Subpopulations of lymphocytes in connective tissue from adolescents with periodontal disease

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Mononuclear cell populations were studied in gingival biopsy specimens from adolescents (n = 10) with at least one periodontal pocket with an increased probing depth (> 5 mm). The marginal bone loss was measured on radiographs, subgingival plaque samples were collected from the lesions, and the microbial flora was identified. Specimens from gingivitis lesions (n = 5) were used as controls. The mononuclear cell populations in the specimens were detected by using monoclonal antibodies defining functional T-lymphocyte subpopulations, B lymphocytes, and monocytes. All gingival specimens from patients with increased probing depth showed large lymphocyte infiltrates, most of which were CD 3-antigen-positive cells (T lymphocytes). Few (2%) infiltrating T cells expressed receptors for interleukin-2. B cells were detected in most specimens from the periodontitis group and varied from less than 1% to 21%. This study indicates that lesions in adolescents with early signs of periodontitis are characterized predominantly by T-cell lesions with relatively few cell aggregates of B cells present. □ *Biopsies; gingivitis; microbiology; monoclonal antibodies; mononuclear cells*

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The most prevalent form of periodontal disease in adolescents is characterized by minimal loss of connective tissue attachment and alveolar bone loss (1). Recently, Aass et al. (2) found that radiographic alveolar bone loss was present in 4.5% of 14-year-old children. However, epidemiologic studies from different countries show great variation in frequency (3–6).

It has been observed that an increase in pocket depth and evidence of alveolar bone loss coincide with a shift in the subgingival microflora, with *Bacteroides gingivalis, Bacteroides intermedius*, and other anaerobic motile rods and spirochetes as the dominant cultured organisms (7, 8). Immunologic mechanisms have been implicated in the pathogenesis of chronic inflammatory periodontal disease. Previous studies (9–11) have shown that gingivitis in children is essentially a T-cell lesion; that is, most of the lymphocytes present in the tissue are T cells. In contrast, Page & Schroeder (12) found that plasma cells and B lymphocytes are predominant in established lesions of gingivitis. The conversion from a stable lesion such as gingivitis to a progressive lesion with alveolar bone loss may involve a shift from a predominantly T-cell infiltrate to a B-cell infiltrate (13). However, according to Schroeder (14), the T- to B-cell conversion is related to the formation of a gingival pocket lined by epithelium rather than to destruction of alveolar bone.

There is still controversy about the relative contribution of humoral and cellular immune reactions in the development of the inflammatory lesion. The aim of the present investigation was to characterize subpopulations of mononuclear cells in gingival biopsy specimens from teenagers with early signs of periodontal disease.

Table 1. Clinical data of the patients

Subject	Sex	Age, years	GBI, %	VPI, %	Pocket depth ≥5 mm, no. of sites	Marginal bone loss, no. of sites
Periodontitis group						
1	F	19	74	60	7	0
2	M	19	100	100	16	7
3	F	19	60	74	9	4
4	M	13	98	94	2	0
5	M	13	100	100	5	0
6	F	12	89	49	1	0
7	M	17	96	94	10	0
8	M	15	75	81	5	0
9	M	14	100	94	4	0
10	F	13	46	69	1	1
Gingivitis group						
11	M	18	27	62	0	0
12	M	12	6	23	0	0
13	M	15	13	60	0	0
14	M	5	7	13	0	0
15	M	9	19	52	0	0

Materials and methods

Patients

The material consisted of 15 patients 5–19 years old (Table 1). All patients were immigrants, mainly from Turkey. The periodontitis group, which had early signs of periodontal disease, consisted of 10 patients with at least one periodontal pocket with an increased probing depth (≥ 5 mm) and subgingival calculus present. The patients had been referred to the Department of Pedodontics, Karolinska Institute, for treatment. The control group comprised five patients who all showed bleeding on probing at a probing depth not exceeding 3 mm and no signs of marginal bone loss. The patients did not have any other systemic disease and had no history of any previous periodontal treatment. The patients had not received any antibiotic treatment for the 12 months before examination.

Clinical examination

The clinical examination included the Visible Plaque Index (VPI) and Gingival Bleeding Index (GBI) in accordance with Axelsson & Lindhe (15). The presence of dental plaque was detected with a disclosing agent on four surfaces of each tooth. The VPI was expressed as the number of surfaces showing plaque divided by the total number of surfaces available. The frequency of surfaces with gingivitis for each individual was based on bleeding on probing at six sites of the tooth. The probing depth was determined to the nearest millimeter with a graded periodontal probe. Microbial analyses were performed in all sites where the periodontal pockets exceeded 5 mm on probing.

Bitewing radiographs were taken by a standardized technique. A distance exceeding 2 mm from the cementoenamel junction (CEJ) to the alveolar bone crest (AC) was recorded as bone loss in accordance with Hansen et al. (6). The measurements were made on premolars, molars, and incisors where the CEJ and AC could be accurately identified. The radiographs were magnified 10 times to make the determination easier. Subgingival calculus was also recorded on the radiographs.

Microbial analysis

Subgingival samples of plaque were collected with the use of paper points from

the periodontal pockets with an increased probing depth (≥ 5 mm). The transport medium described by Möller (16) was used. The presence of *B. gingivalis*, *B. intermedius* (17), and *Actinobacillus actinomycetemcomitans* (18) was determined in the samples at the Department of Oral Microbiology, Karolinska Institutet, Stockholm, Sweden.

Biopsies

Gingival biopsy specimens were taken from all patients. The specimens consisted of the buccal interdental papilla. In patients with gingivitis, evidenced by gingival bleeding on probing in gingival sulcus, the specimens were taken in connection with surgical-orthodontic treatment or surgical extraction of teeth. Specimens from patients in the periodontitis group were taken from one site showing an increased probing depth (≥ 5 mm) from each patient (Table 1). All specimens were excised with the patient under local anesthesia with 2% lidocaine adrenaline, frozen on solid carbon dioxide, and kept at −70°C until further processing. The specimens were then sectioned serially (6 µm) on a cryostat.

Antibodies

The monoclonal antibodies used were anti-leu 4 (CD 3), 5 µg/ml, and anti-leu 3 (CD 4), 6.7 µg/ml (both from Becton-Dickinson, Oxnard, Calif., USA), positive for T helper cells; OKT 8 (CD 8), 1 µg/ml (Ortho Diagnostic System, Raritan, N.J., USA), positive for T suppressor/cytotoxic cells; OKI a 1, 2 µg/ml (Ortho), anti-human HLA-DR framework; OKM 1 (CD 15) (Ortho), positive for macrophages; anti-B1 (CD 20) (Coulter Electronics, Hialeah, Fla., USA), defining B lymphocytes; and anti-interleukin-2 receptor (CD 25) (Becton-Dickinson). The specificity of the monoclonal antibodies was examined with sections of lymph nodes and T- and B-cell-enriched fractions of splenic lymphocytes. Plasma cells were detected using fluorescein isothiocyanate (FITC)-conjugated antisera against IgG, IgM, and IgA obtained from Dakopatts (Copenhagen, Denmark), all with a fluorescein/protein (F/P) molar ratio of 2.3. Monoclonal bone marrow cells constituted positive controls for specificity of these sera.

Biotin-avidin peroxidase staining

After fixation in acetone for 5 min at 4°C, the cryostat sections were rinsed in room temperature in 9.3 mM phosphate-buffered saline (PBS), pH 7.4, for 10 min and oxidized by 0.9 mM H_2O_2 in water for 5 min and subsequently washed in the same buffer for 10 min. The sections were then incubated with various monoclonal antibodies for 30 min. The appropriate concentration was predetermined on additional gingival sections after serial dilutions in PBS supplemented with 2% bovine serum albumin. After being rinsed in PBS, the sections were exposed to affinity-purified biotinylated anti-mouse IgG (25 µg/ml) (Vector Laboratories, Burlingame, Calif., USA) for 30 min, rinsed three times for 7 min in PBS, incubated with a complex of biotinylated peroxidase and avidin DH (17 µg/ml) (Vector Laboratories) for 60 min, and again rinsed in PBS. Finally, the sections were incubated with the peroxidase substrate (3-amino-9-ethylcarbazole) for 5 min, rinsed in PBS, and counterstained with hematoxylin before being mounted in glycerin-gelatin. Control sections were prepared and run in parallel, excluding the monoclonal antibody or exchanging it with normal mouse serum or ascites fluid. In the biopsy specimens belonging to the periodontitis group the percentage of mononuclear cells stained was estimated under a ×40 objective in a Leitz Diavert microscope by counting the total population of mononuclear cells showing membrane staining (> 500 cells/monoclonal antibody type) on two sections for each type representing the area of greatest density of inflammatory cell infiltration. In all diseased specimens this area was subjacent to the pocket and/or junctional epithelia. Consecutive sections were chosen for staining and counting of cells reacting with different monoclonal antibodies. The degree of cell infiltration was classified as scattered cells, scattered cells and aggregates containing less than 25 cells,

moderate aggregates (25–200 cells), and large aggregates containing more than 200 cells.

Results

The clinical data of the patients is shown in Table 1. All patients in the periodontitis group (n = 10) showed loss of attachment, clinically registered by an increased probing depth (≥ 5 mm), and subgingival calculus, which was evident on radiographs. The frequency of gingival units with bleeding varied from 46% to 100% among the subjects. The number of units with an increased probing depth (≥ 5 mm) varied from 1 to 16 (Table 1). Marginal bone loss, characterized by a distance exceeding 2 mm from the CEJ to the alveolar bone crest, was seen in 3 of 10 patients. In one patient marginal bone loss was diagnosed at seven sites. In the gingivitis group the GBI mean value varied from 6% to 27%.

Biopsies

The clinical condition at the biopsy site is shown in Table 2. In the periodontitis group the probing depth in the gingival pockets at the site of the biopsy varied from 5 mm to 8 mm. Seven of 10 subgingival plaque samples were positive for *B. intermedius* and 2 for *B. gingivalis*. In 3 of 10 individuals *A. actinomycetemcomitans* was present (Table 2). In the gingivitis group one patient had *B. intermedius* in the subgingival flora, and one patient had *A. actinomycetemcomitans* at the site of biopsy.

Mononuclear cells

Gingival biopsy specimens from patients with gingivitis contained no mononuclear infiltrates; only scattered CD 3-positive cells were found adjacent to the junctional epithelium. In two patients small infiltrates containing less than 25 cells were found. Most of these cells (95%) were CD 3 antigen-bearing. The CD 4/CD 8 ratio was 2.1 and 2.6, respectively. No plasma cells or B1 antigen-bearing cells were detected in the specimens.

All gingival biopsy specimens from the periodontitis group showed large lymphocyte infiltrates, most of which were CD 3-positive (78–96%) (Table 3). Most of the infiltrating cells were HLA-DR-positive.

Table 2. Clinical data at the site of biopsy

Subject	Examined site	Marginal bone–CEJ (mm)	Probing depth (mm)	Bacterial composition		
				B. gingivalis	*B. intermedius*	*A. actinomycetemcomitans*
Periodontitis group						
1	26 m	2	6	–	–	–
2	16 d	2.5	8	–	+	–
3	36 d	3.7	6	–	+	–
4	46 m	1.8	5	–	+	–
5	35 m	1.5	8	–	–	–
6	26 m	1.9	5	–	+	–
7	26 m	1.7	6	+	+	–
8	26 m	1.3	5	–	+	+
9	16 d	1.9	5	–	+	+
10	45 d	2.9	5	+	–	+
Gingivitis group						
11	45 d		2	–	–	+
12	34 m		2	–	–	–
13	45 b		2	–	–	–
14	85 m		2	–	+	–
15	84 b		1	–	–	–

Table 3. Subpopulations of mononuclear cells in the biopsies from children with early signs of periodontitis

Subject no.	Size of cell infiltrate*	Reactivity with monoclonal antibodies (% of all mononuclear cells)								
							Plasma cells			
		CD3	CD 4/ CD 8	CD 25	CD 15	HLADr	CD20	IgM⁺	IgA⁺	IgG⁺
1	+++	87 (76–95)	3.6	<1	5 (2–9)	97 (92–98)	5 (4–7)	2 (0–5)	3 (0–5)	0
2	++	78 (70–89)	3.2	<1	6 (2–11)	85 (77–93)	<1	0	0	0
3	+++	77 (71–86)	2.5	2	4 (2–7)	91 (76–92)	21 (0–43)	4 (0–6)	6 (0–10)	0
4	+++	82 (65–91)	0.3	<1	7 (3–11)	84 (70–96)	4 (0–12)	2 (1–3)	3 (1–5)	0
5	++	92 (81–95)	1.7	<1	2 (1–5)	80 (75–91)	1 (0–2)	0	0	0
6	+++	86 (72–88)	4.5	<1	15 (10–20)	74 (60–90)	2 5–10	3 (1–5)	7 (5–10)	0
7	+++	96 (90–99)	9.2	<1	<1	84 (76–90)	1 (0–3)	<1	<1	<1
8	+++	91 (78–96)	8.5	<1	<1	50 (38–67)	<1	<1	<1	<1
9	+++	93 (81–97)	3.6	<1	3 (2–5)	85 (79–93)	5 (0–14)	<1	<1	<1
10	++	92 (81–95)	2.5	<1	<1	76 (52–91)	<1	<1	<1	<1

* + = scattered cells and aggregate (<25 cells); ++ = aggregate containing 25–200 cells; and +++ = aggregate containing >200 cells.

The ratio of T lymphocytes with so-called helper and suppressor/cytotoxic phenotype CD 4+/CD 8+ varied between 1.7 and 9.2, with a mean value of 4.0. In all biopsy specimens few T cells (< 2%) expressed receptors for IL-2, although they appeared to be activated, as indicated by HLA-DR expression. CD 20-positive cells could be detected in the tissue from most patients, varying from less than 1% to 21%. The infiltrating CD 20-positive cells were generally found as dense aggregates surrounded by CD 3 antigen-bearing cells.

In four specimens occasional plasma cells were identified in the connective tissue, mostly IgM- and IgA-positive cells. No IgG plasma cells were found in specimens containing large inflammatory cell infiltrates, and there was no clear correlation between the presence of plasma cells in the specimens and the extent of alveolar bone loss at the same site.

No correlation existed between the microbiological flora in the subgingival plaque sample taken from the periodontal pockets and the immunopathological status of the biopsy.

Discussion

The results of the present investigation show that gingival biopsy specimens from adolescents with early signs of periodontitis are characterized predominantly by T-cell infiltration, with few B cells present in the area representing the greatest density of inflammatory cell infiltration. The infiltrating B cells were present in aggregates surrounded by T cells, similar to the distribution of T and B cells in lymphoid tissue. The lymphocytes did not appear to be proliferating locally, since few T cells (<2%) expressed receptors for interleukin-2. However, most of the infiltrating T cells were activated, since they expressed DR antigen. It is relevant in

this context that in vitro findings suggest that expression of HLA-DR and IL-2 receptors is dissociable, but such a dissociable expression is unlikely to exist in patients in vivo unless the infiltrating lymphocytes were synchronized to the same phase of the growth cycle (20).

The gingival specimens were taken from adolescents showing early signs of periodontitis characterized by an apical proliferation of the junctional epithelium, resulting in a periodontal pocket. In three of the patients marginal bone loss was diagnosed from radiographs when the distance from the CEJ to the alveolar crest exceeded 2 mm (6). The lesions studied represent slowly progressing periodontitis, first seen in the interdental areas of posterior teeth, according to Löe & Morrison (19). Rapidly progressing periodontitis, normally termed juvenile periodontitis, is another form of periodontal disease seen in adolescents. However, there is some controversy concerning the nomenclature and classification of the periodontal diseases in children. All subjects studied showed subgingival calculus, indicating a transition from a supragingival to a subgingival infection and representing a critical change in the disease process.

The subgingival flora in our patients consisted mainly of an anaerobic bacteria, with *B. intermedius* as the dominant form seen in 7 of 10 individuals. In three patients *A. actinomycetemcomitans* was detected. This bacterium has been suggested to be a causative agent in the rapidly progressing form of periodontitis known as juvenile periodontitis. Recently, however, Asikainen et al. (21) reported that *A. actinomycetemcomitans* could also be found in adolescents with no evidence of alveolar bone loss. Furthermore, several reports have shown serum antibodies against *A. actinomycetemcomitans* in patients with gingivitis but also in patients with healthy periodontium (22, 23). In our material two of three patients with *A. actinomycetemcomitans* in the subgingival sample did not show any marginal bone loss radiographically. The patient with *A. actinomycetemcomitans* in the subgingival plaque sample showed a moderate marginal bone loss and was not characterized as having juvenile periodontitis with regard to criteria described by Baer (24) and used by Saxén (25).

Monoclonal antibodies were used to identify mononuclear cells in gingival tissue biopsy specimens from adolescents with early signs of periodontitis. These lesions were characterized predominantly by T cells with aggregates of B cells present in the tissue. These results are in contrast to those of Page & Schroeder (26), who showed that the established lesion was characterized by a predominance of plasma cells and B lymphocytes, probably in conjunction with creation of a small gingival pocket without significant marginal bone loss. Our results differ, since very few plasma cells could be detected in the tissue. The failure to detect plasma cells was not caused by insufficient antisera, since positive controls for their reactivity were run in parallel. However, in agreement with Page & Schroeder (26), we found that B cells could be detected in most lesions from adolescents with early signs of periodontitis. The lesions with early signs of periodontitis did not seem to be controlled as in gingivitis, since the ratio between so-called T helper and T suppressor/cytotoxic lymphocytes was increased. This increase has previously been shown in lesions of juvenile periodontitis (27) and chronic periodontitis (28).

The results of our study do not indicate any significant change in lymphoid cell proportion in the periodontal lesions with or without marginal bone loss at the site where the biopsy was taken. This finding supports Schroeder's suggestion (14) that conversion of a stable established lesion to destructive progressive periodontitis is accompanied by an acute inflammation rather than by a change in lymphoid cell proportion in the tissue.

In conclusion, this study indicates that lesions in adolescents with early signs of periodontitis are characterized predominantly by T cells with relatively few aggregates of B cells present in the tissue.

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