Inflammatory cells and their subsets in lesions of juvenile periodontitis

A family study

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The inflammatory cells in the gingival biopsy samples from a total of 9 patients with juvenile periodontitis (JP) and from 10 of their family members (JP_{relat}) belonging to 5 different families were subjected to phenotypic characterization. Plasma cells and their immunoglobulins were stained with immunoperoxidase kits for IgA, IgG, and IgM. B, T, and MPS (cells of the mononuclear phagocyte system) cells were demonstrated by the acid α-naphthyl acetate esterase (ANAE) technique in cryostat sections. The subsets (T helper/inducer and T suppressor/cytotoxic cells) of T cells were detected with monoclonal antibodies OKT4 and OKT8, respectively, using indirect immunofluorescence. Similar studies were completed for 19 age- and sex-matched periodontally healthy subjects. IgG plasma cells far outnumbered the IgA and IgM cells in all three series and were most pronounced in JP_{relat} series. Most of the inflammatory cells were ANAE-negative (B cells) in all series (78–87%). The highest proportions of T and MPS cells were found in the JP series (10% for both). A statistically significantly higher ratio for T_H/T_S ($OKT4^+/OKT8^+$) was found in both the JP and JP_{relat} series as compared with that of the healthy controls. The findings suggest that imbalance (either inherited or acquired) in the immune regulation may play role in the development of JP.

□ *ANAE stain; gingival biopsies; monoclonal antibodies; phenotypic characterization*

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The adverse effects of plaque bacteria on the host's periodontal tissue are well established (1–3). According to current knowledge, both the immune response (both systemic and local) and genetic factors may play an important role in the initiation and progression of periodontal diseases (4, 5). Lately, further evidence that juvenile periodontitis (JP) is a separate clinical entity has been provided by observations indicating failure in the host defense mechanism of JP (6, 7).

Gingival biopsy samples from JP patients have contained markedly increased numbers of lymphocytes and plasma cells as compared with healthy controls (8, 9). Furthermore, a predominance of plasma cells over lymphocytes has been reported in severer cases of JP (4, 6, 8). An increase in proportions of IgA- and IgG-secreting plasma cells has also been reported in these patients (6, 9). On the other hand, the presence of IgM-producing cells in gingival tissue is a more controversial issue (6, 8–10). Attempts have been made to identify T and B cells of the gingival tissue in chronic periodontal disease (11–13). Most lymphocytes in clinically defined progressive gingival lesions are thought to be of B-cell origin (11–13).

In the gingival lesions of JP, however, no previous reports on the composition of the inflammatory cell infiltrate (whether B, T, or MPS cells) seem to be available, as far as we are aware. The same seems to hold true in the analysis of the T-cell subsets (T helper/inducer and T suppressor/cytotoxic) known to regulate the immune responses in tissues. As part of a family study attempting to clarify the etiological aspects of JP, including the role of HLA types, the present communication reports on the phenotypic characterization of the inflammatory cells (plasma cells, B, MPS, and T cells, and the subsets of the latter) in gingival biopsy samples from JP patients and their relatives.

Materials and methods

Patients and controls

The material of the present study consists of the family members of five young subjects (aged 12–18 years), who were initially examined at the Department of Periodontology, University of Kuopio, for suspected JP. The diagnosis JP was based on clinical and radiological examinations. The possibility of any systemic disease was excluded by extensive laboratory tests. The members of the families of these five JP patients (Fig. 1) were invited for further examinations. These included a histological biopsy taken from the interdental papilla between the right mandibular second bicuspid and the first molar, during local anesthesia induced with prilocaine (Citanest-Octapressin®) 8% solution. Six of the subjects invited refused the biopsy, and four others were excluded for prosthetic reasons. Thus, the final series of patients biopsied comprises a total of 9 subjects with JP or post-JP and 10 relatives of the former with no signs of JP. The mean age of the JP patients was 24.7 ± 11.0 years, and the F/M ratio was 1.25:1. The corresponding figures of their relatives (JP_{relat}) were 28.1 ± 13.1 years and 1:1 (F/M). These two test series were supplemented with 19 age- and sex-matched periodontally healthy subjects as controls. Fig. 1 summarizes the family patterns, the occurrence of JP, and the ages of the subjects in the JP and JP_{relat} series.

Biopsies and tissue sections

The interdental papilla biopsy sample was divided into two parts, one half being mounted in Tissue-Tek® II O.C.T. Compound (Lab-Tek Products, Ill., USA) and immediately frozen in isopenthane cooled with liquid nitrogen. The frozen specimens were stored at −80°C until further processed. The other half was fixed in 10% neutral formalin, embedded in paraffin, and cut into 4-μm-thick sections.

Morphological studies were done in hematoxylin and eosin-stained sections, and the findings will be reported separately. Plasma cells were demonstrated in paraffin-embedded sections by staining with commercially available immunoperoxidase kits for IgA, IgG, and IgM (Histoset, Immulok Inc, Calif., USA). In the staining procedure, the specifications of the manufacturer were strictly followed, including the set-up of controls (normal lymph nodes in this case). The plasma cells staining positive for the Igs were recognized by the strong

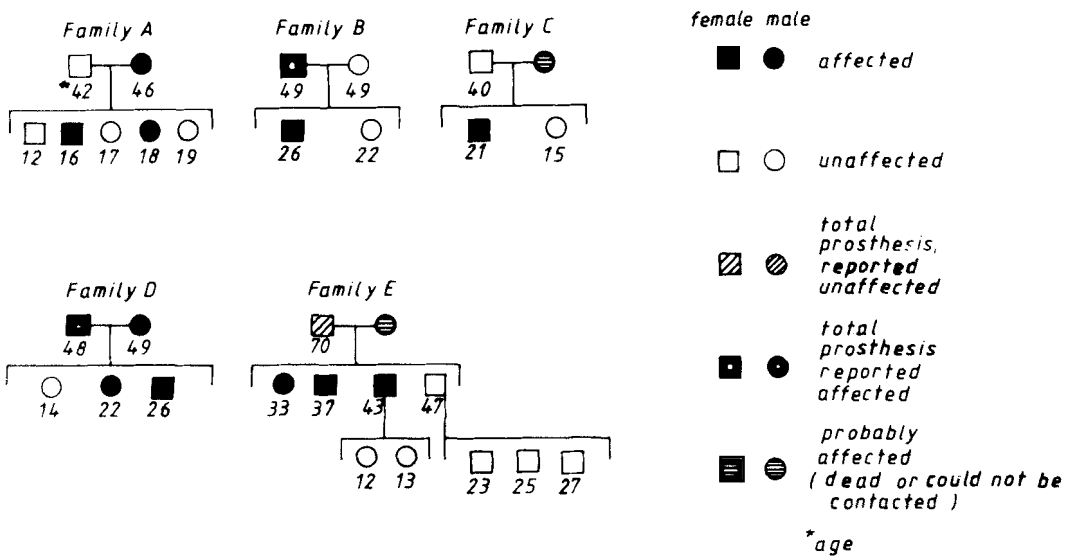

Fig. 1. The family patterns of the patients with juvenile periodontitis and their relatives.

reddish-brown precipitate in their cytoplasm, not detectable in the negative controls (Fig. 2). The percentages of IgA-, IgG-, and IgM-secreting plasma cells and their ratios were established by counting 100 plasma cells (in separate sections) under a ×40 objective, as previously described (15).

ANAE staining

B and T lymphocytes and cells of the mononuclear phagocyte system (MPS cells) were demonstrated in cryostat sections by the acid α-naphthyl acetate esterase (ANAE) technique, as recently detailed (14, 15). In brief, the 6-μm-thick cryostat sections were incubated in a mixture of phosphate buffer, hexazotized pararosaniline, and α-naphthyl acetate in acetone (pH 5.8) for 60 min at room temperature. Hexazotized pararosaniline was prepared as described previously (14). After the incubation, slides were washed in distilled water, counterstained with freshly prepared 1% aqueous toluidine blue for 2 min, and processed into light microscopical specimens.

In ANAE-stained sections, T lymphocytes are recognized by their single (or a few) intracytoplasmic brown spots (T^+ pattern). MPS cells show a diffuse ANAE staining (M^+ pattern), making their distinction from T cells quite easy. In the ANAE technique B lymphocytes (and possibly some activated T cells) remain negative (Fig. 3). As a control of ANAE stain, normal lymph nodes were parallelly processed, as previously suggested (14).

Immunofluorescence technique

The subsets of T lymphocytes were demonstrated by means of monoclonal antibodies OKT3, OKT4, OKT6, OKT8 (Ortho Immunobiology Ltd., Raritan, N.J., USA). The production and characterization of these antibodies have recently been detailed (16, 17). According to these data, OKT3 reacts with all peripheral T cells, OKT4 with inducer/helper T cells, OKT6 with mature thymocytes and Langerhans cells, and OKT8 with cytotoxic/suppressor T cells (16, 17). T cells were stained by an indirect immunofluorescence method in 6-μm cryostat sections. Sections were fixed in cold (+4°C) acetone for 1 min, washed in phosphate-buffered saline (PBS), pH 7.2, and subsequently incubated with the monoclonal antibody (1:10 dilution in distilled water) at +37°C for 15 min and with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (Miles Laboratories, Elkhart, Ind., USA) (1:10 dilution in distilled water) at +37°C for 15 min. In negative controls, the primary antiserum was omitted or substituted with

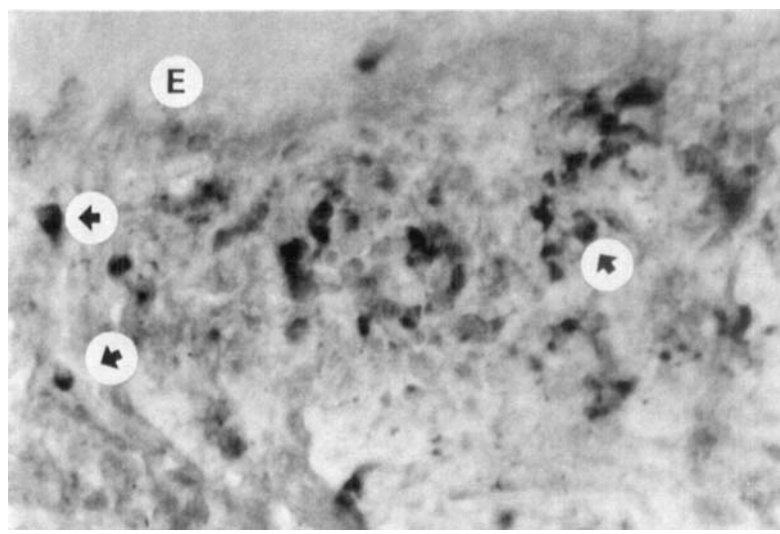

Fig. 2. An infiltrate of inflammatory cells subjacent to gingival epithelium in a section stained for IgG with the immunoperoxidase kit. Many characteristic plasma cells (arrows) with distinct cytoplasmic reactivity for IgG are discernible. E = epithelium. (Immunoperoxidase kit for IgG; original magnification, ×400.)

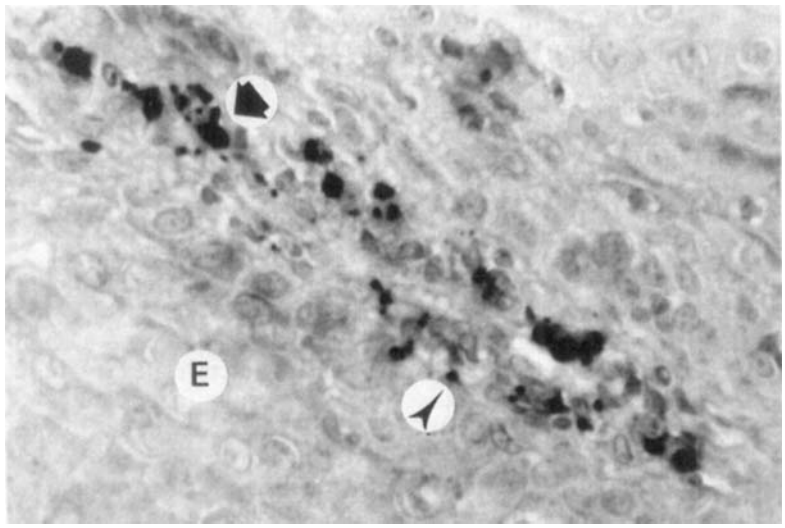

Fig. 3. A high-power detail of a dermal papilla infiltrated with inflammatory cells. In this ANAE-stained section, MPS cells (large arrow) show a diffuse cytoplasmic staining, whereas T cells (arrowhead) contain only one (or a few) cytoplasmic spots. Several ANAE-negative (B) cells are also present. E = epithelium. (Original magnification, ×400.)

normal mouse serum, both giving constantly negative staining. As positive controls, normal lymph nodes were processed in parallel, showing positive T-cell staining mainly in the paracortex (a T-cell region in the lymph node).

The immunofluorescence slides were viewed with a Leitz Orthoplan Fluorescence microscope equipped with an appropriate set of filters. The absolute counts of $OKT3^+$, $OKT4^+$, $OKT6^+$, and $OKT8^+$ T cells were calculated in separate sections and the helper/suppressor ($OKT4^+/OKT8^+$) ratio established, on the basis of analysis of two sections subsequent to each other (Fig. 4).

Statistics

All the cellular calculations were completed in a blind manner, the authors being unable to recognize the specimen as either of JP, JP_{relat}, or control origin at this stage of the study. The results were analyzed with Student's *t* test, as generally applied in materials with normal distribution.

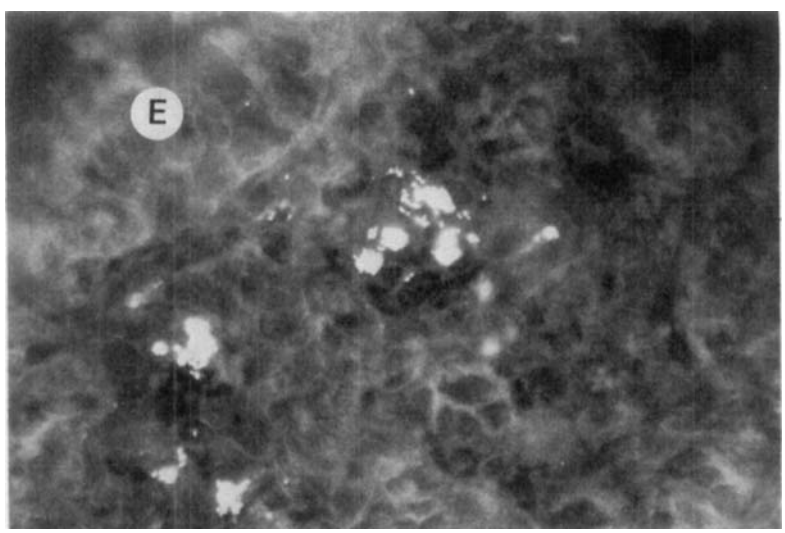

Fig. 4. Immunofluorescence demonstration of $OKT4^+$ (T inducer/helper cells) cells in the gingival tissue from a JP patient. A cluster of cells with positive fluorescence is found within the cellular infiltrate. E = epithelium. (Indirect immunofluorescence with OKT4 monoclonal antibody; original magnification, ×400.)

Results

The percentages of the immunoglobulin-producing gingival plasma cells are given in Table 1. In all three series the number of IgG-positive plasma cells far exceeded that of IgA and IgM plasma cells. In controls the percentages of IgA and IgM plasma cells were higher than in JP and JP_{relat} series.

The calculated ratios of IgG/IgA/IgM plasma cells are listed in Table 2. The proportion of IgA was closest to that of IgG (IgA/IgG, 1:2.4) in the control series and furthest from it in JP_{relat} series.

Table 3 summarizes the percentage distribution of ANAE-positive and -negative lymphocytes and of MPS cells in the biopsy samples. In all three series ANAE-negative lymphocytes far outnumbered the other cell types. The lowest relative counts of ANAE-negative lymphocytes were found in JP lesions, which in turn contained the highest values of T and MPS cells.

The results of T-cell subset enumeration are shown in Table 4. The data on the $OKT6^+$ (Langerhans) cells (a few found in some sections) are not included. The highest numbers of $OKT8^+$ cells were found in the JP series, followed by JP_{relat} and control series. The highest absolute counts were enumerated for $OKT3^+$ cells (all peripheral T cells), the figures fitting those obtained by summing up the $OKT4^+$ and $OKT8^+$ T cells. As calculated from two adjacent sections, the ratio $OKT4^+/OKT8^+$ cells was significantly higher both in JP and JP_{relat} series as compared with that in the control sections. In both cases this elevation was due to an increase of $OKT4^+$ cells and not to decrease of $OKT8^+$ cells.

Table 1. Percentages (mean ± SD) of IgA-, IgG-, and IgM-secreting plasma cells in gingival specimens from juvenile periodontitis (JP) patients, their relatives (JP_{relat}), and individuals with no periodontitis (controls)

Series	No.	IgA (%)	IgG (%)	IgM (%)
JP	9	20.6 ± 15.0	78.8 ± 15.4	0.7 ± 1.2
JP_{relat}	10	14.4 ± 4.1	84.3 ± 5.2	0.3 ± 0.8
Controls	19	29.4 ± 19.6	68.2 ± 19.6	2.9 ± 4.7

Table 2. Calculated ratios of the Ig-producing plasma cells given in Table 1

Series	No.	IgG/IgA/IgM ratio
JP	9	3.8:1:0.02
JP_{relat}	10	5.8:1:0.03
Controls	19	2.4:1:0.10

Discussion

It was recently shown that an inflammatory infiltrate is present both in the gingival biopsy samples of JP and JP_{relat} patients and in those of healthy controls (18). The inflammatory reaction was slightest in the control series and much more intense in the two other groups. Such a mild inflammation even in healthy-appearing gingiva is generally ascribed to a host response against plaque always present in small amounts in the gingival margin (9, 19). The present communication gives the data on the phenotypic characterization of the cells participating in such infiltrates.

Previous articles have suggested that IgG plasma cells dominate in the gingiva regardless of the severity of the inflammatory reaction (8, 10, 19). The present finding of a distinct predominance of IgG plasma cells in all three series of gingival biopsy samples are in alignment with this view. The observed relative increase of IgG plasma cells and the relative decrease in IgA and IgM plasma cells in the gingiva of JP and JP_{relat} series seems to be consistent with the suggestion that there is a shift from IgM to IgG synthesis in the chronically inflamed gingiva (19). This is also substantiated by the reported failure to find IgM plasma cells constantly in the specimens from JP patients (8, 10, 19, 20). This was also confirmed in the present study (Tables 1 and 2), in which the relative number of IgM plasma cells was exceedingly low in JP and JP_{relat} series, as compared with that in controls.

Although a relative decrease in the mean percentages of IgM plasma cells in JP and JP_{relat} patients as compared with the controls was observed, the absolute counts for these cells were almost equal in all three series.

Table 3. Distribution (mean ± SD) of ANAE-positive lymphocytes, ANAE-negative lymphocytes, and macrophages in gingival biopsies from patients with juvenile periodontitis (JP), their relatives (JP_{relat}), and individuals with no periodontitis (controls)

Series	No.	ANAE-positive lymphocytes, %	ANAE-negative lymphocytes, %	Macrophages, %
JP	9	10.3 ± 10.1	78.3 ± 13.9	10.3 ± 8.5
JP_{relat}	10	8.1 ± 10.4	85.4 ± 11.8	6.6 ± 6.0
Controls	19	5.5 ± 6.8	86.7 ± 11.7	7.2 ± 6.4

The distribution pattern of the Ig-synthesizing cells observed in the present study could be interpreted as a characteristic profile of Ig production in the gingival lesions of JP patients and of their relatives as well. The absolute plasma cell counts detectable in hematoxylin and eosin-stained sections and in those stained by the immunoperoxidase kits for the three Igs were not completely equal. Similar observations have previously been reported by other workers as well (8, 21, 22). This is most probably due to the different techniques and the intersectional cell count variation (8, 21, 22). The possibility that the Ig-negative plasma cells might be those secreting IgE or IgD cannot be completely ruled out, however. Yet another possibility would be the switch-off of their genes controlling the Ig synthesis at any one time.

Most workers agree that B lymphocytes outnumber T and MPS cells in the gingival tissue of periodontitis (11–13). This could be clearly confirmed in the present study. The number of macrophages increased concomitantly with the degree of inflammation, which is in agreement with the previous findings (5, 13). The role of MPS cells in the gingiva is still incompletely understood. As the antigen-processing and -presenting cells, MPS cells conduct important functions in protective immunological reactions. On the other hand, they seem to contribute to tissue destruction by elaborating enzymes and prostaglandins (5). The observed relative increase of MPS cells in JP might be an indication of the latter type of activity in these gingival lesions. The possibility that MPS cells, by synthesizing prostaglandins, could activate T suppressor cells and thus depress the immune response (24) merits a further study of MPS cells in periodontitis lesions as well.

Table 4. T-lymphocyte subsets in gingival specimens expressed as T helper/T suppressor ($OKT4^+/OKT8^+$) ratio (mean ± SD)

Series	No.	$OKT4^+/OKT8^+$	Significance (comp. with contr.)
JP	9	3.2 ± 2.4	$p > 0.01$
JP_{relat}	10	3.4 ± 2.1	$p > 0.001$
Controls	19	0.8 ± 0.6	

The small number of ANAE-positive T cells present in the gingival tissue of the healthy subjects (both T helpers and T suppressors almost equally represented (Table 4): $OKT4^+/OKT8^+ = 0.8$) is an indication of a well-balanced regulation of the local humoral antibody response to the continuous stimuli of oral antigens. On the other hand, the increase of the relative proportions of ANAE-positive T cells in JP (and less so in JP_{relat}) might indicate the activation of the cell-mediated immune mechanisms including the release of lymphokines in such situations (13, 23). The dynamic nature of these reactions is further emphasized by the recent observations of a shift from T-cell dominance to B-cell dominance in stable and in progressive periodontitis, respectively (22).

A more detailed analysis of the immunoregulatory mechanisms in patients with JP is possible by enumerating the T-cell subsets and their relative proportions by means of monoclonal antibodies generally available (16, 17). This was accomplished in the present study by counting the $OKT4^+$ and $OKT8^+$ cells in two subsequent sections, a technique commonly used in such assessments. Owing to the small number of $OKT6^+$

cells (Langerhans cells) found in some specimens, further discussion about their role (auxiliary cells in immune response) is outside the scope of this communication. Despite the two-section technique used, the number of $OKT3^+$ cells (all T cells) quite closely paralleled that of $OKT4^+$ and $OKT8^+$ counted together. Both in JP and JP_{relat} series the ratio of T helper/T suppressor cells was significantly elevated when compared with that in controls (Table 4). In both series this elevation was due to an increased number of $OKT4^+$ cells. This elevated $OKT4^+/OKT8^+$ ratio both in the diseased individuals and in their apparently healthy relatives could indicate two things: either an increased demand of T helper activity for the B-cell response or a genetically (HLA) determined imbalance in immune regulation. Whether any evidence of the latter possibility is obtainable remains to be shown by our HLA analysis data for these patients. There is some evidence that also enhanced T suppressor activity could be detectable in more severe forms of periodontitis (24). Thus, activation of T suppressor cells by antigen–antibody complexes in the inflamed gingiva and dissemination of the latter into the systemic circulation may play an important role in the development of periodontitis as a whole (24). Circumstantial evidence along this line could provide the present finding of the minor differences in $OKT4^+/OKT8^+$ ratios between JP and JP_{relat} patients. In the JP series $OKT8^+$ (T suppressor) cells were also somewhat increased, leading to a slightly less elevated $OKT4^+/OKT8^+$ ratio than in the JP_{relat} series. Thus, the immunopathological basis for the morphological similarities emphasized between JP and adult periodontitis (8–10) could be the activation of T suppressor cells, genetically regulated in the former and acquired in the latter. Studies are in progress to elucidate the role of HLA antigens in the development of JP.

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