The composition of gingival inflammatory cell infiltrates in children studied by enzyme histochemistry


Abstract. Gingival biopsies were obtained from 23 children, aged 5-11 years (8.6±1.8 years). Specimens were taken from areas of the gingiva adjacent to the teeth which were to be extracted because of caries or its sequelae and which clinically had a gingival index score of at least 1. Staining for α-naphthyl acetate esterase with unspecific esterase at pH 5.8 (ANAE) permitted identification of T lymphocytes, monocytes/macrophages, plasma cells and non-reactive (ANAE-negative) cells. Cells which tentatively were identified as “natural killer” (NK) cells were also observed. Differential cell counting was performed for 10 specimens, selected on the basis of the presence of a well-defined inflammatory infiltrate, clear morphology throughout and good ANAE staining. Cell counts confirmed earlier studies showing that lymphocytes predominate in the inflammatory infiltrates in children’s gingivitis. T lymphocytes dominated particularly in the periphery of the most densely infiltrated areas. Relatively few plasma cells were seen. It was concluded that T lymphocytes dominate in the inflammatory infiltrate in children’s gingivitis.

Chronic inflammatory periodontal disease (CIPD) is not uncommon in children (Jamison 1960). Although it may clinically produce more dramatic superficial changes than commonly seen in adults, it usually develops more slowly and does not have the same tendency to produce irreversible destructive defects (Mattson 1978). Mackler & Crawford (1973) and Cox et al. (1974) suggested that in the preschool child, gingivitis would develop much more slowly than in the adult for a given plaque amount. These findings were confirmed by Mattson (1978). He compared the development of gingivitis in preschool children and young adults and found that the latter group had a higher propensity to develop gingivitis. It has been suggested (Longhurst et al. 1977) that the histological appearance of the tissue of a biopsy taken from a preschool child with gingivitis resembles an “early lesion” (Page & Schroeder 1976).

Established gingival inflammatory infiltrates in adults contain a large proportion of plasma cells (Brandtzaeg 1966, Lindhe et al. 1980). The proportion of plasma cells appears to increase with increasing severity of inflammation (Lindhe et al. 1980). Immunohistochemical studies indicate that there is a gradually increasing proportion of plasma cells producing IgG with increasing severity of the inflammatory changes (Brandtzaeg & Tolo 1977).

Longhurst et al. (1977, 1980) and Seymour et al. (1981) have presented studies based on morphological methods which may indicate that in children, the gingival inflammatory infiltrate contains a higher proportion of lymphocytes than usually seen in CIPD in adults. Seymour et al. (1979), on the basis of histochemical and immunofluorescence studies, have presented evidence suggesting that the change from a stable to a destructive lesion may involve a shift from a T cell to a B cell (plasma cell) dominated lesion. However, Gillett et al. (1986) held that small B lymphocytes dominate when the inflammatory lesion is small and quiescent, whilst on activation, the lesion increases in size and many B lymphocytes are transformed into plasma cells. This finding was refuted by Walsh et al. (1987) on the basis of an extensive study, using a panel of monoclonal antibodies and histochemical markers.

Thus, the gingival inflammatory reaction in children in some respects is similar to the reaction described in adults with gingival inflammation, but without marked or active destruction of supporting tissues (stable lesion) (Seymour et al. 1979). Other authors (Page & Schroeder 1982) have expressed doubts concerning the validity of the hypothesis of Seymour et al. (1979).

Several methods which may be helpful in characterizing the various cells in inflammatory infiltrates have become available. Receptors have been used as markers to study the inflammatory infiltrates in various diseases of the skin and other tissues (Bjerke 1982, Nyland & Nilsen 1982) including periodontal disease (Kristoffersen et al. 1985). Monoclonal antibodies have proved valuable in the study of gingival inflammatory infiltrates (Johannessen 1986, Seymour & Greenspan 1979, Seymour et
al. 1982, Okada et al. 1983, Walsh et al. 1987). This method has the advantage of a high degree of specificity. It requires very gentle handling of the tissues in the laboratory, preferably using frozen sections, and only 1 or 2 cell types can be marked with such specificity in the same section. Staining for α-naphthyl acetate esterase (ANAE) (Müller et al. 1975, Ranki & Häry 1979) has also proved to be valuable in differentiating between various cell types found in inflammatory infiltrates, and this method has been used to study cell types involved in periapical granulomas (Johannessen 1986, Johannessen et al. 1984) and in adult CIPD (Johannessen et al. 1990). The method has the advantages that several cell types can be marked with a certain specificity in the same section, fixed tissues can be used and tissue and cell morphology are well preserved.

The aim of this study was to characterize the cell populations in gingival inflammatory infiltrates in children, particularly with respect to lymphoid cell types, by means of well-established enzyme histochemical methods which allow for the identification of several cell types in the same section.

Material and Methods

Tissue

Biopsies were obtained from 23 patients, with a mean age of 8.6 years (SD = 1.8, range 5-11 years), undergoing extraction of primary or permanent teeth, and having a gingival index (GI), (Löe & Silness 1963) of at least score 1 adjacent to the tooth to be extracted. Cases of juvenile periodontitis were not included in this study. Informed consent from a guardian was obtained in each case.

Biopsies were taken under local anesthesia and prior to the extraction, using a disposable no. 15 scalpel, utilizing 2 vertical incisions and 1 horizontal incision at the level of the bottom of the periodontal pocket. For comparison, some biopsies were taken from patients undergoing dental extraction and having a GI score 0 at the site. In addition, specimens of human spleen and lymph node obtained at autopsy were used as controls.

Fixation, sectioning, staining

The biopsies were cut axially in several pieces in such a way that the junctional or pocket epithelium, the connective tissue and the oral gingival epithelium would be present in the same section.

The specimens were fixed overnight in a solution of 1% glutaraldehyde diluted in 0.1 M sodium cacodylate buffer, pH 6.3, with 1% sucrose (Bozdech & Bainton 1981) at 4°C. The fixed tissues were then kept in 0.1 M cacodylate buffer (pH 6.3) with 7% sucrose for 24 h, dehydrated with acetone, cleared in toluene and embedded in paraffin. Sections were cut at 3–4 μm, collected on gelatin-coated glass slides and deparaffinized in toluene.

Sections were stained for ANAE at pH 5.8 according to Müller et al. (1975). The sections were incubated in a moist chamber for 3 h at room temperature. After incubation, the sections were washed in phosphate-buffered saline pH 7.2 (PBS) for 20 min, dipped in distilled water, counterstained for 10–15 s with methyl green, washed in PBS, dehydrated in ethanol and cleared in toluene. The sections were then mounted in Depex® mounting medium (Difco Laboratories, England) and examined by transmitted light.

Sections from all specimens taken at various levels during sectioning were stained with hematoxylin, eosin and safranin (HES) for the purpose of orientation.

Differential cell counts

Differential cell counts were performed on 1 or more sections from each of 10 biopsy specimens stained for ANAE. The sections were selected on the basis of the presence of a well-defined inflammatory infiltrate, clear morphology and good quality of the ANAE staining throughout. Sources of specimens selected for counting are given in Table 2. The mean age of these patients was 9.0 years, range 5.0–11.0 years. Non-inflammatory cells such as fibroblasts, fibrocytes and endothelial cells were not included. Counting was done “blind” and was performed at a magnification of 400 x . The microscope was connected to a digitizer (Bit Pad One TM, Summographics, Connecticut, USA) which was in turn connected to a computer (Commodore CBM, Model 3032, Santa Clara, CA, USA). The computer was programmed (Medizinisch Technische Apparate, Tübingen, FRG) to record the number of ANAE positive T lymphocytes, plasma cells and monocytes/macrophages as well as ANAE negative cells. Results are reported as mean %s of each cell type for the respective areas.

The counting was performed separately in 2 different areas of the section (Fig. 1):

ICT the inflammatory infiltrate subjacent to the junctional or pocket epithelium

SOGE connective tissue immediately subjacent to the oral gingival epithelium

The various cell types were identified according to the criteria given in Table I and all cells within each specified area were counted.

Statistical methods

Statistical significance of differences between the ICT and the SOGE in the % distribution of various cell types was evaluated by the appropriate t-test or by analysis of variance, using the SPSS statistical package (SPSS Inc., Chicago, III.).
Table 1. Characterization of mononuclear cells according to their ANAE staining pattern

<table>
<thead>
<tr>
<th>Cell types</th>
<th>ANAE staining patterns</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T lymphocytes</td>
<td>one or a few discrete cytoplasmic spots</td>
<td>Müller et al. (1975), Ranki et al. (1976), Knowles &amp; Holck (1978)</td>
</tr>
<tr>
<td>monocytes/macrophages</td>
<td>medium to strong diffuse cytoplasmic staining</td>
<td>Ranki &amp; Häyri (1979), Kulenkampff et al. (1977)</td>
</tr>
<tr>
<td>plasma cells</td>
<td>multiple cytoplasmic granules or diffuse cytoplasmic staining with spearing of a perinuclear area</td>
<td>Hermansky et al. (1980), Müller et al. (1981)</td>
</tr>
<tr>
<td>large granular lymphocytes (NK cells)</td>
<td>multiple cytoplasmic granules</td>
<td>Timonen et al. (1979), Huhn et al. (1982)</td>
</tr>
</tbody>
</table>

Results

The areas to be differentially counted (Fig. 1) and the various types of cells (Fig. 2) were readily distinguished. T lymphocytes, containing 1 to 3 reddish dots, were readily recognized (Fig. 3), and so were monocytes/macrophages (Fig. 3). The latter showed considerable variability both with respect to size and morphology and to staining intensity. Plasma cells showed variable intensity of staining. Scattered cells with the morphology and ANAE staining pattern characteristic of NK cells were occasionally observed along the periphery of the ICT (Fig. 5).

In some discrete regions immediately subjacent to the junctional or pocket epithelium, T lymphocytes were particularly numerous, and these areas also contained many cells identified as macrophages or monocytes.

T lymphocytes were also frequently seen within the pocket epithelium (Fig. 4). However, the inflammatory infiltrate in most regions contained many cells which did not give a staining reaction with the ANAE reagents. Such cells appeared to be somewhat more numerous along the periphery of the ICT. In this area, plasma cells, although few in number, were more frequently encountered.

In the connective tissue subjacent to the oral gingival epithelium (SOGE), the scattered inflammatory cell infiltrate exhibited a predominance of macrophages or monocytes (Table 3). These cells were most often relatively small and round, lacking dendritic projections. Cells of similar appearance were also seen within the oral gingival epithelium. Clusters of T lymphocytes also occurred in this area, but both T lymphocytes and ANAE-negative cells were proportionally fewer than in the ICT.

The density of inflammatory cells (number of cells per microscopic field) was approximately twice as high in the

Fig. 2. ANAE positive cells in the infiltrated connective tissue subjacent to the pocket epithelium. The infiltrate is predominated by T lymphocytes with 1 or a few dots (arrows) (×800).

Fig. 3. T lymphocytes (open arrows) and monocytes/macrophages (arrow) in the infiltrated connective tissue subjacent to the pocket epithelium (×1120).
central portion of the ICT compared to the peripheral portion and the SOGE. Different microscopic fields in the various areas often exhibited remarkably similar distribution of the various types of inflammatory cells, and repeated blind countings of related areas showed no significant variability of results.

In Table 3, the mean %s of the 4 types of cells in the different areas are presented. Statistical significance of differences in proportions of the various cell types between the 2 main areas are also noted.

Discussion

The ANAE staining reaction together with counterstaining and good cellular morphology permitted evaluation of the localization and distribution of the various cell types. Some cells which were slightly larger than lymphocytes but which exhibited a granular ANAE staining pattern of their cytoplasm or had more than 3 ANAE reactive dots, could possibly represent NK cells. Such cells are difficult to identify with certainty on the basis of morphology and staining reaction and were not included during counting. Thus, some cells classified as plasma cells or T lymphocytes may have been NK cells. Preliminary studies in our laboratory using monoclonal antibody to NK cells indicate that they are present as scattered cells in gingival inflammatory infiltrates.

Only cells with the characteristic morphology and a positive ANAE staining reaction were counted as plasma cells. The number of plasma cells recorded may therefore be somewhat low, since it is known that some plasma cells do not give positive ANAE staining (Johannessen, Nilsen & Skaug 1984).

The majority of inflammatory cells counted as "ANAE negative cells" exhibited lymphocyte morphology and probably represent B lymphocytes. However, some of these ANAE negative cells may represent plasma cells. A proportion of the ANAE negative cells may actually have been polymorphonuclear leukocytes. In their study of lymphoid cell subpopulations in gingivitis in children, Seymour et al. (1981) reported that less than 1% of the infiltrating cells were monocytes, polymorphonuclear leukocytes or plasma cells. Their estimate appears to be in reasonably good agreement with the findings in the present study.

Our findings using enzyme histochemical markers have corroborated and extended the observations of Longhurst et al. (1977). Using methyl green-pyronin stain on sections of inflamed gingiva from adults and children, they found that small and medium-sized lymphocytes predominated in the latter group. Our findings are, however, at some variance with the observations of Longhurst et al. (1980). On the basis of electron microscopic observations which demonstrated a relative lack of transforming T lymphocytes

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Area of biopsy</th>
<th>GI</th>
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<td>M</td>
<td>74 – buccal</td>
<td>2</td>
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<td>2</td>
<td>11</td>
<td>M</td>
<td>53 – buccal</td>
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<td>3</td>
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<td>4</td>
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<td>F</td>
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</tr>
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<td>11</td>
<td>F</td>
<td>55 – buccal</td>
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</tr>
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<td>6</td>
<td>8</td>
<td>F</td>
<td>85 – buccal</td>
<td>1</td>
</tr>
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<td>M</td>
<td>46 – buccal</td>
<td>2</td>
</tr>
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<td>8</td>
<td>5</td>
<td>F</td>
<td>75 – buccal</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>F</td>
<td>54 – lingual</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>M</td>
<td>74, 75 – buccal</td>
<td>2</td>
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</table>
and a rather high proportion of plasma cells, they compared the inflammatory lesion of children's gingivitis with a stage between the early and established lesion as seen in adults (Page & Schroeder 1976). Our findings are also apparently in conflict with the recent report by Gillett et al. (1986), which indicated that the lymphocytes associated with gingivitis in children are primarily B lymphocytes. They based their findings on the use of a monoclonal anti-HLADr antibody. This antibody would mark both (certain) T and (certain) B lymphocytes and their results would consequently not be directly comparable with ours.

To a large extent, our observations corroborate the findings of Seymour et al. (1981) and Walsh et al. (1987) with respect to the composition of the lymphoid cell population in gingivitis in children. Seymour et al. (1981) also found that lymphocytes predominated in these lesions and estimated that approximately 70% of these lymphocytes were T cells as evidenced by their non-specific esterase activity. Using a monoclonal antibody reacting with mature T cells and some thymocytes on sections from 2 individuals, Seymour et al. (1982) found that 75–80% of the lymphocytes population were T cells. Walsh et al. (1987) using a panel of anti-T-cell antibodies on gingival sections from 10 children found that the gingival inflammatory lesions consisted largely (80–88%) of T lymphocytes. In our study, the proportion of lymphocytes recorded as T cells was somewhat lower, and ANAE negative cells were actually equally numerous in the most densely infiltrated area. However, these ANAE negative cells also include a few polymorphonuclear cells, plasma cells and possibly mast cells. Our observations that plasma cells constituted a very small proportion of the inflammatory infiltrate in the inflamed gingiva of the children studied, are also in accordance with the observations of Seymour et al. (1981, 1982) and Walsh et al. (1987).

Although direct comparisons can not be made, we got the impression that the variability from section to section and from biopsy to biopsy was less pronounced in children than in adult CIPD (Johannessen et al. 1986, 1990). Further studies of gingival inflammatory infiltrates in children are called for. Such studies should aim to elucidate both their cellular composition with more specific markers and their variability from individual to individual and from section to section in the same specimen.

Zusammenfassung

Die Zusammensetzung des gingivalen entzündlichen Zellinfiltrats bei Kindern, studiert mittels Enzymhistochemie


Résumé

Composition des infiltrats de cellules inflammatoires dans la gencive chez les enfants – étude histochnomique enzymatique

Des biopsies gingivales ont été prélevées chez 23 enfants âgés de 5-11 ans (moyenne 8.6, écart-type 1.8 ans). Les spécimens ont été prélevés dans des zones où la gencive atteignait à des dents devant être extraites pour cause de carie ou de complications de la carie, et où les scores de l'indice gingival étaient ≥ 1. Une coloration pour α-naphtyl acétate esterase avec esterase non spécifique à pH 5.8 (ANAE) a permis d'identifier des lymphocytes T, des monocyes/macrophages, des plasmocytes et des cellules sans reaction (ANAE-négatif). On observait aussi des cellules qu'on classait comme cellules NK dans une tentative d'identification. Des numérations différentielles ont été pratiquées pour 10 spécimens, choisis parce qu'ils présentaient un infiltrat inflammatoire bien défini, une morphologie nette dans tout le spécimen, et qu'ils présentaient bien la coloration ANAE. Les résultats des numé-

Table 3. Mean %s of various cell types in the gingival (ICT) and in the area underlying the oral gingival epithelium (SOGE) for the 10 patients for whom differential countings were performed; total mean (± standard deviation = SD) and ranges

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>ICT M/M</th>
<th>T cells</th>
<th>Pl. cells</th>
<th>neg. cells</th>
<th>SOGE M/M</th>
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<td>38.0–64.8</td>
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<td>21.9–39.1</td>
<td>0.2–5.7</td>
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</tbody>
</table>

M/M = monocytes, macrophages; T cells = T lymphocytes; Pl. cells = plasma cells; neg. cells = ANAE negative cells, mainly B lymphocytes.

* Significant difference (P < 0.05) between ICT and SOGE.
rations montraient que, dans la gingivite de l'enfant, les lymphocytes pré dominaient dans les infiltrats inflammatoires. Les lymphocytes T dominaient particulièrement à la périphérie des zones présentant les infiltrats les plus denses. On observait relativement peu de plasmaocytes. En conclusion, l'infiltrat inflammatoire dans la gingivite de l'enfant est dominé par les lymphocytes T.

References


Brandtzaeg, P. & Tolo, K. (1977) In situ characterization of α-naphthyl acetate esterase. Laboratory Investigation 39, 70-76.


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