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Enzyme differentiation of lymphocyte subpopulations in sections of human lymph nodes, tonsils and periodontal disease

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SUMMARY

Five enzymes have been studied to differentiate between T and B lymphocytes in sections of human lymph nodes, tonsils and chronic inflammatory periodontal disease. The presence of acid phosphatase, β-glucuronidase N-acetyl-β-glucosaminidase, non-specific esterase and fluoride-resistant esterase activity was determined histochemically.

The results indicate that cells in the B area of both lymph nodes and tonsils are negative for enzyme activity, while those in the T area show a single intense granule of activity. These enzymes were unable to differentiate between T blasts, B blasts and plasma cells in the sections studied. The majority of the lymphoid cells in the lesions of chronic inflammatory periodontal disease are enzyme-negative and probably of B-cell origin.

INTRODUCTION

The use of cell surface characteristics to differentiate between T and B-cells depends upon the *in vitro* manipulation of living cells (Brown & Greaves, 1974), therefore spontaneous sheep red blood cell rosettes to detect T cells cannot be used on tissue sections. Although rosetting techniques to detect Fc and complement receptors give consistent results with lymphoid tissue (Dukor, Bianco & Nussenzweig, 1970; Mendes, Miki & Peixinho, 1974), individual cells cannot be identified because of the heterogeneity of cell types exhibiting these receptors (Brown & Greaves, 1974; Smith & Haegert, 1974; Gyöngyössy *et al.*, 1975). Therefore the use of such techniques in the study of chronic inflammatory lesions must be considered as being of only very limited value (Claudy *et al.*, 1976). Similarly, there are limitations on the use of immunofluorescent techniques employing anti-thymus and anti-immunoglobulin antisera, which have been used in the study of human connective tissue diseases such as rheumatoid arthritis and systemic lupus erythematosis (Williams *et al.*, 1973), as well as in the study of the mononuclear cell infiltrates present in NZB/NZW mice (Greenspan *et al.*, 1974a, b). These techniques require the use of specific antisera which are not readily available, especially for the study of human diseases.

A straightforward means of differentiating between T and B cells in tissue sections is therefore required and would be of great value in the study of inflammatory lesions characterized by a mononuclear cell infiltrate. Recently, Mueller *et al.* (1975) have described the use of non-specific acid esterase staining as a means of differentiating between T and B cells in mouse lymph node sections. This technique has also been applied to the study of human peripheral blood lymphocytes, infantile thymocytes and tonsil lymphoid cells (Kulenkampff, Janossy & Greaves, 1977). The present paper reports on the use of other enzyme markers to differentiate between T and B cells in sections of human lymph nodes and tonsils, together with the application of these enzymes to differentiate cell types *in situ* in the lesions

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of chronic inflammatory periodontal disease, which can be considered as a human model of chronic inflammation.

MATERIALS AND METHODS

Tissues. Clinically normal adult human lymph nodes were obtained from the inguinal region of twelve patients undergoing arterial surgery. Tonsils were obtained from seven patients undergoing tonsilectomy. Tissues exhibiting chronic inflammatory periodontal disease were obtained from twelve patients undergoing periodontal surgery.

Fixation procedure. Tissue preparation was as previously described (Seymour, Dockrell & Greenspan, 1976).

Sections. Cryostat sections 6μm in thickness were cut and picked up on formol gelatinized cover-slips or slides and stored at 0–4°C before staining.

Staining. The sections were stained with haematoxylin and eosin (H & E), and methyl green pyronin (MGP).

Enzyme histochemistry. Simultaneous azo dye coupling techniques, using naphthol AS-BI substrates and hexazotized new fuchsin as the dye, were used to stain for acid phosphatase (Barka & Anderson, 1962), β-glucuronidase (Hayashi, Nakajima & Fishman, 1964) and N-acetyl-β-glucosaminidase (Hayashi, 1965). In each case the sections were incubated at 37°C for 2 hr. Staining was also carried out for non-specific esterase at pH 7·3 (Davis, 1959) using α-naphthyl acetate as the substrate, and again incubating at 37°C for 2 hr. Fluoride-resistant esterase staining was done using the same technique as for non-specific esterase, but with the addition of sodium fluoride (1·5%) to the incubating medium. Peroxidase staining was done using a diaminobenzidine (DAB) technique (Graham & Karnovsky, 1966) incubating for 10 min at room temperature. Control sections for each enzyme were prepared by incubating in the respective medium without the specific substrate. All sections were counterstained with 1% methyl green.

RESULTS

Lymph nodes and tonsils

Cells showing a single intense granule of activity for all enzymes except peroxidase were found in the parafollicular cortex of both lymph nodes and tonsils (Figs 1 and 2). On the other hand, cells comprising the small lymphocyte mantle of the germinal centres and of the secondary follicles lacking a distinct germinal centre were negative for all enzymes (Fig. 3). However, it was observed that in the tonsils a single granule of very weak acid phosphatase activity occurred in some of the cells of the mantle of the germinal centres. This was not observed with the other enzymes, nor was it observed in the clinically normal lymph nodes.

In the tonsils, pyroninophilic cells larger than the small lymphocytes were found immediately beneath the tonsillar epithelium. These cells showed increased enzyme activity for acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase, whereas there was no substantial increase with the non-specific esterase nor fluoride-resistant esterase (Fig. 4). Cells with the morphological appearance of plasma cells showing similar activity for acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase were found in the medullary tissue of the lymph nodes (Fig. 5). These cells also showed no substantial increase in the enzyme activity with the non-specific nor fluoride-resistant esterase.

Large cells were found associated with the germinal centres and secondary follicles, as well as being scattered throughout both the lymph nodes and tonsils. These cells showed very intense staining, both in discrete granules and diffusely in the cytoplasm, for all enzymes studied except peroxidase (Fig. 6).

Peroxidase staining was found mainly in the red blood cells and in a few mononuclear cells surrounding the blood vessels in both the lymph nodes and tonsils.

Chronic inflammatory periodontal disease

Large cells showing intense enzyme activity were found immediately beneath the epithelium lining the periodontal pocket. Away from this epithelium the inflammatory cells had the morphological appearance of lymphocytes and were non-pyroninophilic. Cells showing a single granule of enzyme activity, as well as cells negative for all enzymes, were found in this area (Fig. 7). However, in all instances the majority of cells were negative for enzyme activity, although the relative numbers were variable from case to case. Cells showing intense activity were also found in this area, together with cells showing

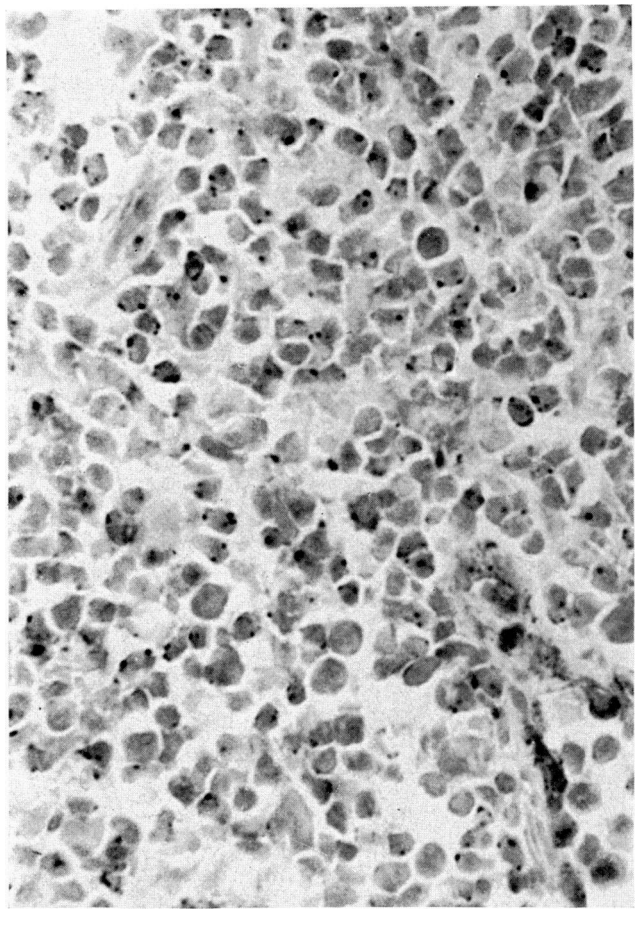

FIG. 1. Lymph node section showing intense granule activity in cells of the parafollicular cortex (T cells) for β-glucuronidase. (Magnification × 800.)

increased activity for acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase. On the advancing front of the lesion, cells with the morphological appearance of plasma cells were found. These were intensely pyroninophilic and showed increased activity for acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase. All control sections were negative for enzyme activity.

DISCUSSION

The two major classes of lymphocytes, T and B cells, are found in different regions of the peripheral lymphoid organs. Thus B cells are found in the primary and secondary follicles, with or without germinal centre formation, while T cells are found in the parafollicular cortex (Parrot, de Sousa & East, 1966; Gutman & Weissman, 1972). Preliminary results, using an anti-monkey thymus antiserum (Greaves & Janossy, 1976) and an anti-human immunoglobulin (Nordic Immunochemicals) in an indirect immunofluorescent technique, have confirmed this segregation of cell types (Figs 8 and 9). The present study further indicates that the two classes of lymphocytes can be differentiated in sections of human nodes and tonsils using a standardized enzyme histochemical technique. These results confirm those of Tamaoki & Essner (1969), who found that in human lymph nodes the cells of the parafollicular cortex stained for acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase, while the cells in the mantle of the germinal centres were negative for these enzymes. Similar results were found in the spleen, where

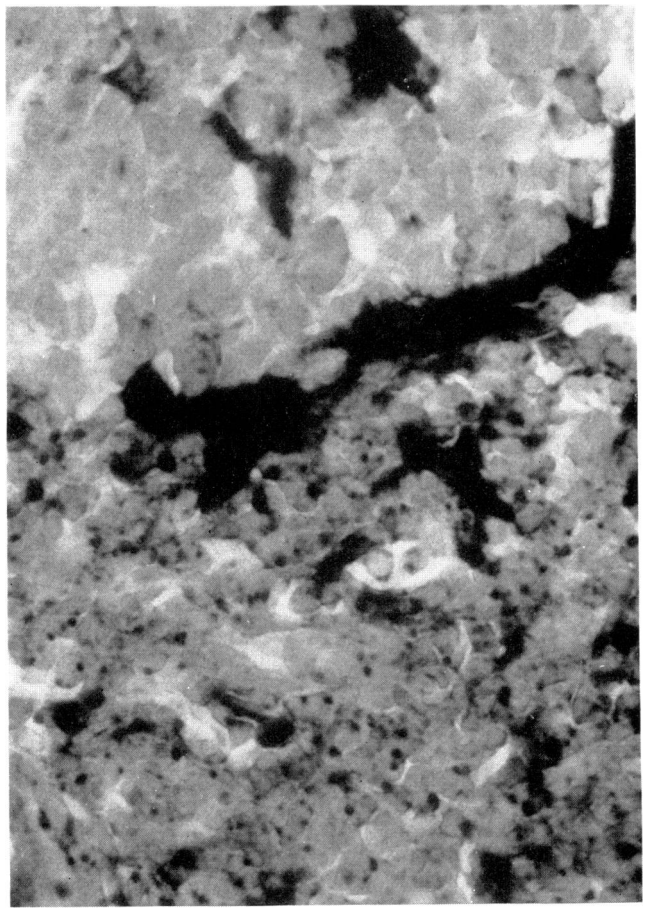

FIG. 2. Tonsil section showing junction between secondary follicle (top) and parafollicular cortex (bottom) stained for nonspecific esterase. The very intense staining of the macrophages can also be seen. (Magnification × 800.)

the cells surrounding the central arteriole of the white pulp (a T cell area) were positive while those in the nodules of the white pulp (B cell area) were negative. On the other hand, these results and those of the present study conflict with the findings of Li, Yam & Crosby (1972) and Müller-Hermelink, Heusermann & Stutte (1974), who failed to demonstrate acid phosphatase or β-glucuronidase activity in human lymphoid cells. This apparent conflict can be explained in terms of technique, as both Li *et al.* (1972) and Müller-Hermelink *et al.* (1974) used relatively short incubation times (30–60 min), whereas in the present study and in that of Tamaoki & Essner (1969), the sections were incubated at 37°C for 2 hr. We also failed to detect enzyme activity for all the enzymes studied using short incubation times and/or incubating at room temperature.

In peripheral blood, T cells can also be distinguished from B cells by the use of the non-specific acid esterase technique of Mueller *et al.* (1975), Kulenkampff *et al.* (1977) and Ranki, Totterman & Hayry (1976). Furthermore, Barr & Perry (1976) claim that on the basis of β-glucuronidase and acid phosphatase activity, T cells can be distinguished from non-T cells (i.e. B cells and null cells) in human peripheral blood. It therefore appears from these studies and the present study that in both the peripheral blood and tissue sections the lysosomal enzymes offer a convenient marker of T cells, provided a standardized technique involving long incubation times at 37°C is employed.

Mitogen-stimulated cells show increased lysosomal enzyme activity (Allison & Malucci, 1964;

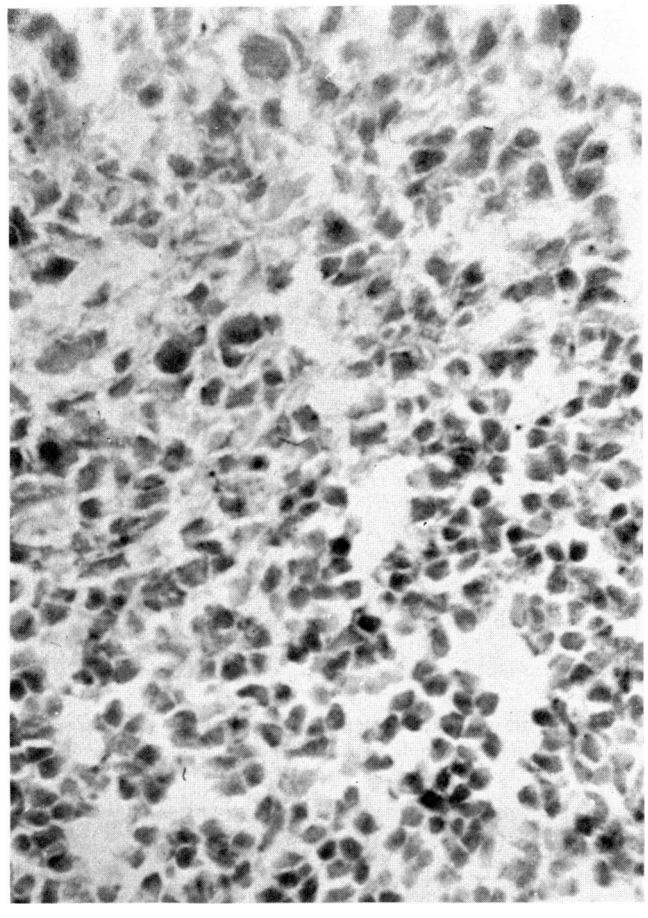

FIG. 3. Lymph node section of secondary follicle (B-cell area) showing absence of staining for β-glucuronidase. Some positive cells can be seen and probably represent T-cell infiltration of the B-cell area. (Magnification $\times 600$.)

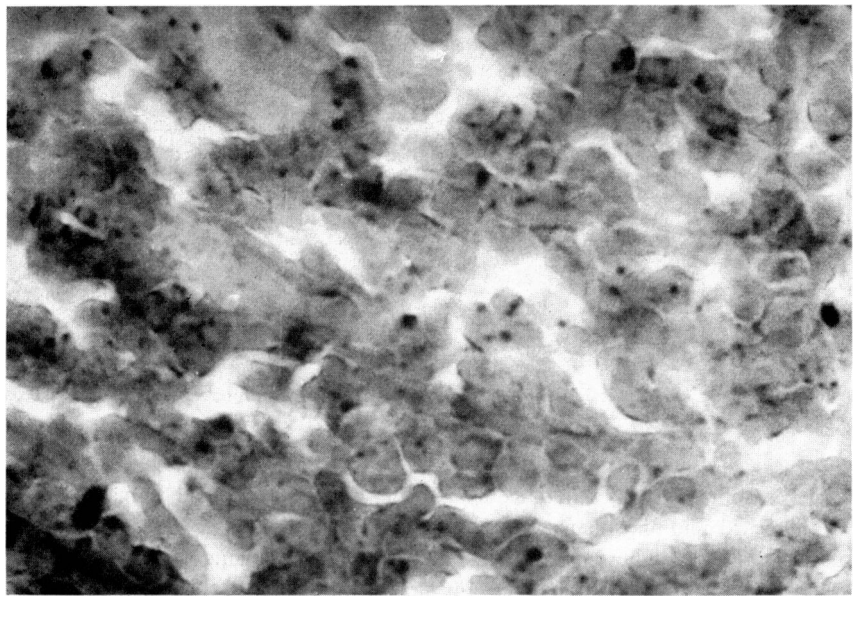

FIG. 4. Tonsil section showing blast cell population immediately beneath the epithelium. No substantial increase in activity can be seen here with fluoride-resistant esterase. (Magnification $\times 600$.)

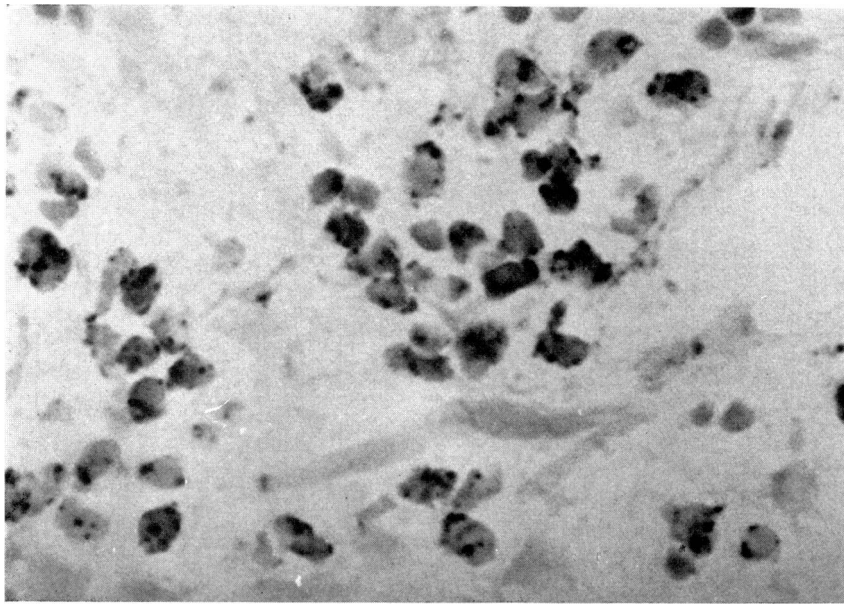

FIG. 5. Lymph node section showing morphological plasma cells in the medulla, seen here with increased enzyme activity for acid phosphatase. (Magnification × 600.)

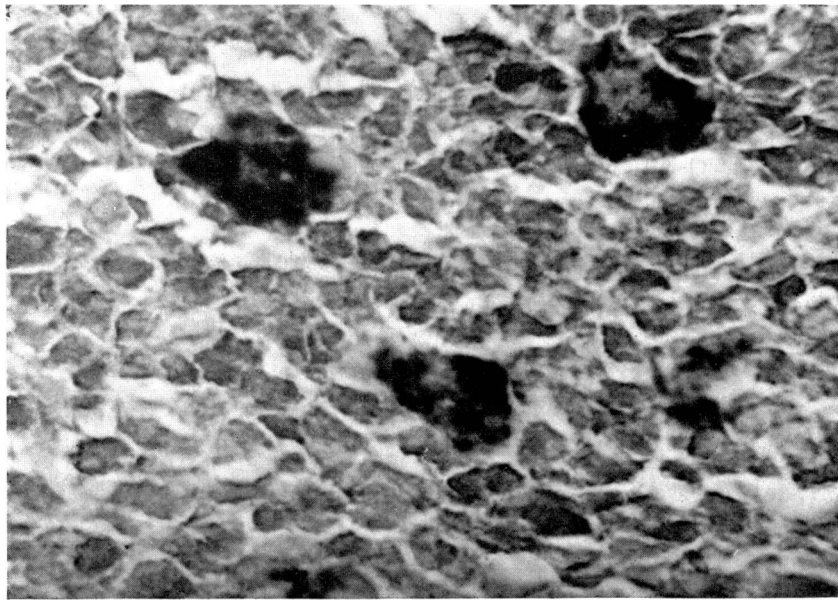

FIG. 6. Tonsil section showing very intense staining pattern of the macrophages seen here in a secondary follicle. (Magnification × 2000.)

Konig, Brittinger & Cohnen, 1973). Phytohaemagglutinin (PHA) stimulated human lymphoid cells, for instance, show increased acid phosphatase activity (Hirschhorn *et al.*, 1965) and β-glucuronidase activity (Yam & Mitus 1968). On the other hand Kulenkampff *et al.* (1977), using the non-specific acid esterase technique (Mueller *et al.*, 1975), failed to detect any significant change in the enzyme activity in mitogen-activated human peripheral blood T and B cells.

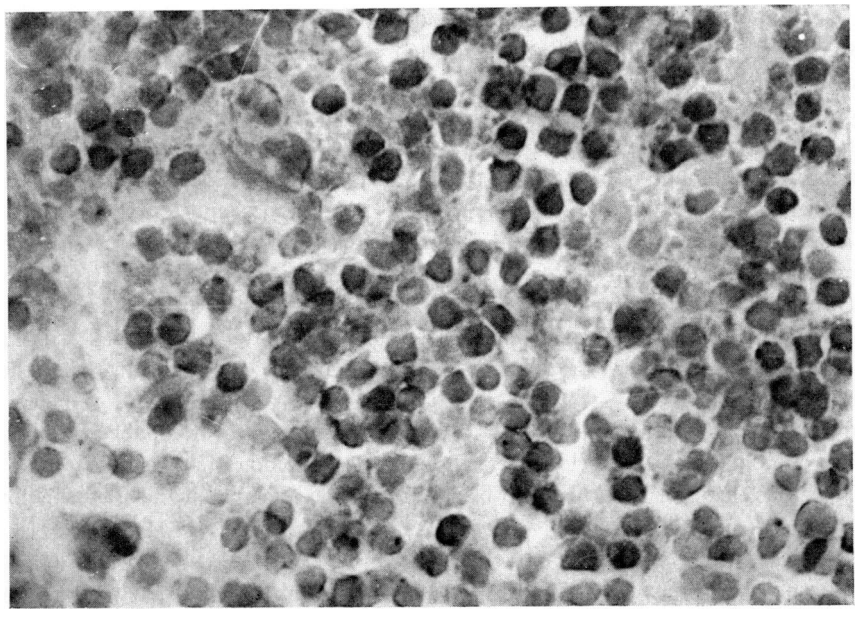

FIG. 7. Chronic inflammatory periodontal disease showing both positive and negative cells, with the majority of cells being negative here with β-glucuronidase. (Magnification × 800.)

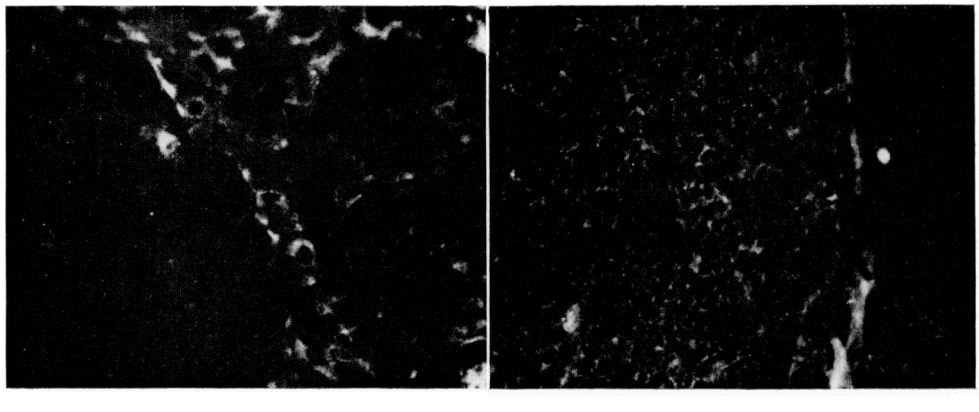

FIG. 8. FIG. 9.

FIG. 8. Tonsil section using anti-monkey thymus antiserum (1 : 5 dilution) showing staining confined to cells of the parafollicular cortex (T cells). The B-cell area (left) is negative. (Magnification × 385.)

FIG. 9. Tonsil section using anti-human immunoglobulin antiserum (1 : 50 dilution) showing staining in B-cell area (left). Some cells in the T-cell area are also staining. (Magnification × 385.)

In the present study, the pyroninophilic blast cells seen beneath the tonsillar epithelium may represent blast cells of either T or B origin, or both (Curran & Jones, 1977). In our preliminary immunofluorescent study, cells with the surface phenotype of T cells, together with cells showing cytoplasmic Ig, were both found beneath the tonsillar epithelium. All these cells appeared to show increased enzyme

activity for acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase, but no substantial increase with either the non-specific or fluoride-resistant esterases. These results are therefore in keeping with those of Hirshhorn *et al.* (1965) and Yam & Mitus (1968), as well as with those of Kulenkampff *et al.* (1977). However, the results do not indicate that these enzymes can be used to distinguish blast cell populations in human tissue sections. Cells with the morphological appearance of plasma cells and showing intense pyroninophilia were found in the medullary tissue of the lymph nodes. These cells also showed increased enzyme activity for acid phsophatase, β-glucuronidase and N-acetyl-β-glucosaminidase, but no substantial increase in non-specific nor fluoride-resistant esterase. That they were plasma cells can, at the present time, only be inferred from their morphology and location in the tissues. However, our preliminary results using immunofluorescence seem to indicate that they contain cytoplasmic Ig. Recently, Lennert, Kaiserling & Müller-Hermelink (1975) have described a cell with the electron microscopic appearance of a plasma cell, but being of putative T cell origin. This they called a T-associated plasma cell.

Thus in the present study, human T blasts, B blasts and plasma cells could not be readily differentiated in terms of pyroninophilia and histochemistry. It is of interest to note that preliminary work (Dockrell *et al.*, 1978) suggests that murine T blasts, B blasts and plasma cells may be distinguished using similar enzyme techniques.

Some cells in the B cell areas of the reactive tonsils displayed weak acid phosphatase activity. This was not seen in the clinically normal lymph nodes. This may be a reflection of the state of activity of these cells, as plasma cells with increased activity for acid phosphatase presumably acquire the enzyme at some stage in differentiation. Barr & Perry (1976) have demonstrated acid phosphatase activity in EAC-rosetting cells of human peripheral blood. However, as this population of cells could contain both B cells and a population of T cells bearing C3 receptors, the results are inconclusive. A population of both murine (Gyöngyössy *et al.*, 1975) and human (Smith & Haegert, 1974) T cells have been shown to bear C3 receptors.

The present study does indicate that acid phosphatase alone, using the technique described, may not differentiate T and B cells in tissue sections. It must be used in conjunction with other enzymes to enable cell phenotypes to be established.

The large cells seen in both lymphoid organs and showing intense activity for all enzymes except peroxidase were thought to represent the macrophage population. The few peroxidase-positive cells are probably monocytes which have not yet differentiated into tissue macrophages.

In chronic inflammatory periodontal disease, the large cells with intense enzyme activity seen immediately beneath the epithelium, lining the periodontal pocket, are probably macrophages. While both enzyme-negative (B) and enzyme-positive (T) cells could be identified, the majority of cells within the lesion appeared to be B cells. This is supported by the findings of Mackler *et al.* (1977), who were able to demonstrate, using immunofluorescence, that the majority of cells showed membrane-bound immunoglobulin. In the present study both T cells and macrophages were also found. Their relatively fewer numbers could indicate that the role of the T cells is one of helper activity, in conjunction with the macrophages, co-operating with the B cells in antibody production. However, this view does not preclude the involvement of cell-mediated immune mechanisms in the pathogenesis of the lesion.

That chronic periodontitis is indeed a B cell lesion is supported by the finding of a large number of cells with the morphological appearance of plasma cells, although the morphological and histochemical findings of the present study do not permit the definite identification of the cells as such. They may, in fact, be cells of either the B cell or T cell lineage or, indeed, of neither cell line, the so-called 'null' cells. Further identification of these cells using surface and cytoplasmic markers is therefore essential.

Enzyme markers, used with a standardized technique, can differentiate between T and B cells in human tissue sections. The technique does not permit the differentiation of T blasts, B blasts and plasma cells.

In periodontal disease, the enzyme marker technique demonstrates that the majority of the lymphoid cells of the inflammatory infiltration are non-T cells and probably B cells. It seems probable that other connective tissue diseases may be fruitfully investigated by this method.

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