung der

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allgemeinen variablen Immundefizienz (CVID, Freiburger Klassifikation) herangezogen werden – wahrscheinlich nicht die Immunglobulinspiegel im Serum oder die klinische Anamnese unserer Patienten mit agP beeinflussen. Wahrscheinlicher ist, dass die durch LPS induzierte Erhöhung der Spiegel von IL-1β und IL-8 sowie die reduzierte IL-4-Freisetzung einen proinflammatorischen Kreislauf auslösen, der für die Lymphozytenmigration zu den Sites mit klinischen Zeichen der agP sorgt.

Schlüsselwörter: Aggressive Periodontitis; Knochenverlust; Primärer Immundefekt; Memory-B-Zellen.

Introduction

Aggressive periodontitis (AgP) represents a type of periodontitis with a rapid rate of disease progression that affects people who, in most cases, appear otherwise healthy. Epidemiological surveys have shown that the prevalence rates for AgP vary from 0.1% to 17.6%, whereas the disease is less common in Caucasian populations and more frequent in India and Africa [1, 2]. AgP is a multifactorial disease that tends to have a familial aggregation [3]. Pathogenic bacteria like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, and *Fusobacterium nucleatum* represent the primary triggers in the etiology of periodontitis [4]. However, most of these bacteria are part of the normal oral microflora. Thus, additional host susceptibility factors such as single-nucleotide polymorphisms within inflammatory genes, epigenetic regulation, and also smoking behaviour, race, age, and the environment may play a role [5]. The aim of this investigation was to elucidate a connection between periodontal condition in patients with AgP and their possible state of immunodeficiency.

Patients and methods

Subjects

This investigation covers 10 females (ages 18–46, mean age 29.8±8.62 years) who were included following an established diagnosis of aggressive periodontitis based on full periodontal examination and radiographs [6]. All patients had to fulfil the following inclusion criteria: (i) age ≤35 years at time of diagnosis, (ii) radiographic bone loss ≥50% at a minimum of two different teeth, and (iii) being otherwise clinically healthy on examination [7].

The absence of systemic conditions associated with the onset of the periodontitis (e.g., diabetes mellitus or HIV infection) completed the diagnosis of aggressive periodontitis. Thus, we proceeded with immunodiagnostics to exclude the oral manifestations that result from primary immunodeficiency diseases. All patients agreed with adjunctive immunodiagnostics to identify additional factors for the disease (informed consent).

Clinical and microbiological investigations

A full-mouth clinical examination was performed in all patients. Clinical attachment level (CAL), probing pocket depth (PD), and bleeding on probing (BOP) were measured at six sites per tooth. Radiographic bone loss was recorded by Orthopantomograph images. Microbiological culture of subgingival plaque samples, taken at four pocket sites, was done immediately according to a protocol previously described [6, 8].

Immunological investigation

Eighteen millilitres of peripheral blood was collected to analyse the composition of lymphocyte subpopulations and the peripheral B cells by flow cytometry (FCM) and to stain T cells intracellularly for IFNγ and IL-4 (ICS) [9–11]. Furthermore, we determined the level of serum IgM, IgG, and IgA and their Ig-subclasses and cultured B cells for 7 days in the presence of IL-10 and anti-CD40 mAb to analyse their corresponding IgG and IgA production (Figure 1). The release of IFNγ, IL-10, IL-1β, IL-8 (CXCL8), and RANTES (CCL5) by peripheral blood mononuclear cells (PBMC) upon stimulation with either phytohaemagglutinin, lipopolysaccharide (LPS, *E. coli* 055:B5), tetanus toxoid, pneumococcal cell wall polysaccharide (*Streptococcus pneumoniae* strain), or mumps virus lysate (Enders strain) was investigated using single-cell enzyme-linked immunospot (ELISPOT) technique as previously described and is shown in Figure 1 [12]. All data from FCM, intracellular staining (ICS), and ELISPOT analyses were subjected to heatmap row-normalization and visualization using Heatmap Builder v1.1 (E. A. Ashley, J. M. Spin, and C. Watt: Quertermous Lab, Stanford University, Stanford, CA, USA). Blood samples for ELISPOT assay were taken in 8 of 10 patients. In addition, eight healthy blood donors who did not differ in terms of age and sex were included as experimental controls.

Results

In general, dental and radiographic examination revealed increased clinical attachment levels (mean 3.7 mm, normal=0 mm) corresponding to an extensive radiographic bone loss and PD (mean 3.6 mm, normal ≤2 mm) in all patients, as indicated in Figure 2 and Table 1. Major periodontal pathogens were detected by microbiological culture of subgingival plaque samples, as shown in Table 1. The patient's clinical reports delivered no evidence for increased susceptibility to infection or frequent need for antibiotic treatment. Two of the 10 patients had received antibiotics within a former periodontal therapy

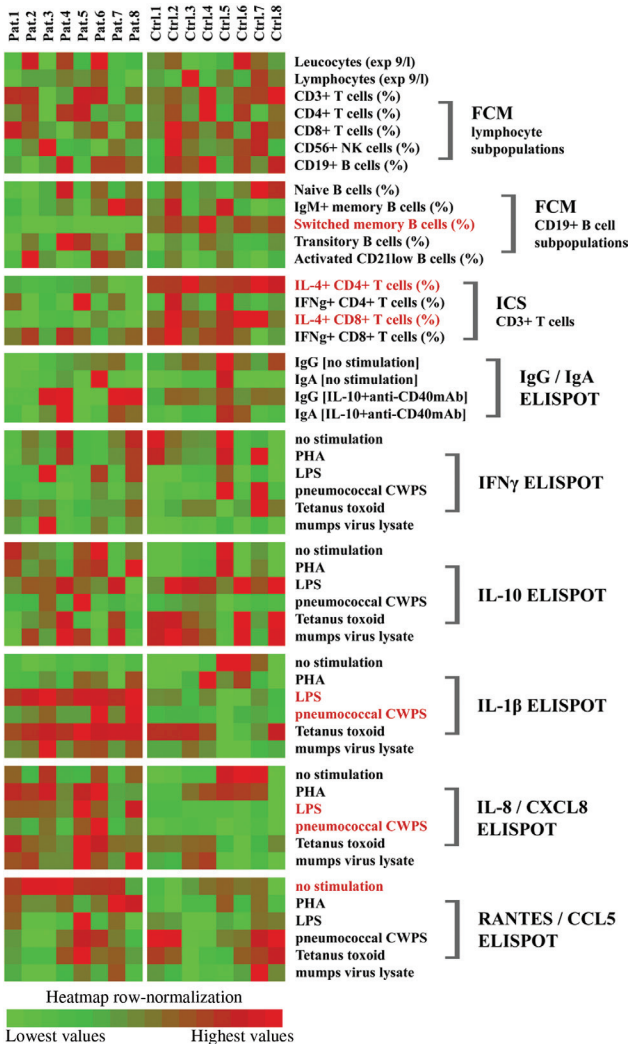

Figure 1: Heatmap visualization of the patient's immunological findings obtained by flow cytometry, intracellular cytokine staining, and ELISPOT assays.

22 months before taking blood samples for ELISPOT assay.

FCM analysis revealed a substantial reduction of IgM^- IgD^- $CD27^+$ switched memory B cells in the peripheral blood of all patients presented herein, whilst the proportions of naïve, IgM^+ memory, transitional, and activated B cells were all normal, as well as the composition of other lymphocyte subpopulations (Figure 1). Serum levels of IgM, IgG, IgA, and subclasses were all normal (data not shown), and the amount of IgG and IgA produced upon in vitro differentiation of B cells was comparable to that of healthy individuals. The percentage of both intracellular $IL\text{-}4^+$ $CD4^+$ and $CD8^+$ T cells was considerably decreased in the peripheral blood of all analysed patients, whilst the percentages of $IFN\gamma^+$ T cells were normal (Figure 1). In the ELISPOT assay, highly elevated IL-1β and IL-8 (CXCL8) responses to LPS and pneumococcal cell wall polysaccharide (CWPS) were discovered in all 8 of 10 analysed patients (Figure 1). Furthermore, the baseline production of RANTES (CCL5) was found to be elevated in the PBMC of patients with AgP.

Discussion

Many primary immunodeficiency diseases clinically present with oral manifestations such as periodontal inflammation, including diseases of phagocytic deficiency or T- and B-cell deficiency, and are commonly caused by the impairment of oral defence mechanisms against candidiasis and herpetic or bacterial infections [13]. In this regard, our finding of severely reduced numbers of switched memory B cells in the peripheral

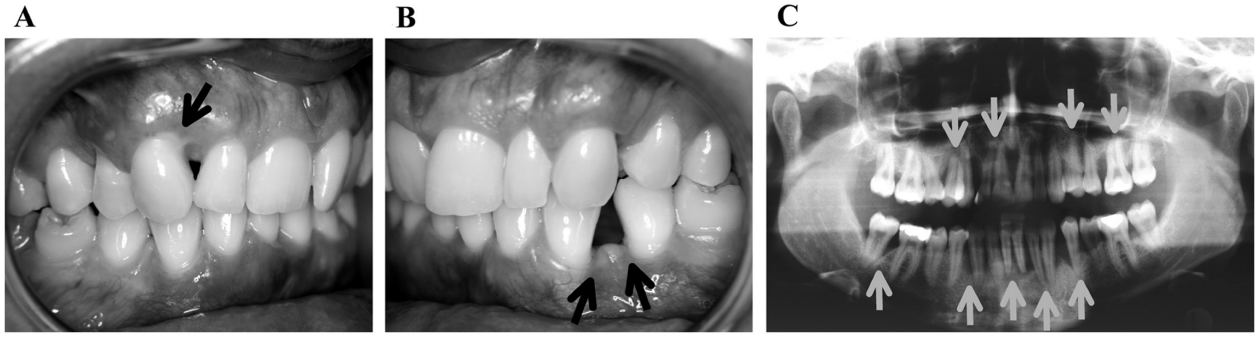

Figure 2: Clinical aspect and Orthopantomograph image of a representative patient diagnosed with AgP.
(A) and (B): Photographic documentation of the clinical aspect; arrows point at displaced tooth and suppurations in several pockets. (C) Orthopantomograph image; arrows point at severe periodontal destruction sites with radiographic bone loss.

Table 1: Results of the periodontal examination of included patients with AgP.

	Age, years	API, %	BOP, %	Mean CAL, mm	Max CAL, mm	Mean PD, mm	Max PD, mm	Max radiographic bone level, %	Identification of periodontal pathogens
Patient 1	29	24	41.60	3.91	12.00	3.90	12.00	90	A.a., C.spp..
Patient 2	36	12	55.95	3.94	10.00	3.76	10.00	80	P.i.
Patient 3	46	18	36.96	3.44	7.00	2.84	6.00	50	A.a., P.g., P.i.
Patient 4	36	36	55.80	4.13	13.00	4.29	12.00	90	P.i., F.n.
Patient 5	39	32	56.25	4.19	12.00	3.78	10.00	90	P.i.
Patient 6	26	48	51.23	3.41	7.00	3.41	7.00	50	A.a., P.i.
Patient 7	23	18	42.86	4.37	9.00	4.42	9.00	80	A.a., P.g., T.f., T.d.
Patient 8	20	56	79.73	3.61	7.00	3.62	7.00	60	A.a.
Patient 9	25	24	48.00	3.68	11.00	3.35	8.00	90	P.g.
Patient 10	18	28	44.60	2.31	12.00	2.46	10.00	80	P.g.

API, interproximal plaque index; normal=0; BOP, bleeding on probing; normal=0; CAL, clinical attachment level, normal=0 mm; PD, probing pocket depth, normal ≤2 mm; radiographic bone level=distance between the cemento enamel junction and the alveolar crest or the most apical extension of an intrabony defect, normally up to a cut-off point in a distance of approximately 2 mm of the cemento enamel junction; A.a., *Aggregatibacter actinomycetemcomitans*; P.g., *Porphyromonas gingivalis*; T.f., *Tannerella forsythia*; T.d., *Treponema denticola*; P.i., *Prevotella intermedia*; F.n., *Fusobacterium nucleatum*; C.spp., *Capnocytophaga* species.

blood of patients with AgP is of certain importance. Whilst IgM^- IgD^- $CD27^+$ switched memory B cells are the main source for isotype-switched serum immunoglobulins, such as IgG, IgA, and IgE, their exclusive reduction is typically referred to the state of common variable immunodeficiency type I syndrome (CVID), according to the classification system proposed by Warnatz et al. [14], with an association between the percentage of switched memory B cells and the frequency of infectious complications. However, as serum levels of IgM, IgG, IgA, and subclasses were all normal and no increased susceptibility to infection or frequent need for antibiotic treatment was found in our patients with AgP, the diagnosis of CVID is very unlikely. We would rather propose that elevated monocytic secretion of both IL-1β and IL-8 and lowering of IL-4 mediated macrophage inhibition triggers a pro-inflammatory circle that attracts lymphocytes from the peripheral blood to local pockets of aggressive periodontitis. Besides their strong chemotactic effects on leukocytes, both IL-1β and IL-8 are known stimulators and regulators of B cells, promoting their proliferation and the synthesis of immunoglobulins.

In accordance with the results of a study by Fiebig et al. [15], our data show that the increased secretion of IL-1β is activation-dependant in terms of a hyper-responsive macrophage phenotype, implying that polymorphisms in the IL-1 gene cluster may not generally increase IL-1β production in patients with AgP. Whilst several IL-4 promoter and intronic polymorphisms have been shown to correlate with the onset of periodontitis and the patient's serum levels of IL-4, little is known about their impact on the peripheral lymphocyte compartment in AgP [16]. IL-4 is a potent down-regulator of macrophage function and prevents the production of IL-1β, TNFα, and prostaglandins in response to activation of monocytes. As shown in agreement with our results from intracellular IL-4 staining, IL-4 serum levels are typically reduced in patients with AgP, most likely unleashing the unfavourable chemotactic effects of IL-1β and IL-8 [16]. Finally, Sigusch et al. [17] have shown that $CD20^+$ B cells are present 13 times more frequently in the periodontal pockets of patients with AgP than in healthy controls, supporting our hypothesis of B-cell migration from the peripheral blood to active sites in AgP.

In summary, our data shed light on the potential interplay of IL-1β, IL-8, and IL-4 to trigger a pro-inflammatory circle that attracts lymphocytes to local pockets in aggressive periodontitis.

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