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The American Academy of Pediatric Dentistry
Volume 9 Number 1

The immunohistology of chronic gingivitis in children

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Abstract

Cell populations in the lesion of childhood gingivitis (CG) associated with the primary dentition were analyzed in 10 children using enzyme histochemistry together with a panel of 11 monoclonal antibodies. Immunohistological analysis revealed that the CG lesion consisted largely of T lymphocytes (80–88%) with a helper:suppressor (T4:T8) ratio of approximately 2.1:1. Activated macrophages (HLA-DR+, acid phosphatase+) comprised 7–16% of the infiltrating cells, while interdigitating cells (HLA-DR+, adenosine triphosphatase+), migrated Langerhans cells (T6+), neutrophils, B lymphocytes, and natural killer (NK) cells accounted for < 4% of the total infiltrating population. The gingival epithelium contained Langerhans cells and T lymphocytes, and the keratinocytes expressed HLA-DR but not HLA-DQ antigens in 5 of the 10 cases. The T lymphocyte profile demonstrated that the cells were activated (> 90% DR+ and DQ+) but not proliferating (< 5% IL-2 receptor and transferrin receptor positive). These results demonstrate that CG is a well controlled immune response in which T cells predominate, and which strongly resembles the delayed-type hypersensitivity response.

Gingivitis associated with the primary dentition in children is nonprogressive and does not produce periodontal bone loss (Jamison 1963). Morphologically this lesion has been shown to consist of large numbers of lymphocytes with relatively few plasma cells (Longhurst et al. 1977; Longhurst et al. 1980). More recently, the lymphoid cell subpopulations present in gingivitis in children (CG) have been analyzed using enzyme histochemical markers (Seymour et al. 1981) and surface antigen markers (Seymour et al. 1982). These studies suggested that the majority of lymphoid cells were T lymphocytes, but because of the lack of absolute specificity of the enzyme markers (Seymour et al. 1981; Poore et al. 1981) and the small number of cases in which definitive T cell analysis was carried out (Seymour et al. 1982), conclusive evidence for the T cell nature of the CG lesion is still lacking.

The development of techniques using monoclonal antibodies to identify specific lymphocyte populations in tissue sections has greatly improved the precision of histological analysis in normal and pathological tissues (Poulter et al. 1982). For example, helper/inducer (CD4+) and suppressor/cytotoxic (CD8+) T lymphocyte subpopulations can be identified using a panel of monoclonal reagents[1] while activated T cells can be detected by antibodies recognizing products of the major Histocompatibility Complex (MHC), the Class II antigens HLA-DR and HLA-DQ (Engleman et al. 1981; Chen et al. 1984). Proliferating T cells can be identified by their expression of receptors for Interleukin-2 (IL-2) and transferrin (Goding and Burns 1981).

A preliminary study has demonstrated that CD4+ and CD8+ lymphocytes are present in the CG lesion (Armitt 1986). The aim of the present study was to characterize the subpopulations of lymphocytes and macrophages present in the CG lesion and to analyze the functional microenvironments of these cells in situ (Poulter et al. 1982) so as to obtain some insight into the immunoregulatory mechanisms operating therein.

Methods and Materials

Tissue used in this investigation was obtained from a previous study (Armitt 1986). Briefly, gingival tissue (2 mm × 2 mm) was excised from the buccal aspect of primary teeth being extracted for orthodontic reasons from 12 children under the age of 10 years. The group contained equal numbers of male and female subjects. No subjects suffered from systemic disease or from localized juvenile periodontitis. Biopsies were taken from sites on those teeth with a gingival index (Löe and Silness 1963) of > 1 and a probing

[1] Engleman et al. 1981; Committee on Human Leukocyte Differentiation Antigens, 1984.

TABLE 1. The Specificity of Monoclonal Antibody Reagents

Antibody	*Specificity**	*Reference*
Anti-Leu 4	Pan T cells (CD3)	Engleman et al. 1981
Anti-Leu 2a	Suppressor/cytotoxic T lymphocyte subset (CD8)	Engleman et al. 1981
Anti-Leu 3a+b	Helper/inducer T lymphocyte subset (CD4)	Engleman et al. 1981 Ledbetter et al. 1981
Anti-Leu 14	Pan B cells	Chen et al. 1984; Schwarting et al. (in press)
Anti-Leu 7	Natural Killer (NK) cells	Abo and Balch 1981
Anti-Leu 11b	NK Fc IgG receptor	Lanier et al. 1983
Anti-IL-2 R	Interleukin-2 receptor	Uchiyama et al. 1981
Anti-Leu 10	HLA-DQ framework determinants	Chen et al. 1984
Anti-HLA-DR	HLA-DR antigen (non-polymorphic)	Lampson and Levy 1980
OKT6	Cortical thymocytes, Langerhans cells (CD1)	Terhorst et al. 1981; Murphy et al. 1981
OKT9	Transferrin receptor	Goding and Burns 1981; Terhorst et al. 1981

* Cluster of differentiation as defined by the Committee on Human Leukocyte Differentiation Antigens.

depth not exceeding 2 mm. All sites had displayed gingival inflammation for more than 8 weeks prior to biopsy.

Tissues were embedded in OCT,[a] quenched in liquid nitrogen and stored at −70°C until sectioning. Serial 6 μm cryostat sections were picked up on numbered uncoated slides, fixed in chloroform-acetone for 5 min (Poulter et al. 1982) then stored at −20°C in cling film until stained by immunoperoxidase or processed for histochemistry.

Sections were stained using monoclonal antibodies in a 3-layer immunoperoxidase technique (Walsh et al. 1986; Hsu and Raine 1981). The first layer antibodies are detailed in Table 1. OKT reagents were obtained from Ortho,[b] while the remaining antibodies were purchased from Beckton-Dickinson.[c] The second layer was biotin-conjugated affinity-purified goat anti-mouse immunoglobulin,[d] while the final layer was horseradish peroxidase-conjugated streptavidin.[e] All incubations were for 30 min, and finally the sections were developed using the diaminobenzidine technique (Graham and Karnovsky 1966). Sections were counterstained lightly with hematoxylin before mounting. Dilutions of all reagents were determined previously by titration so as to obtain optimum specific staining with minimal background reaction. Negative controls consisted of substitution of various layers with phosphate buffered saline, while sections of human tonsil provided positive controls.

Since the available anti-monocyte/macrophage monoclonal antibodies cross react with granulocytes (Committee on Human Leukocyte Differentiation Antigens 1984), enzyme histochemistry was used to identify macrophage populations (Poulter 1983). Sections were stained for adenosine-5'-triphosphatase (ATP) and acid phosphatase (ACP) using established methods (Seymour et al. 1981; Walsh et al. 1985) and lightly counterstained with methyl green. Sections from each block also were stained with hematoxylin and eosin (H&E) using conventional techniques.

The cell profile of CG lesions was determined by the following method. Where possible, approximately 200 cells in characteristic fields of the connective tissue lesion were counted. Lymphocytes were identified by their reactivity with the various monoclonal antibodies, while macrophages were identified by their characteristic enzyme profile (Seymour et al. 1981; Poulter et al. 1982). Polymorphonuclear neutrophils (PMN) were recognized by their morphology in H&E stained sections. Lesion profiles were determined by expressing the number of positive cells as a percentage of the total infiltrating cell count. The latter was considered to consist of the sum of T lymphocytes (Leu 4+), B lymphocytes (Leu 14+), natural killer (NK) cells (Leu 7+, Leu 11b+), macrophages (ACP+, ATP+), and PMN. For both NK cells and macrophages, the 2 contributing subpopulations were considered separately in view of the paucity of cells expressing both the markers used.[2]

Results

Lymphocytes

The majority of the lymphocytes present in the lesion were CD3+ (T cells) and only very occasional B cells (Leu 14+) were seen (Figs 1 & 2). The latter generally were distributed on the periphery of the

[a] Miles Laboratories; Naperville, IL.
[b] Ortho Diagnostics; Raritan, NJ.
[c] Beckton Dickinson Monoclonals; Mountain View, CA.
[d] Sigma Chemical Co.; St. Louis, MO.
[e] Amersham Corp.; Arlington Heights, IL.

[2] Seymour et al. 1981; Poulter et al. 1982; Poulter 1983; Wynne et al. (1986).

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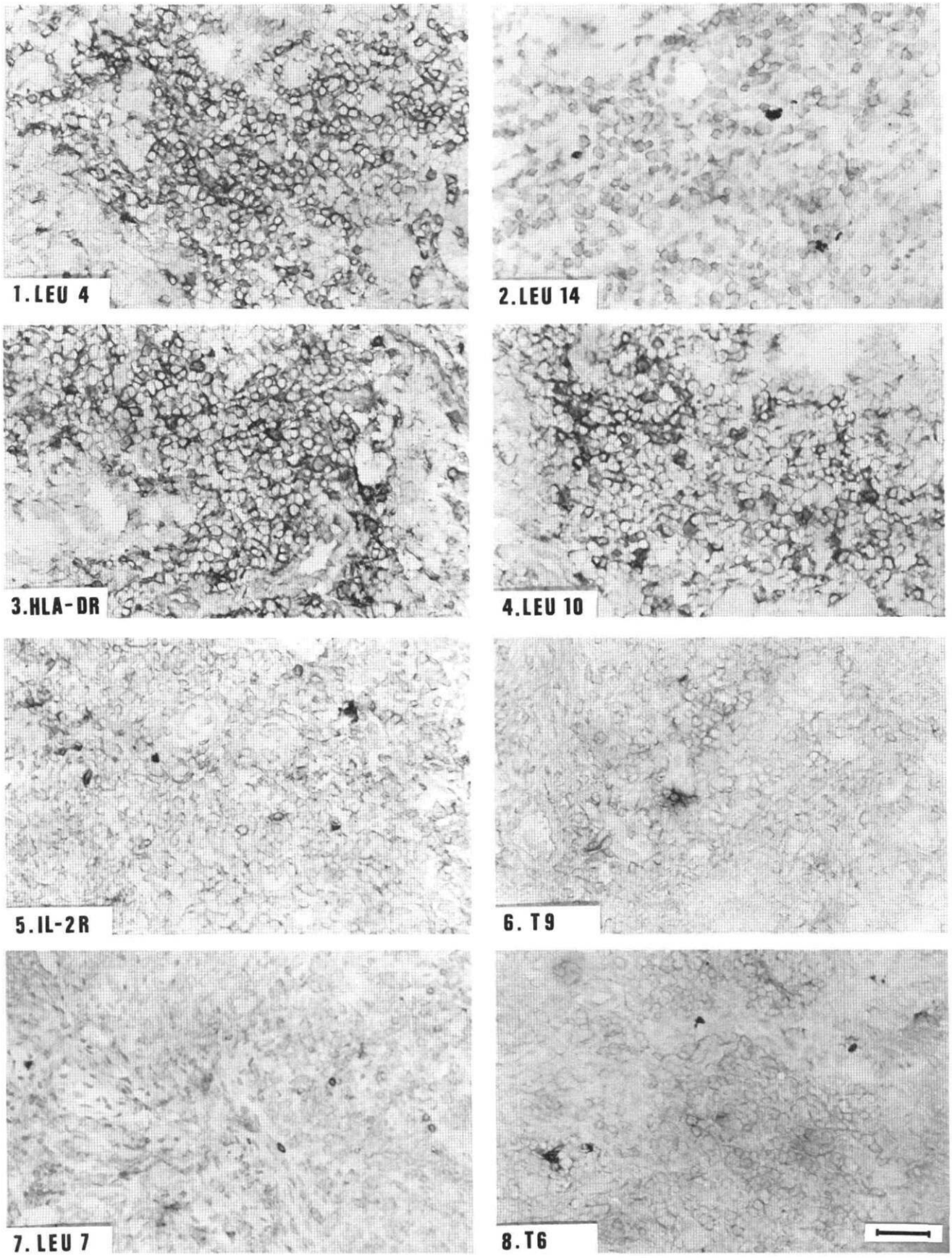
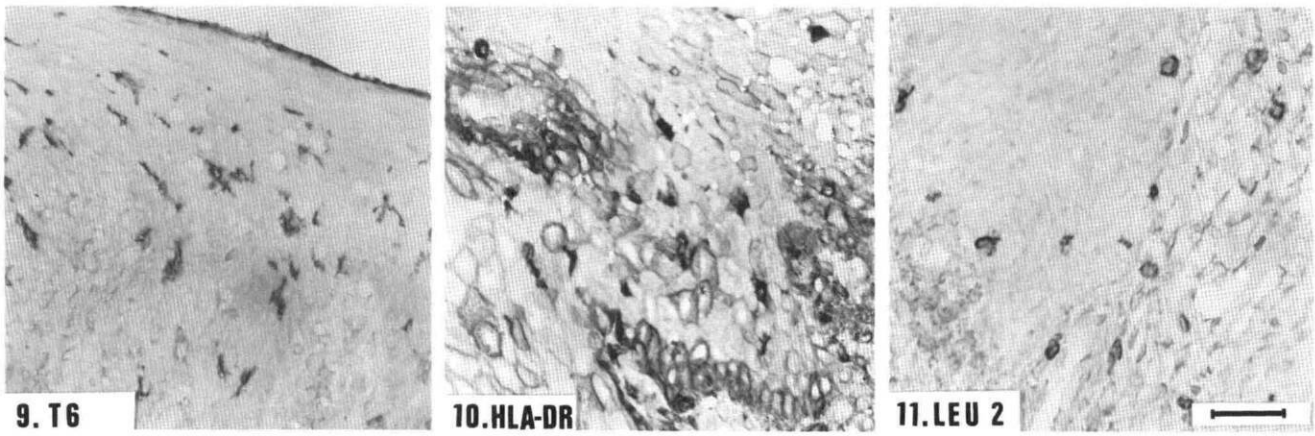
FIGS 1–8 *(next page).* Representative immunohistology of the connective tissue lesion of gingivitis in children. All sections are from the same lesion, and all sections have been stained using immunoperoxidase, with a hematoxylin counterstain. All photomicrographs are the same magnification, and the scale bar indicates a dimension of 25 μm. **Fig 1.** T lymphocytes; **Fig 2.** B lymphocytes; **Fig 3.** HLA-DR antigen expression by T cells and macrophages; **Fig 4.** HLA-DQ expression; **Fig 5.** Expression of Interleukin-2 receptors on T cells; **Fig 6.** Expression of transferrin receptors; **Fig 7.** Natural killer cells; and **Fig 8.** T6+ cells (possibly Langerhans cells) within the lesion.


1. LEU 4
2. LEU 14
3. HLA-DR
4. LEU 10
5. IL-2R
6. T9
7. LEU 7
8. T6



FIGS 9–11. Immunohistology of the gingival epithelium in gingivitis in children. Sections are from the same lesion as Figs 1–8. The scale bar indicates 25 μm. **Fig 9.** Dendritic Langerhans cells and cross-sections of dendrites are seen in the epithelium; **Fig 10.** HLA-DR antigens are expressed by both keratinocytes and LC; and **Fig 11.** CD8+ cells are present in the epithelium.

lesion. In the connective tissue CD8+ T cells were located beneath the crevicular epithelium and singly throughout the lesion while CD4+ cells were clustered around small blood vessels (Figs 13 & 14). The mean helper (CD4) to suppressor (CD8) ratio was 2.1 ± 0.3 SD. Staining of serial sections with anti-Leu 4, anti-Leu 10, and anti-HLA-DR demonstrated that the majority (> 90%) of the lymphocytes expressed DR and DQ antigens (Figs 3 & 4). In contrast, few T cells (< 5%) expressed receptors for IL-2 or for transferrin (Figs 5 & 6). Where present, receptor-positive cells were seen occasionally in the midst of small clusters of lymphocytes which were in close contact with the positive cell (Fig 6).

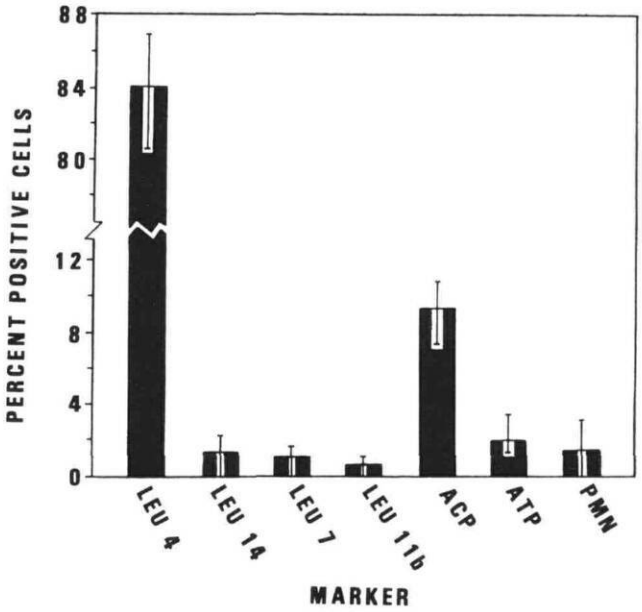


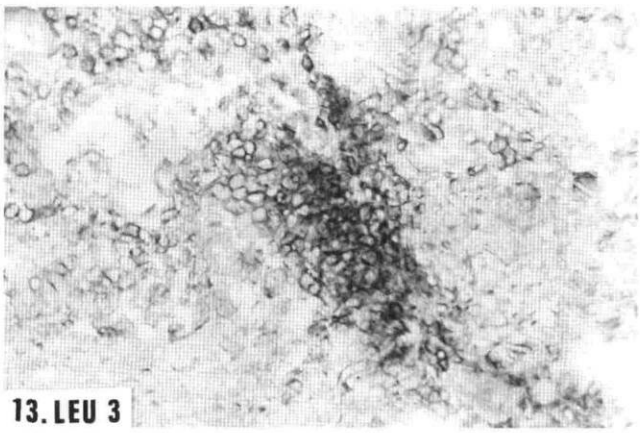
FIG 12. The cellular composition of the connective tissue lesion of gingivitis in children. The vertical axis indicates the percentage contribution of each of the cell populations to the lesion. Bars indicate the range of values recorded in the 10 lesions analyzed.

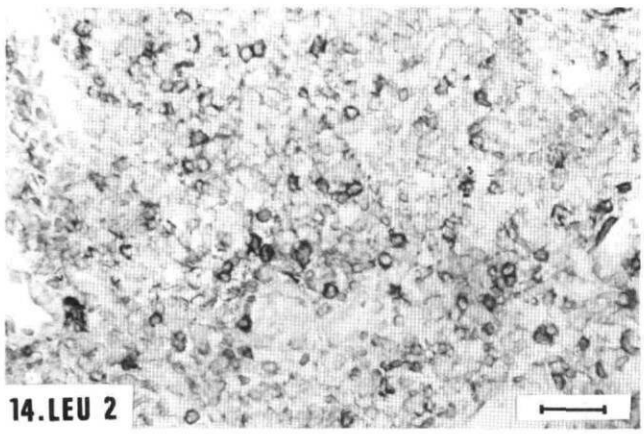
NK Cells and Macrophages

Occasional NK cells were seen scattered throughout the lesion (Fig 7). Leu 7+ cells were more numerous than Leu 11b+ cells, although the small counts obtained did not permit statistical analysis of this difference. CD1+ cells also were observed within the lesion. These cells were dendritic and were seen in close contact with clusters of lymphocytes (Fig 8). Activated macrophages (ACP+) were more numerous than interdigitating (ID) type macrophages (ATP+, Fig 12). The position of large nonlymphoid cells which expressed both DR and DQ antigens (Figs 3 & 4) corresponded with that of macrophages in adjacent sections, indicating that both ATP+ and ACP+ macrophages expressed MHC Class II antigens. Reactivity for ATP also was seen in endothelial cells and in Langerhans cells within the gingival epithelium. While very few PMNs were detected within the lesion, PMNs occasionally were seen in the crevicular epithelium.

Epithelium

Langerhans cells, identified within both the oral and crevicular epithelium by their CD1 staining, were highly dendritic and often located in the more superficial layers of the epithelium (Fig 9). Staining of serial sections demonstrated that the majority of LC (60–80%) expressed DR and DQ antigens, however, this interpretation was complicated by the presence of DR+ T cells in the epithelium. Keratinocyte DR expression was seen in 5 of the 10 CG specimens. Where present, DR was most strongly expressed on the first 2 cell layers, and occasionally extended into





FIGS 13, 14. T lymphocyte subsets in the CG lesion. Sections are from the same lesion as Figs 1–8. The scale bar indicates 25 μm. **Fig 13.** CD4+ cells in the CG lesion. **Fig 14.** CD8+ cells are scattered throughout the lesion.

the stratum spinosum (Fig 10). No keratinocyte reactivity with anti-Leu 10 was seen. CD8+ cells were present in the deeper layers of the oral (Fig 11) and crevicular epithelia, whereas CD4+ cells were rarely present. Dendritic CD4+ cells were detected in a single specimen and in this case could only be stained by using the Leu 3a+b antibody neat in the immunoperoxidase technique.

Lesion Profiles

The largest component of the CG lesion (Fig 12) was T lymphocytes (> 80%). Macrophages (ACP+ or ATP+) were a sizeable component of all lesions. B lymphocytes, NK cells and PMNs were absent in some cases. The lesion profile was consistent with regard to the T cell and macrophage populations.

Discussion

The results of the present study both confirm previous findings and extend considerably the available data on the immunohistology of gingivitis in children. Using monoclonal antibodies and histochemistry to identify cell subpopulations, the present study has established that the CG lesion is predominantly a T lymphocyte lesion, a result consistent with the hypothesis that a stable, nonprogressive response to dental plaque is dominated by T cells.[3] Furthermore, the T cell population would appear not to be undergoing significant local proliferation since few T cells express receptors for either IL-2 or transferrin. That the majority of the T cells express DR and DQ antigens suggests that they nevertheless are activated in situ (Chen et al. 1984), but the site of this activation has not been identified. The concept that the CG lesion is well controlled (Seymour et al. 1981) is supported by the finding that the T4:T8 ratio in CG (2.1:1) is essentially the same as that in peripheral blood (Okada et al. 1984). The close similarity between the immunohistology reported for CG and that of delayed type hypersensitivity (DTH) (Poulter et al. 1982) is further evidence that the immune response in CG is controlled properly by the immune system and by efficient feedback mechanisms.

With regard to other cell types present in the lesion, the results of this study support the hypothesis that NK cells are predominantly associated with B cell-dominated ("progressive") lesions of adult periodontitis (Wynne et al. 1986). The relative paucity of B cells in CG confirms previous studies (Seymour et al. 1981; Poulter 1983) and is again comparable to the situation in DTH (Poulter et al. 1982). The presence of CD1+ cells (possibly Langerhans cells) within the T lymphocyte infiltrate is consistent with the view that gingival LC have the capacity to migrate from the epithelium and present bacterial antigens locally (Walsh et al. 1985). It was not possible in the present study to determine whether these CD1+ cells were migrating from the epithelium or into the epithelium. In addition to LC, other cells can function in an antigen presenting capacity. Interdigitating (ID) cells (ATP+, HLA-DR+) and activated macrophages (ACP+, HLA-DR+) were present within the lesion, and the ratio of ACP+ to ATP+ macrophages (~ 3:1) is similar to that seen in DTH (Poulter et al. 1982). While both ATP+ and ACP+ macrophages have the ability to present bacterial antigens (Poulter 1983), within the limits of the present study it was not possible to determine whether both populations were functioning as antigen presenting cells.

The cellular profiles obtained for the 10 CG lesions analyzed in the present study demonstrate that the lesion consists primarily of activated T cells (DR+, DQ+) while sizeable numbers of macrophages (DR+, DQ+) are also present. This result is in sharp contrast

[3] Seymour et al. 1982; Armitt 1986; Seymour et al. 1979.

with a recent report (Gillet et al. 1986) which described the infiltrate in 5 subjects (aged 3-12 years, GI = 1). Using a monoclonal antibody and morphologic criteria, these workers suggested that the majority of lymphocytes in the CG lesion were nontransformed B cells. In view of the results of the present study, it is likely that these lymphocytes were in fact T cells. DR antigens are not B-cell specific and are expressed on activated T cells.[4] In the present study the T cell nature of the DR positive lymphocytes was established using a panel of anti-T cell antibodies, while enzyme histochemistry was used to demonstrate the presence of other DR positive cell populations such as macrophages. On this basis the authors refute the claim that the CG lesion is dominated by B cells. The paucity of lymphocytes which express the B cell markers Leu 14 or FMC1 (Seymour et al. 1982) is further evidence for the T cell nature of the CG lesion.

The detection of T cells within the epithelium confirms recent reports of CD4+ and CD8+ cells within gingival epithelium (Reibel et al. 1985). The presence of CD8+ cells within the basal layers is a feature of noninflamed gingiva, as is the occasional presence of very weakly staining CD4+ dendritic cells (Reibel et al. 1985). The latter are most probably Langerhans cells (Wood et al. 1983) and not the human equivalent of murine Thy 1 cells (Cooper et al. 1985), as has been suggested (Reibel et al. 1985). Technical factors are involved in the demonstration of Leu 3+ LC (Cooper et al. 1985), and for this reason a modified staining protocol was used to demonstrate these cells in the present study, specifically the use of neat Leu 3a+b antibody in the first layer of the staining technique. Further studies are required to determine the significance of Leu 3 antigen expression by LC.

Conclusion

In conclusion, the results of this study have established the cellular profile of the lesion of gingivitis in children, and demonstrated that the lesion is a well controlled T cell response resembling the DTH reaction. It should now be possible to compare the immunohistology of CG with that of adult gingivitis and periodontitis, as has been suggested (Ranney et al. 1981). Insight into the cell population shifts thought to occur in the development of adult periodontitis (Seymour et al. 1979) may thereby be gained.

This study was supported by the National Health and Medical Research Council of Australia and by the Australian Society of Periodontology.

[4] Engleman et al. 1981; Chen et al. 1984; Uchiyama et al. 1981.

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