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Experimental Gingivitis in a Down's Syndrome Child and Sibling

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Biopsies were taken from a child with Down's syndrome and her sibling under experimental gingivitis conditions and analyzed with monoclonal antibodies. At first, plaque caused a shift from a predominance of T cells to a predominance of B cells. Later, a gradual increase of T cells and a looser arrangement of B cells were observed.

Experimental gingivitis studies have shown that after cessation of oral hygiene for 21 to 28 days, the clinical signs of inflammation are controlled and a new steady state is established (2, 12). This control mechanism is clearly manifest in the deciduous dentition of normal children (5, 9). Also the gingival lesion related to permanent dentition has been found to be controlled (G. J. Seymour, L. J. Walsh, and E. Gemmel, Abstract, J. Dent. Res., **65**:983, 1986). In the present study, we investigated the cellular composition of the gingival lesion related to deciduous dentition in a Down's syndrome (DS) child (11-year-old female) and her sibling (9.5-year-old female) under experimental gingivitis conditions. The study was performed with the informed consent of parents and children, in agreement with the report of the Landelijk Werkverband (1).

During the 4 weeks before the experimental phase, the teeth of both children were professionally cleaned every day. Upon initiation of the experimental phase, all oral hygiene procedures were discontinued for 35 days. The amount of plaque (assessed with the Plaque Index [11]) and the degree of gingival inflammation (assessed with the Gingival Index [3]) were recorded immediately before oral hygiene was interrupted (day 0) and then on days 6, 13, 20, 27, and 35. On day 0, the percentage of plaque sites was 0 for both children, and the percentages of gingivitis sites were 4.2 for the DS child and 0 for the sibling. From days 0 to 35, these values gradually increased. No difference in plaque score arose between the children. On day 35, the percentage of plaque sites was 93 for both. The percentage of gingivitis sites was 93.8 for the DS child and 56.3 for the sibling. The values given are the mean percentages of plaque sites (scores 2 and 3 versus scores 0 and 1) and gingivitis sites (scores 1, 2, and 3 versus score 0) of the total number of surfaces scored (9).

On days 0, 6, 13, 20, and 35, gingival biopsies were taken, as described by Payne et al. (8), from areas near different elements (cuspids and molars) in the deciduous part of the dentition, minimizing the possible influence of stress caused by the biopsies. All samples were oriented on filters with OCT compound (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.), subsequently snap frozen in liquid nitrogen, and stored at −70°C until they were analyzed. From each biopsy, 12 serial sections of 5-μm-thick frozen tissue were cut and air dried with a ventilator for 10 to 15 min before use. The sections were fixed in acetone for 10 min at room temperature. After washing with phosphate-buffered saline, the 12 tissue sections of every biopsy were incubated for 30 min with 25 μl each of one of the following monoclonal antibodies: Leu 1 (peripheral mature T cells and a subpopulation of B cells; Becton Dickinson & Co., Paramus, N.J.); Leu 5 (E rosette receptor cells; Becton Dickinson); Leu 3a (helper-inducer cells; Becton Dickinson); WT32 (mature T lymphocytes; Institute for Public Health, Bilthoven, The Netherlands); B-pan (pan B cells; MCA Development, Groningen, The Netherlands); T8 (cytotoxic-suppressor cells; MCA); B_1 (pan B cells; a gift of Lee Nadler, Dana-Farber Cancer Institute, Boston, Mass.); D, M, A, G (heavy-chain immunoglobulins D, M, A, and G; L. Nadler); and OKIa (activated T cells, B cells, and macrophages; Ortho Diagnostics, Inc., Raritan, N.J.). Subsequently, the slides were washed in phosphate-buffered saline for 5 min. As a second step, we used peroxidase-conjugated rabbit anti-mouse antibodies (P 161; Dakopatts). After another 5-min wash in phosphate-buffered saline, peroxidase activity was demonstrated with 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, Mo.). Nuclear counterstaining was obtained with Mayer's Hemalum.

Cell counts of positive cells were performed on the slides obtained. The results are given in Table 1. Since the size of the infiltrate in the biopsies showed some variation, the

TABLE 1. Monoclonal antibody-identified cells in gingival biopsies of a DS child and her sibling

Child and cell type[a]	% of cells on day(s):			
	Zero	6	14	20 and 35
DS				
T	59	10	20	38
B	41	90	80	62
Th	45	65	ND[b]	53
Ts	55	35	ND	47
Sibling				
T	63	20	31	40
B	47	80	69	60
Th	46	56	70	46
Ts	54	44	30	54

[a] Abbreviations: T, T lymphocytes; B, B lymphocytes; Th, T helper-inducer cells; Ts, T suppressor-cytotoxic cells.
[b] ND, Not done.

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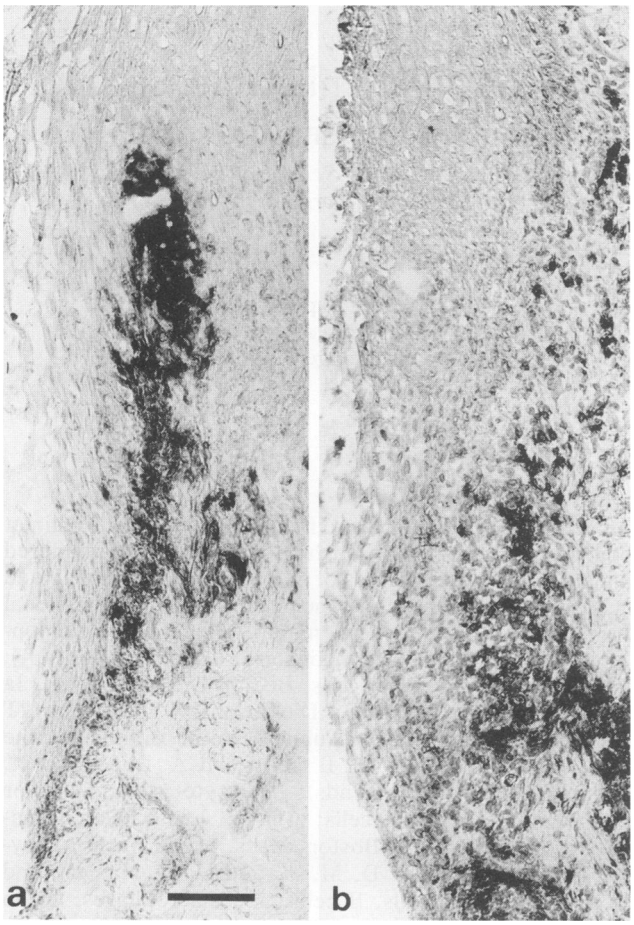

FIG. 1. Illustration of the B-cell density (monoclonal antibody B-pan identified) in the gingival tissue area behind the margin of the JE (magnification, ×115). The scale mark in the figure is 100 μm. In panel a, the situation on day 6 is shown; in panel b, the looser arrangement of cells on day 20 is seen.

results are represented as percentages rather than absolute values. In none of the biopsies were any immunoglobulin-producing cells found. In both children, an increase in the percentage of B cells over that of T cells was seen from days 0 to 6, but after day 14 a reduction was observed. The B cells were located directly behind the junctional epithelium (JE) (Fig. 1a). On day 20, the B cells in this area had become arranged more loosely (Fig. 1b). On both days 0 and 20, the percentage of cells with helper-inducer and suppressor-cytotoxic phenotypes were in the same range in the two children. In the DS child, an increase in the percentage of helper-inducer cells was noted on day 6. In the sibling, there was no such increase before day 14. On day 0, the helper-inducer cells were mainly located parallel to the JE. Later on, these cells were also located in the marginal part of and directly behind the JE. The JE of both children showed suppressor-cytotoxic cells on all days. Even on day 0, there was positive staining of human leukocyte antigen class II cells ($OKIa^+$) located directly behind the JE and at some distance parallel to the JE. On day 6, an increase in these cells was observed. In the sibling, there were relatively fewer human leukocyte antigen class II^+ cells on day 20 than on days 6 and 14. However, in the DS child these cells retained the density they had had on day 6. At all days after counting of B cells, an abundance of human leukocyte antigen class II^+ cells remained. The helper-inducer and suppressor-cytotoxic cells described above were part of this activated-cell area.

It was found that, under identical environmental conditions and with the same amount of plaque, the DS child developed more extensive gingival inflammation than did her sibling. The development of inflammation showed a pattern described earlier (9). The infiltrate in the gingival tissues of the children was a lymphocyte-dominated lesion with few plasma cells. This agrees with previous studies (4, 10). On day 0, the lesion was dominated by T cells. Helper-inducer and suppressor-cytotoxic cells were located at some distance behind the JE. On days 6 and 14, however, excessive migration of B cells was found located directly behind the JE (Fig. 1a). There was a shift from a predominance of T cells to a predominance of B cells, apparently caused by accumulation of plaque. Such a migration of B cells has not previously been reported.

It was also found that the helper-inducer cells reacted earlier in the DS child. On day 20, there was a decrease in the human leukocyte antigen class II^+ cells directly behind the JE in the sibling, whereas this was not the case in the DS child. This may indicate that in the DS child there were still more activated T cells, macrophages, or both. The density of B cells was diminishing by then. The ratio of helper-inducer to suppressor-cytotoxic cells in the gingiva on days 0 and 20 was in the same range for the sibling and the DS child. These findings are in accordance with earlier results (6, 7; Seymour et al., Abstract). The fact that helper-inducer cells moved toward the JE in the later stages of the study suggested a key role for these cells. The results indicated that T-cell regulation over B cells may lead to a new steady state in gingival inflammation during experimental gingivitis in the deciduous dentition. Extension of this study to a larger group is envisaged.

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