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Human T Lymphocyte Subpopulations in Chronic Periapical Lesions

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Seven human chronic periapical lesions were examined for the presence of T lymphocyte subpopulations with monoclonal antibodies using a biotin-avidin-horseradish peroxidase method. Six of the seven specimens stained positively for the presence of T cytotoxic/suppressor and T helper/inducer lymphocytes. The remaining specimen diagnosed as an apical scar contained no T lymphocytes.

Bacteria and altered host tissue substances are potential antigens capable of initiating immunological reactions in periapical tissues via the root canal system (1, 2). These reactions include humoral immune responses mediated by B lymphocytes and plasma cells, and cell-mediated immune responses through the actions of T lymphocytes and lymphokines. Different classes of immunoglobulins have been identified in chronic periapical lesions by several investigators (3–7), but the presence of T lymphocytes has not been demonstrated.

It is possible to identify T lymphocytes, in serum suspensions, by their capacity to form rosettes with sheep erythrocytes (8). However, this technique cannot be easily applied to the study of these cells in tissue sections. The advent of monoclonal antibody techniques has allowed for differentiation of subpopulations of T lymphocytes in frozen tissue sections (9–12). Kohler and Milstein (9) developed a method to fuse mouse myeloma cells with lymphocytes from the spleens of mice immunized with sheep red blood cells as antigen. The cells derived from this process can produce large quantities of specific antibodies. Reinherz et al. (10) produced monoclonal antibodies to human peripheral blood T cells and used these antibodies to identify T lymphocyte subpopulations. Warnke and Levy (11) used a biotin-avidin-horseradish peroxidase method to study frozen tissue sections of reactive lymph nodes, spleen, B cell lymphomas, and T cell lymphomas. They found that this monoclonal antibody technique effectively identifies T and B cell types.

It is the intent of this study to identify human T lymphocyte subpopulations in chronic periapical lesions of endodontic origin.

MATERIALS AND METHODS

Seven biopsy specimens from seven individual patients were obtained during periapical surgery. Endodontic surgery was performed on teeth exhibiting pain, recurrent swelling, persistent drainage, or fistula combined with periapical radiolucencies. Medical histories of all patients were unremarkable.

One-half of each periapical specimen was placed into neutral buffered formalin for routine histological examination using hematoxylin and eosin stain. The remaining half was placed in normal saline for transport to the immunology laboratory. The specimen was then embedded in gelatin, quick frozen by immersion in liquid nitrogen, and stored in a freezer at −86°C.

Frozen sections were obtained from two distinct areas of each specimen at least 150 μm apart. Serial sections 6-μm thick were collected for testing with each of five monoclonal antibodies and one section was stained with hematoxylin and eosin. Both areas of the specimen were treated using the methods described above. The hematoxylin and eosin sections were air dried for 1 h, fixed in neutral buffered formalin for 10 min, and then rinsed in distilled water for 5 min and stained routinely. The sections used for immunological testing were air dried for 1 h, fixed in acetone at room temperature for 10 min, and then air dried an additional 30 min prior to staining.

These sections were then rehydrated by incubating the slides with phosphate-buffered saline for 3 min. One drop of the specific monoclonal antibody was added to the sections and incubated at room temperature for 15 min. At the end of this time period, the sections were rinsed in phosphate-buffered saline for 3 min. The tissue was then incubated with biotin-conjugated antimouse IgG for 15 min and washed with phosphate-buffered saline for 3 min. This was followed by addition of avidin-horseradish peroxidase for 15 min. The specimen was then washed in phosphate-buffered saline for 6 min. The section was subsequently incubated with diaminobenzidine-peroxide solution for 5 min, washed two times with phosphate-buffered saline for 3 min, and rinsed by dipping 10 times into distilled water. This was followed by placing copper sulfate solution on the spec-

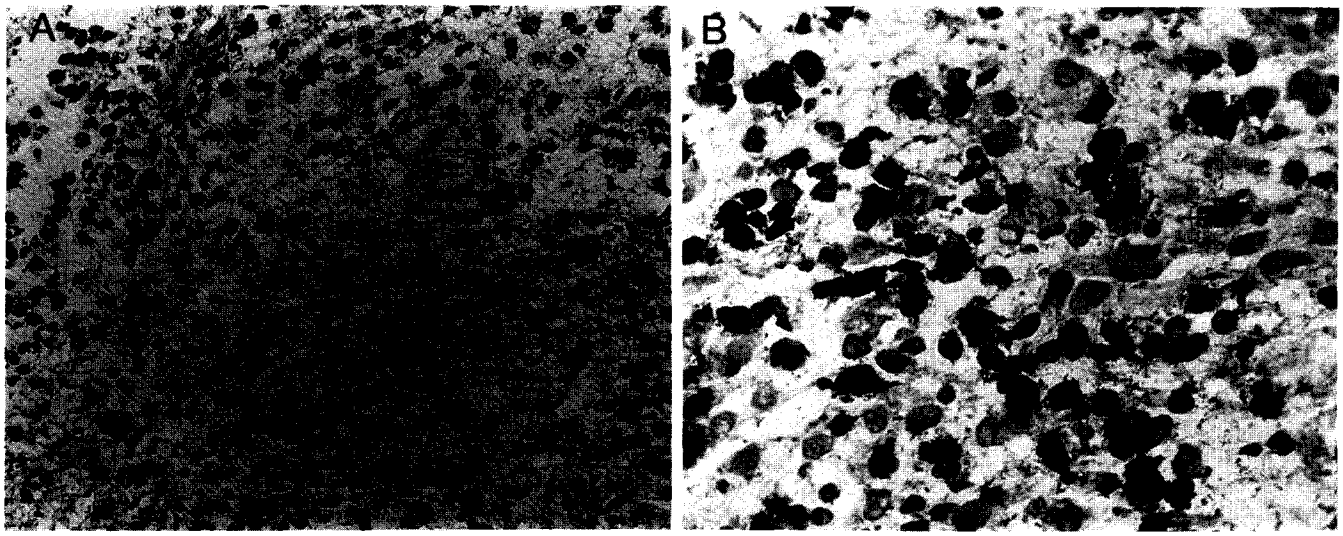

FIG 1. Frozen section of chronic periapical lesion stained with Anti-Leu-1 (specific for all T lymphocytes). *A*, original magnification ×200; *B*, original magnification ×400. Positively staining T lymphocytes appear black in color, whereas negatively staining cells appear as a light shade of gray.

TABLE 1. Monoclonal antibodies and their reactivity

Antibody	Reactive Cell Population
Anti-Leu-1	All T cells
Anti-Leu-2a	T cytotoxic/suppressor cells
Multiclone anti-Leu-3a + 3b	T helper/inducer cells
Anti-Leu-4	All T cells
Anti-HLA-DR	B cells, macrophages, and activated T cells

imen for 5 min, rinsing with phosphate-buffered saline, and dipping in distilled water five times. Finally, the section was counterstained with hematoxylin, rinsed with phosphate-buffered saline, dehydrated, and mounted.

The sections obtained were examined under light microscopy. The presence of T lymphocytes was confirmed by the appearance of brown rings surrounding cells with the morphology of human lymphocytes. Cells which did not have the tested antigens only displayed the hematoxylin counterstain (Fig. 1).

The staining procedure and reagents that were used were supplied by the Becton-Dickinson Co. (Mountain View, CA) in their T Cell Panel Kit. This technique uses a panel of T lymphocyte-specific monoclonal antibodies (Table 1) with a biotin-avidin-horseradish peroxidase method. This kit is based upon the indirect immunoenzyme technique described by Warnke and Levy (11) and Wood and Warnke (12).

RESULTS

Seven periapical lesions were prepared with paraffin-embedded hematoxylin and eosin staining, frozen hematoxylin and eosin staining, and a monoclonal antibody technique. Five of the seven specimens were diagnosed as granulomas by both the paraffin-embedded and frozen hematoxylin and eosin staining procedures. These specimens stained positively for all five monoclonal antibodies in each of two areas tested. This finding confirms the presence of T helper/inducer cells and T cytotoxic/suppressor cells in these lesions (see Fig. 1).

One of the seven specimens was diagnosed as an apical scar with both the paraffin-embedded and frozen hematoxylin and eosin staining techniques. This specimen was devoid of inflammatory cells and reacted negatively with all five monoclonal antibodies in both areas of the lesion tested.

The seventh specimen had conflicting results. This lesion was diagnosed as a granuloma from the paraffin-embedded hematoxylin and eosin section. However, the frozen hematoxylin and eosin staining procedure revealed one area of the lesion as a granuloma and the second area of the same specimen as an apical scar. The area of the specimen diagnosed as a granuloma stained positively for all of the five monoclonal antibodies. Conversely, the second area diagnosed by the frozen hematoxylin and eosin as an apical scar, stained negatively for each of the five antibodies.

Although the number of T lymphocytes detected in this study were not quantitated, it was the author's impression that the number of T helper/inducer cells was similar to the number of T cytotoxic/suppressor cells within the same specimens. All T lymphocytes were grouped in discrete foci which were nonuniformly distributed throughout the lesions tested.

DISCUSSION

Other investigations of chronic periapical lesions have implied the delayed hypersensitivity mechanism (13–

15). These have been based on routine light microscopic evaluation, revealing a large lymphocyte population and the presence of macrophages. Our study confirms the presence of T cytotoxic/suppressor and T helper/inducer lymphocytes in periapical granulomas. In the specimen diagnosed as an apical scar, no inflammatory cells or T lymphocytes were detected. This afforded a natural control for our study.

With a continued source of antigen from the root canal system a chronic periapical lesion can be maintained. Previously sensitized lymphocytes contacting antigenic materials produce lymphokines. These substances are capable of recruiting inflammatory cells, activating them, and keeping them at the site (8). In addition to the functions described above, T lymphocytes regulate production of immunoglobulins by plasma cells. T helper cells enhance the immune response by stimulating B lymphocytes to differentiate into fully mature antibody-secreting plasma cells. T helper cells also stimulate precursors of T lymphocytes to become cytotoxic T cells. T suppressor cells depress the immune response by down-regulating antibody production by plasma cells and by exerting a negative influence on precursors of T lymphocytes to become cytotoxic T cells. Cytotoxic T cells function to lyse cells infected by non-self antigens, particularly viruses.

In this study we did not attempt to quantitate our findings, but rather to conclusively demonstrate the presence of T lymphocyte subpopulations in chronic periapical lesions. Despite the limited sample size, the techniques delineated should prove beneficial in further immunological studies of inflammatory lesions of this type.

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