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Identification of cells expressing T and p28,33 (Ia-like) antigens in sections of human lymphoid tissue

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SUMMARY

A method has been developed for identifying human lymphocyte populations in tissue sections using hetero-antisera to T cells and to the B cell associated Ia-like, p28,33 polypeptide complex. The differential topographical localisation of T and B cells is revealed by simultaneous staining with antibodies tagged with different fluorochromes; this approach also identifies a small population of T cells bearing Ia-like antigens. Preliminary studies on lymphomatous tissue suggests this approach will be of value in the study of malignancy and cellular infiltration in disease.

INTRODUCTION

In recent years the ability to identify and enumerate T and B lymphocytes in man has led to a better understanding of many disease processes especially in the immunodeficiencies and in haemopoietic malignancies (reviewed in Cooper & Seligmann, 1976; Greaves 1975; Hayward & Greaves, 1977). The application of cell surface markers in the study of inflammatory diseases has in general been limited to peripheral blood lymphocytes (Messner, Lindström & Williams, 1973; Papamichail, Sheldon & Holborow, 1974), or to cells extracted from mononuclear cell infiltrates (Strom *et al.*, 1975; Abrahamsen *et al.*, 1975; Claudy *et al.*, 1976). The activity of blood lymphocytes may not reflect accurately the activity of the cells present in peripheral inflammatory lesions and it would clearly be desirable to be able to study the cell types present *in situ* in the lesion. If lymphoid cell lineages and subsets could be reliably identified in tissue sections then when interfaced with histopathological and histochemical techniques this would greatly improve the rational interpretation of the observed pathology in relation to likely cellular function.

Various attempts have been made to identify T and B cells in sections. Although rosetting techniques to detect Fc and complement receptors give consistent results with sections of lymphoid tissue (Dukor, Bianco & Nussenzweig, 1970; Mendes, Miki & Peixinho, 1974) their use in the study of heterogeneous mononuclear cell infiltrates may be considered as being of limited value. Immunofluorescent techniques have previously been used to detect mouse T cells in tissue sections (Gutman & Weissman, 1971) whilst both immunofluorescence and immunoperoxidase methods have been used to demonstrate immunoglobulin bearing or containing cells in sections of human lymphoid tissue (Levine & Polliac, 1975; Curran & Jones, 1977). More recently Lamelin *et al.* (1978) have reported the use of anti-human T cell sera on sections of tonsillar tissue.

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MATERIALS AND METHODS

Tissue. Clinically normal adult lymph nodes were obtained from the inguinal region of nine patients undergoing arterial surgery. Similarly, tonsils were obtained from nine infant or juvenile patients undergoing tonsillectomy for recurrent streptococcal infections. Three lymph nodes were obtained from patients with suspected lymphoma. A testicular biopsy was obtained from a boy with a testicular relapse of T cell acute lymphoblastic leukaemia.

Tissue preparation. The tissues were cut in small pieces and were either fixed in formol-sucrose as previously described (Seymour, Dockrell & Greenspan 1976), fixed in cold 95% ethanol according to the method of Saint-Marie (1962) or immediately quenched in liquid nitrogen.

Twenty-five to thirty serial sections were cut from each block of tissue.

Staining. An haematoxylin and eosin (H & E) stain was carried out on a section from each block. Some of the formol-sucrose fixed sections were stained for non-specific esterase at pH 7·3 using a naphthyl acetate as substrate and incubating for 2 hr at 37°C (Seymour, Dockrell & Greenspan, 1978).

Antisera. The rabbit anti-monkey thymocyte antiserum has been previously described (Greaves & Janossy, 1976; Janossy *et al.*, 1977). It was raised by injecting 2×10^8 viable rhesus monkey thymocytes twice intravenously into a rabbit. The resultant antiserum was then absorbed three times with human AB red blood cells, three times with chronic lymphocytic leukaemia cells (CLL) having B-cell phenotype membrane. This serum was specific in immunofluorescence binding tests for T lineage cells (i.e. thymocytes and blood or lymphoid tissue cells) and did not react with monocytes, B cells or with the so called 'null' or 'L' cells in normal blood (Horwitz *et al.*, 1978). In cell suspension studies $f(ab')_2$ antibody fragments have to be used to maximise the cellular specificity (i.e. by abolishing binding to Fc receptors). This was not found to be necessary for studies on tissue sections providing the reagent was ultracentrifuged (100,000 *g*, 90 min) prior to use. The cells which react with this antiserum are referred to as HuTLA$^+$. Recent studies indicate that this serum interacts with at least two distinct T cell membrane polypeptides (Niaudet & Greaves 1978).

Rabbit anti-p28,33 serum (Schlossman *et al.*, 1976) was made by immunising rabbits with immune complexes (Freund's incomplete adjuvant) formed between human spleen extracts and anti-p28,33 serum (Welsh & Turner, 1976; Janossy *et al.*, 1977). For double labelling experiments with anti-T serum a chicken anti-p28,33 serum was made by an essentially similar procedure. The 'p28,33' complex consists of two non-covalently linked glycoprotein chains in SDS polyacrylamide gels of apparent mol. wt 28,000 and 33,000 Daltons (Springer *et al.*, 1977; Snary *et al.*, 1977). Human alloantibodies (i.e. anti-DRW) react with this same structure (Giphart *et al.*, 1977). The p28,33 complex is referred to as Ia-like by analogy with Ia antigens coded by the I region of mouse H-2 and with which they share structural similarity and sequence homology (McMillan *et al.*, 1977; Allison *et al.*, 1978).

These sera sometimes require some absorption with T cells (thymocytes) and myeloid tissue (e.g. chronic granulocytic leukaemia). Thereafter they stain B cells and some monocytes (Schlossman *et al.*, 1976; Welsh & Turner, 1976; Janossy *et al.*, 1977; Winchester *et al.*, 1976). More recent studies indicate that anti-p28,33 sera also react with myeloid precursor cells, myeloblasts and CFUc cells (Winchester *et al.*, 1977; Janossy *et al.*, 1977) and with a subset of normal blood T cells (Greaves *et al.*, 1979).

The cells which react with these anti-p28,33 sera are referred to as p28,33$^+$.

Immunofluorescence. A two layer indirect technique was used. The first layer consisted of one of the following: (a) rabbit anti-monkey thymocyte antiserum at a 1/5 dilution; (b) rabbit anti-p28,33 serum at a 1/5 dilution; (c) chicken anti-p28, 33 serum at a 1/5 dilution. The second layer of this indirect technique was also one of the following:

(a) Fluorescein (FITC) conjugated sheep anti-rabbit immunoglobulin (Ig) antiserum (Institut Pasteur, Paris) at a 1/200 dilution;

(b) Rhodamine (TRITC) conjugated goat anti-rabbit Ig antiserum (Nordic Immunochemicals) at a 1/100 dilution;

(c) Fluorescein conjugated sheep anti-chicken Ig antisera (kindly supplied by Dr Y. Ivanyi, Wellcome Research Laboratory, England) at a 1/100 dilution.

These antisera were absorbed extensively with pig liver powder to reduce non-specific or electrostatic binding of the conjugate to tissue components.

The concentrations of the primary antisera used as well as those of the conjugates were derived by serial dilutions of the sera to allow optimum specific staining with minimal background fluorescence.

The sections were incubated for 30 min in the primary antiserum, washed in phosphate buffered saline (pH 7·4) (PBS) for 30 min before reincubation with the second layer. Following 30 min incubation with the conjugates the sections were again washed for 30 min in PBS, mounted in PBS/glycerine (50/50) and examined using a Zeiss Standard 16 microscope with an IV/F epi-fluorescence condenser and narrow band filters for fluorescein and rhodamine excitation and observation.

Double labelling was carried out by first incubating the sections in the rabbit anti-monkey thymus antiserum, washing, then incubating with chicken anti-p28,33 serum, washing, reincubating with TRITC goat anti-rabbit Ig, washing, and finally incubating in FITC sheep anti-chicken Ig.

Control sections included normal rabbit serum or PBS as the first layer with the FITC and TRITC conjugates as the second layer.

Photographs were taken on Kodak high speed ektachrome film at 400 ASA.

RESULTS

Histologically both the clinically normal lymph nodes and the tonsils showed reactive hyperplasia with secondary follicle formation being evident. Furthermore, large blast-like cells were seen immediately beneath and in some cases within the tonsillar epithelium.

Although different techniques were used, the immunofluorescence results appeared to be similar irrespective of the type of tissue preparation. Subjectively, however, the unfixed or acetone post-fixed sections may have been superior.

Anti-immunoglobulin reagents gave bright staining of follicular regions as previously reported (Lamelin *et al.*, 1978; Seymour *et al.*, 1978). Under high power the staining was often diffuse and at least partly intercellular whereas in other samples clear cellular staining was observed (Fig. 1).

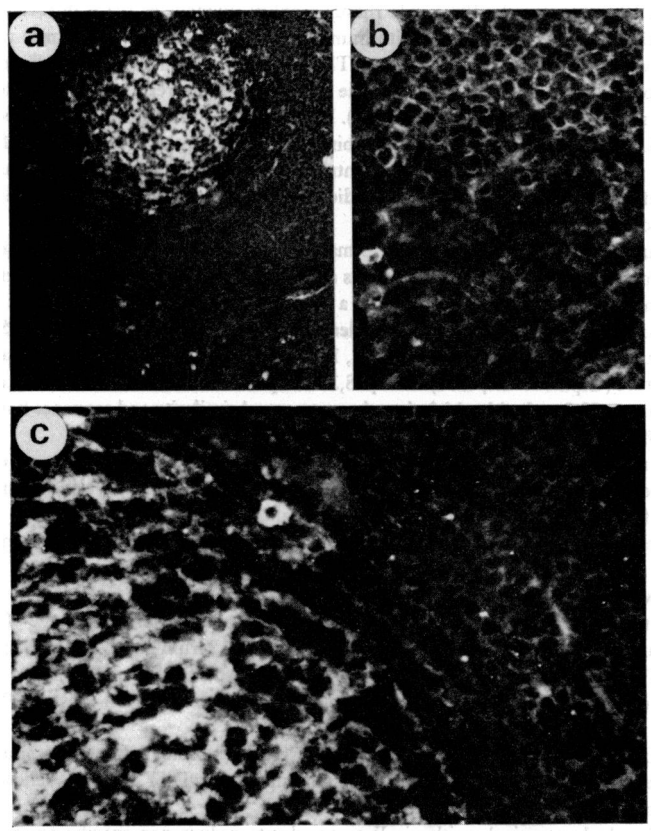

FIG. 1. Staining of lymphoid tissue with anti-Ig. Tonsil tissue, formol-sucrose fixed. (a) Low magnification showing positively stained follicle. (b) and (c) High power field of edge of follicle showing discrete (b) or diffuse (c) staining with anti-Ig.

HuTLA$^+$ cells were in general confined to the parafollicular and interfollicular areas (Fig. 2). A small number of antigen positive cells were seen in the small lymphocyte mantle of the secondary follicles. However, the majority of cells in these regions were HuTLA$^-$. Very faint staining of some cells within the germinal centres was observed with this anti-monkey thymus serum while at the same time intense fluorescence of the endothelial cells of blood vessels was noted (Fig. 2a).

Both HuTLA$^+$ and HuTLA$^-$ cells were seen immediately beneath the tonsillar epithelium, whereas very few HuTLA$^+$ cells were found in the medullary tissues of the lymph nodes.

Staining with the rabbit anti-p28,33 serum showed that antigen positive cells were mainly confined to the secondary follicles. The p28,33$^+$ cells seemed to include both the larger germinal centre cells and the

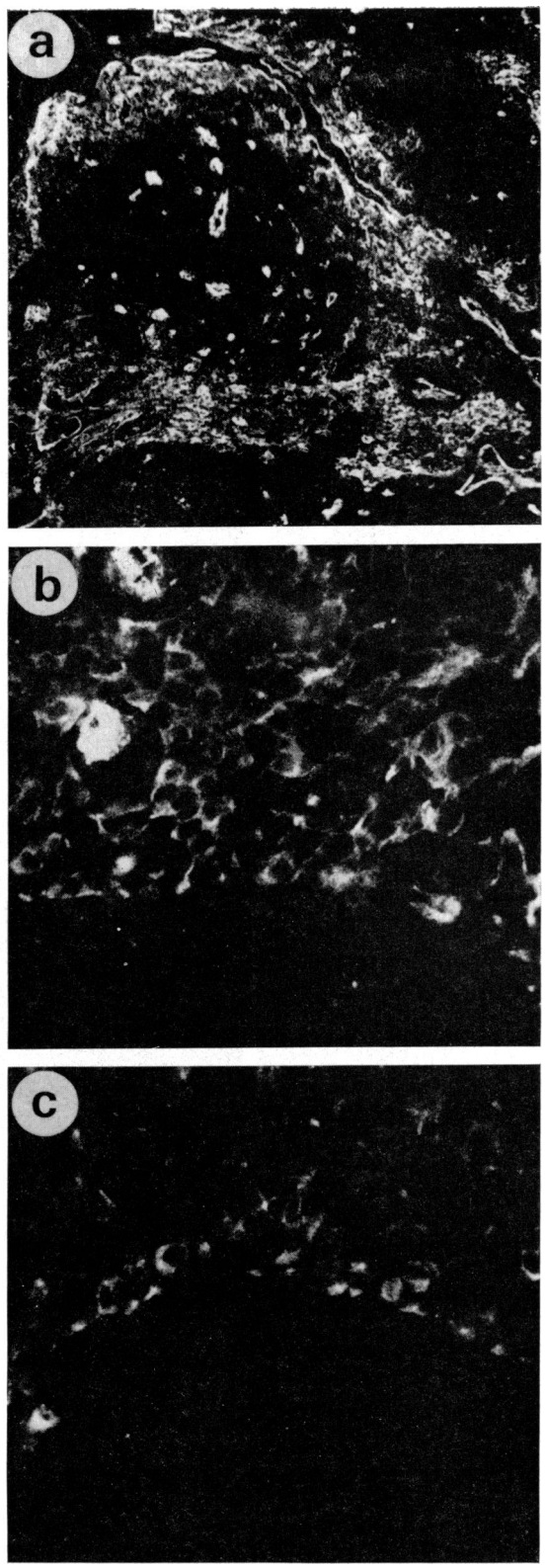

FIG. 2. Staining of lymphoid tissue with anti-T cell serum. Tonsil tissue, formol-sucrose fixed. (a) Interfollicular areas staining with anti-T serum showing additional reactivity of blood capillary endothelial cells. (b) High power view showing edge of follicle (in which cells are unstained) and surrounding perifollicular T cells. (c) As in (b).

smaller lymphocytes in the lymphocyte corona (Fig. 3). A considerable proportion of p28,33$^+$ cells were also found in the parafollicular areas especially in the tonsils and also the medullary areas of the lymph nodes. A small number of p28,33$^-$ cells were found in the follicles. Reactivity with endothelial cells was not seen with this reagent. Staining with p28,33 was largely reciprocal with non-specific esterase staining (Fig. 3d), which was anticipated from previous work (Seymour *et al.*, 1978).

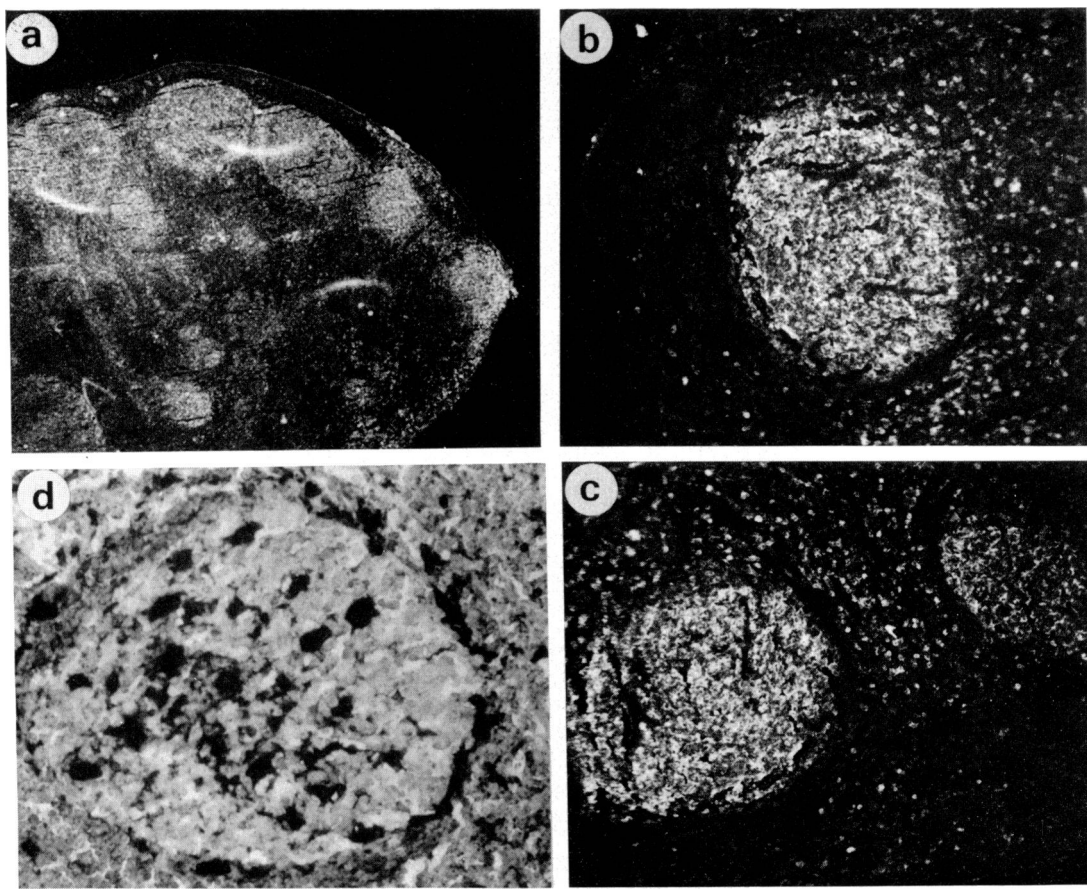

FIG. 3. Staining of lymphoid follicles with anti-Ia (p28,33) and a comparison with acid, non-specific esterase staining. Tonsil tissue fixed in formol sucrose. (a) Low power field showing a number of follicles. Note: 2 crescent shaped areas of bright staining are artefacts. (b) and (c) High power fields of stained follicles. (d) Follicle in section of same tissue stained with acid, non-specific esterase. Note: strong staining of intrafollicular and perifollicular macrophages, dot-like reaction of T cells in the surrounding paracortex and the lack of staining of follicular B lymphocytes.

Double labelling

The tissue used consisted of three lymph nodes and three tonsils. The specimens were either unfixed or acetone post-fixed cryostat sections.

By alternating filter combinations it was possible to establish that the majority of cells in the secondary folliculi were p28,33$^+$, HuTLA$^-$, whilst the parafollicular and interfollicular areas mainly consisted of HuTLA$^+$, p28,33$^-$ cells with a small admixed population of p28,33$^+$, HuTLA$^-$ cells. In addition, with this method it was possible to detect a few cells staining for both HuTLA and p28,33. These cells were found both in the secondary follicles as well as in the parafollicular areas. They were not infrequent in the tonsils but absent or very scarce in the lymph node sections.

These observations are illustrated in Fig. 4. When double exposure is used HuTLA$^+$/p28,33$^-$ cells

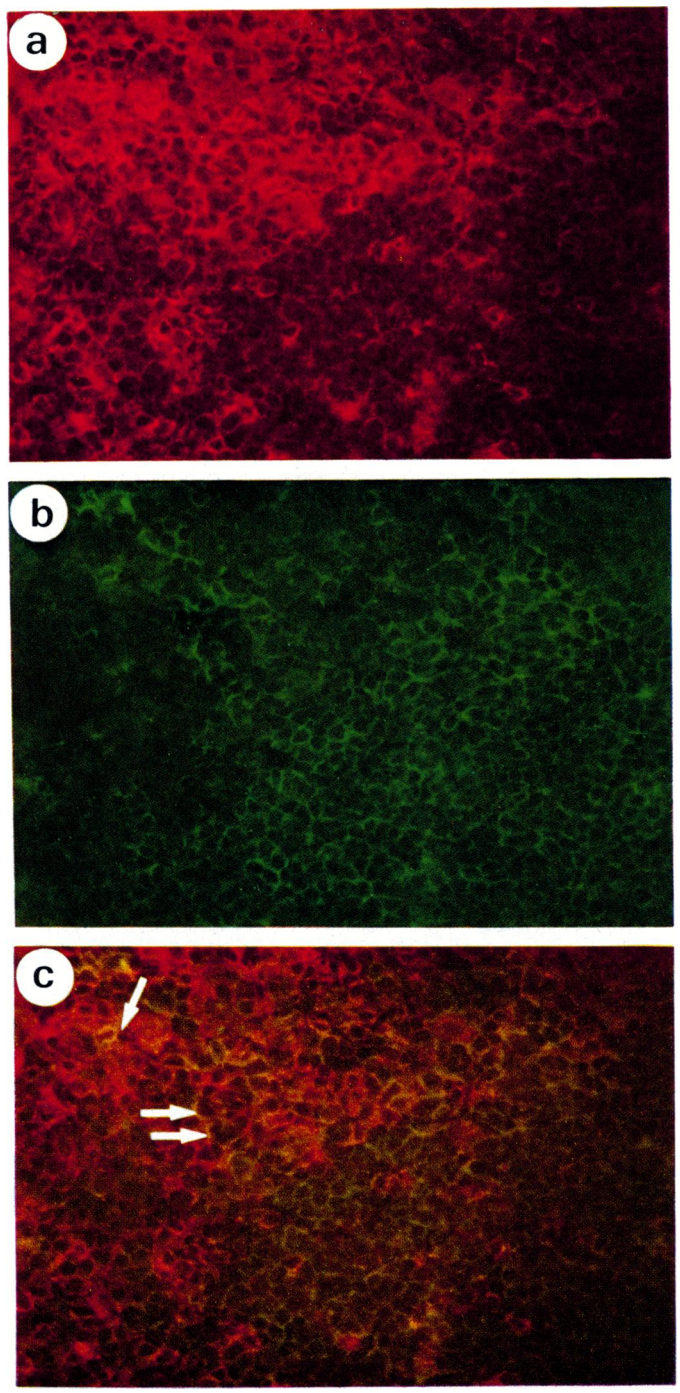

FIG. 4. 'Double' staining of tonsil tissue sections for T and Ia (p28,33) antigens. Unfixed section of tonsil. The section was stained with both rabbit anti-T and chicken anti-p28,33 as described under methods. (a) Observed and photographed with excitation and barrier filters for rhodamine excitation and observation. Shows anti-T cell serum stained perifollicular area and a small number of interfollicular cells. (b) Observed and photographed with excitation and barrier filters for fluorescein excitation and observation. Shows anti-p28,33 serum staining most cells in the follicle plus a few cells outside. (c) Double exposure using, sequentially, excitation and observation filter combinations for fluorescein and rhodamine. Cells staining with both anti-p28,33 and anti-T appear orange-yellow (see arrow).

are red and p28,33$^+$/HuTLA$^-$ cells are green. The few cells which stain with both antisera (HuTLA$^+$/p28,33$^+$) are distinguished by their orange-yellow colour.

Not all the HuTLA$^+$ cells within the secondary follicles were p28,33$^+$ and likewise not all the p28,33$^+$ cells in the parafollicular area were HuTLA$^+$. Tonsils and normal lymph nodes gave similar results although double stained cells (HuTLA$^+$, Ia$^+$) were rarer in the latter.

Preliminary studies on tissue from lymphomas and leukaemias

Three nodes from patients with suspected or confirmed lymphoma were studied. One node was from a patient with an equivocal diagnosis of non-malignant immunoreactive hyperplasia. In sections from this node both HuTLA$^+$ and p28,33$^+$ cells were observed (Fig. 5a). The HuTLA$^+$ cells were found in the parafollicular areas while the p28,33$^+$ cells were in general confined to the secondary follicles, i.e. an essentially normal picture.

In one node from a patient with confirmed non-Hodgkin's lymphoma only p28,33$^+$ cells were observed with a general destruction of tissue architecture (Fig. 5b) and in the third case studied (an

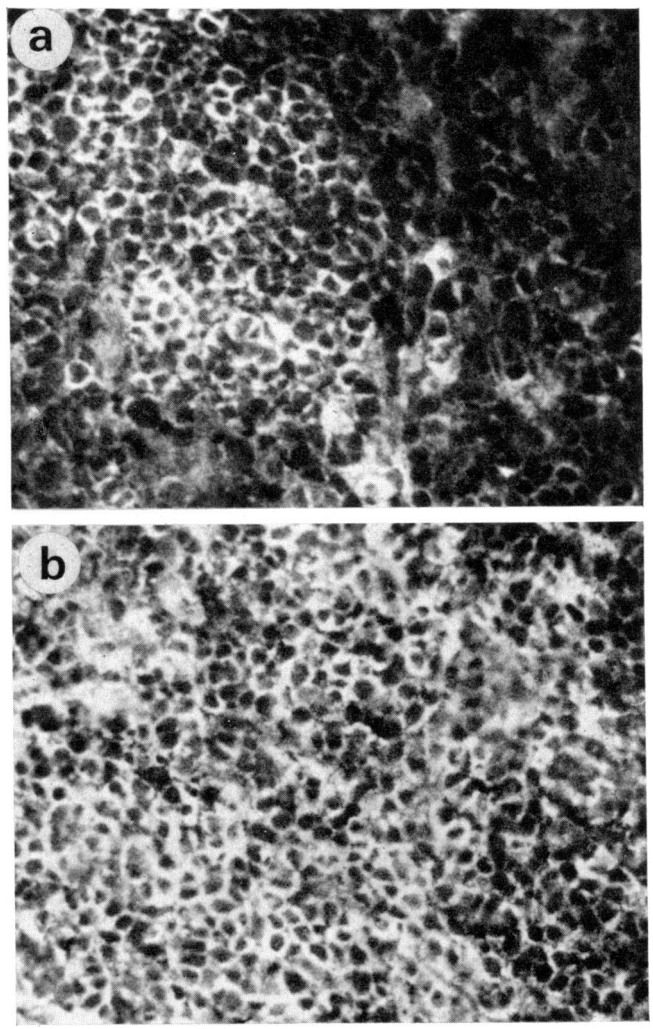

FIG. 5. Staining of clinically reactive node sections with anti-Ia (p28,33). (a) Non-malignant immuno-reactive node, edge of follicle shown with staining (B) cells and non-staining (T) cells. (b) 'Poorly differentiated' lymphocytic lymphoma with a B cell phenotype. Complete loss of normal tissue, follicular architecture; almost all cells are stained with anti-Ia (p28,33).

infiltrate of Sezary lymphoma) the enlarged node showed complete replacement by sheets of large lymphoid cells all of which stained with anti-T but not anti-p28,33, as anticipated from the usual T cell phenotype of Sezary lymphoma. A single testicular biopsy specimen from a case of T cell ALL in testicular relapse was also studied. In this instance large sheets of T^+, $p28,33^-$ cells were observed with considerable loss of normal tissue structure. Lymphoid cells teased from this tissue specimen were $HuTLA^+$, p28, 33^- and 90% made E rosettes.

DISCUSSION

The results of the present study indicate that the detection of lymphocyte differentiation antigens by an immunofluorescent technique in tissue sections provides a reliable approach to establish cellular identity *in situ*.

Murine T and B lymphocytes are found in different regions of the peripheral lymphoid organs; T lymphocytes are found predominantly in the parafollicular areas and B cells in the follicles and germinal centres (Parrott & De Sousa, 1971). Depletion of circulating T cells by neonatal thymectomy or thoracic duct drainage leads to a selective loss of cells from the paracortex (Gowans & Knight, 1964; Davies *et al.*, 1969; Parrott, De Sousa & East, 1966). T and B cells radiolabelled *in vitro* and traced *in vivo* home to their selective compartments of lymphoid tissue (Parrott & De Sousa, 1971; Howard, Hunt & Gowans, 1972). Immunofluorescent labelling of cells in murine lymph nodes has furthermore indicated that the majority of cells in parafollicular areas are thymus antigen positive, immunoglobulin negative. This is in contrast to the phenotype of most cells in the primary and secondary follicles which are thymus antigen negative, immunoglobulin positive (Gutman & Weissman, 1972; Greenspan *et al.*, 1974a, b).

The present study using two well defined hetero-antisera to lymphocyte differentiation antigens (anti-monkey thymus serum and anti-'Ia' or p28,33 serum) has demonstrated that the topographical localisation of human T and B cells in peripheral lymphoid organs is essentially the same as that described for the mouse and as suggested by the studies on human tissue referred to above. An important limitation of the anti-p28,33 (Ia) reagent is that in addition to B cells it may react with some macrophages, a subset of T cells as well as haemopoietic precursors (see under Materials and Methods). We cannot be sure, therefore, that all T^-, $p28,33^+$ cells are necessarily B cells.

Yata *et al.* (1970) have described the presence of a human thymus-lymphoid tissue antigen in the cytoplasm of human T cells; it is possible that the antisera used in the present study were defining such an antigen since much of the staining observed did not appear to be restricted to cell surfaces. It is also possible that the fixation and drying procedure allowed the surface antigens to diffuse into the cytoplasm of the cell. This might explain some or all of the cytoplasmic staining seen with both the anti-T and anti-p28,33 sera.

As anticipated from previous studies in mice (Gutman & Weissman, 1972) the topographical compartmentalisation of T and B cells was not absolute, some T^+, $p28,33^-$ cells being observed in the secondary follicles of tonsils and T^-, $p28,33^+$ cells detected in the paracortical, T cell dominant region. We cannot exclude that some of the latter cells were macrophages.

The 'blast-like' population seen beneath the tonsillar epithelium contained both $HuTLA^+$, $p28,33^-$ and $p28,33^+$, $HuTLA^-$ cells. This population might therefore represent cells of T and B origin, respectively.

In addition, double labelling experiments, revealed the presence of a few $HuTLA^+/p28,33^+$ cells in both the follicles and paracortex of tonsils; these cells were absent or extremely scarce in normal lymph nodes. A subpopulation of murine T cells expresses Ia antigens coded for by the Ij sub-region and are thought to be suppressor cells (Murphy *et al.*, 1976). A subset of normal human blood T cells also expresses the p28,33 hetero-antigen (Greaves *et al.*, 1979). The possible suppressor function of Ia $(DRW)^+$ human blood and tonsil T cells should now be investigated.

It has often proved difficult to distinguish between benign hyperplasia of lymphoid tissue and neoplastic lymphomas in terms of morphological criteria alone. Of the three enlarged lymph node biopsies examined in the present study one showed a normal topographical distribution of $HuTLA^+$ and $p28,33^+$

cells, while the others showed only p28,33$^+$ cells in one case and only HuTLA$^+$ cells in the other. These cases were diagnosed as benign hyperplasia, non-Hodgkin's lymphoma and Sezary syndrome respectively.

Finally, it is interesting to note that cross-reactivity existed between the anti-monkey thymus antiserum and the endothelial cells of the blood vessels. This may reflect a shared antigenic determinant between human T cells and endothelial cells in the same way as the Thy-1 antigen of murine T cells is also expressed on normal brain tissue (Reif & Allen, 1964), epidermal cells from skin (Scheid *et al.*, 1972) and normal fibroblasts (Stern, 1973).

The present study has therefore shown that human lymphoid tissue has well defined T and B cell areas. These areas are similar to those previously described for murine tissue (Gutman & Weissman, 1972) and can be demonstrated using antisera to lymphocyte differentiation antigens in an immunofluorescence technique. This method may provide a valuable tool in the study of neoplastic lymphomas and sites of chronic inflammation.

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REFERENCES

Abrahamsen, T.G., Froland, S.S., Natvig, J.B. & Pahle, J. (1975) Elution and characterisation of lymphocytes from rheumatoid inflammatory tissue. *Scand. J. Immunol.* **4**, 823.

Allison, J.P., Walker, L.E., Russell, W.A., Pellegrino, M.A., Ferrone, S., Reisfeld, R.A., Frelinger, J.A. & Silver, J. (1978) Murine Ia and human DR antigens: homology of amino-terminal sequences. *Proc. Natl. Acad. Sci. USA.* **75**, 3953.

Claudy, A.L., Schmitt, D., Viac, J., Alario, A., Staguet, M.J. & Thivolet, J. (1976) Morphological, immunological and immunocytochemical identification of lymphocytes extracted from cutaneous infiltrates. *Clin. exp. Immunol.* **23**, 61.

Cooper, M. & Seligmann, M. (1976) B and T lymphocytes in immunodeficiency and lymphproliferative diseases. *B and T Lymphocytes in Immune Recognition.* (Ed. by F. Loor and G. Roelants), p. 377. J. Wiley, London.

Curran, R.C. & Jones, E.L. (1977) Immunoglobulin containing cells in human tonsils as demonstrated by immunochemistry. *Clin. exp. Immunol.* **28**, 103.

Davies, A.J.S., Carter, R.L., Leuchars, E., Wallis, V. & Koller, P.C. (1969) The morphology of immune reactions in normal, thymectomised and reconstituted mice. I. The response to sheep erythrocytes. *Immunol.* **16**, 57.

Dukor, P., Bianco, C. & Nussenzweig, V. (1970) Tissue localisation of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. *Proc. Natl. Acad. Sci., USA.* **67**, 991.

Giphart, M.J., Kaufman, J.F., Fuks, A., Albrechtsen, D., Solheim, B.G., Bruning, J.W. & Strominger, J.L. (1977) HLA-D associated alloantisera react with molecules similar to Ia antigens. *Proc. Natl. Acad. Sci. USA.* **74**, 3533.

Gowans, S.L. & Knight, E.S. (1964) The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond.* [*Biol*]. **159**, 257.

Greaves, M.F. (1975) Clinical applications of cell surface markers. *Progr. Hematol.* **9**, 255.

Greaves, M.F. & Janossy, G. (1976) Antisera to human T-lymphocytes. *In vitro Methods in Cell-mediated and Tumor Immunity* (Ed. by B.R. Bloom and J.R. David) p. 89. Academic Press, New York.

Greaves, M.F., Verbi, W., Festenstein, H., Papasteriadis, Chr., Jaraquemada, D. & Hayward, A. (1979) 'Ia-like' antigens on human T cells. *Eur. J. Immunol.* **9**, 356.

Greenspan, J.S., Gutman, G.A., Weissman, I.L. & Talal, N. (1974a) Thymus-antigen- and immunoglobulin-positive lymphocytes in tissue infiltrates of NZB/NZW mice. *Clin. Immunol. Immunopathol.* **3**, 16.

Greenspan, J.S., Gutman, G.A., Talal, N., Weissman, I.L. & Sugai, S. (1974b) Thymus-antigen- and immunoglobulin-positive cells in lymph nodes, thymus and malignant lymphomas of NZB/NZW mice. *Clin. Immunol. Immunopathol.* **3**, 32.

Gutman, G.A. & Weissman, I.L. (1971) The bone marrow origin of lymphoid primary follicle small lymphocytes. *Morphological and Functional Aspects of Immunity.* (Ed. by K. Lindahl. G. Alm and M. Hanna) p.595. Plenum Press, New York.

Gutman, G.A. & Weissman, I.L. (1972) Lymphoid tissue architecture: experimental analysis of the origin and distribution of T-cells and B-cells. *Immunology.* **23**, 465.

Hayward, A. & Greaves, M.F. (1977) Human T- and B-lymphocyte populations in blood. *Recent Advances in Clinical Immunology.* Vol. 1. (Ed. by R.A. Thompson) p. 149. Churchill Livingstone, Edinburgh.

Horwitz, D.A., Niaudet, P., Greaves, M.F., Dorling, J. & Deteix, P. (1978) Surface markers and electron microscopy of human blood L cells. A comparison with T and B lymphocytes. *J. Immunol.* **121**, 678.

Howard, J.C., Hunt, S.V. & Gowans, J.L. (1972) Identification of marrow-derived and thymus-derived small lymphocytes in the lymphoid tissue and thoracic duct lymph of normal rats. *J. exp. Med.* **135**, 200.

JANOSSY, G., GOLDSTONE, A.H., CAPELLARO, D., GREAVES, M.F., KULENKAMPFF, J., PIPPARD, M. & WELSH, K. (1977) Differentiation linked expression of p28,33 (Ia-like) structures on human leukaemic cells. *Br. J. Haematol.* **37**, 391.

LAMELIN, J.-P., THOMASSET, N., ANDRE, C., BROCHIER, J. & REVILLARD, J-P. (1978) Study of human T and B lymphocytes with heterologous antisera. III. Immunofluorescence studies on tonsil sections. *Immunology.* **35**, 463.

LEVINE, G.D. & POLLIAC, A. (1975) The T-cell nature of the lymphocytes in two human epithelial thymomas: a comparative immunologic scanning and transmission electron microscope study. *Clin. Immunol. Immunopathol.* **4**, 199.

MCMILLAN, M., CECKA, J.M., MURPHY, D.B., MCDEVITT, H.O. & HOOD, L. (1977) Structure of murine Ia antigens: partial NH_2-terminal amino acid sequences of products of the I-E or I-C subregion. *Proc. Natl. Acad. Sci. USA.* **74**, 5135.

MENDES, N.F., MIKI, S.S. & PEIXINHO, Z.F. (1974) Combined detection of human T- and B-lymphocytes by rosette formation with sheep erythrocytes and zymosan-C3 complexes. *J. Immunol.* **113**, 531.

MESSNER, R.P., LINDSTRÖM, F.D. & WILLIAMS, R.C. JR. (1973) Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosis. *J. Clin. Invest.* **52**, 3046.

MURPHY, D.B., HERZENBERG, L.A., OKUMURA, K. & MCDEVITT, H. (1976) A new I sub-region (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T-lymphocytes. *J. Exp. Med.* **144**, 699.

NIAUDET, P. & GREAVES, M.F. (1978) Identification of T lineage associated membrane proteins. *Human Lymphocyte Differentiation: Its application to Cancer.* (Ed. by B. Serrou and C. Rosenfeld) p. 117. North-Holland, Amsterdam.

PAPAMICHAIL, M., SHELDON, P.J. & HOLBOROW, E.J. (1974) T- and B-cell subpopulations in infectious mononucleosis. *Clin. exp. Immunol.* **18**, 1.

PARROTT, D.M.V. & DE SOUSA, M.A.B. (1971) Thymus-dependent and thymus-independent populations: origin, migratory patterns and lifespan. *Clin. exp. Immunol.* **8**, 663.

PARROTT, D.M.V., DE SOUSA, M.A.B. & EAST, J. (1966) Thymus-dependent areas in the lymphoid organs of neonatally thymectomized mice. *J. Exp. Med.* **123**, 191.

REIF, A.E. & ALLEN, J.M.V. (1964) The AKR thymic antigen and its distribution in leukaemias and nervous tissue. *J. Exp. Med.* **120**, 413.

SAINT-MARIE, G. (1962) A paraffin-embedding technique for studies employing immunofluorescence. *J. Histochem. Cytochem.* **10**, 250.

SCHEID, M., BOYSE, E.A., CARSWELL, E.A. & OLD, L.J. (1972) Serologically demonstrable alloantigens of mouse epidermal cells. *J. Exp. Med.* **135**, 938.

SCHLOSSMAN, S.M., CHESS, L., HUMPHREYS, R.E. & STROMINGER, J.L. (1976) Distribution of Ia-like molecules on the surface of normal and leukaemic human cells. *Proc. Natl. Acad. Sci. USA.* **73**, 1288.

SEYMOUR, G.J., DOCKRELL, H.M. & GREENSPAN, J.S. (1976) Fixation for immunofluorescence and enzyme histochemistry on serial sections. *J. Histochem. Cytochem.* **24**, 1112.

SEYMOUR, G.J., DOCKRELL, H.M. & GREENSPAN, J.S. (1978) Enzyme differentiation of lymphocyte subpopulations in sections of human lymph nodes, tonsils and periodontal disease. *Clin. exp. Immunol.* **32**, 169.

SNARY, D., BARNSTABLE, C.J., BODMER, W.F., GOODFELLOW, P.N. & CRUMPTON, M.J. (1977) Cellular distribution, purification and molecular nature of human Ia antigens *Scand. J. Immunol.* **6**, 439.

SPRINGER, T.A., KAUFMAN, J.F., TERHORST, C. & STROMINGER, J.L. (1977) Purification and structural characterisation of human HLA-linked B-cell antigens. *Nature* **268**, 213.

STERN, P.L. (1973) θ alloantigen on mouse and rat fibroblasts. *Nature* **246**, 76.

STROM, T.B., TILNEY, N.L., CARPENTER, L.B. & BUSCH, G.J. (1975) Identity and cytotoxic capacity of cell infiltrating renal allografts. *N. Engl. J. Med.* **292**, 1257.

WELSH, K.I. & TURNER, M.J. (1976) Preparation of antisera specific for human B-cells by immunization of rabbits with immune complexes. *Tissue Antigens* **8**, 197.

WINCHESTER, R.J., WANG, C.Y., HALPER, J. & HOFFMAN, T. (1976) Studies with B-cell allo- and heteroantisera: parallel reactivity and special properties. *Scand. J. Immunol.* **5**, 745.

WINCHESTER, R.J., ROSS, G.D., JAROWSKI, C.I., WANG, C.Y., HALPER, J. & BROXMEYER, H.E. (1977) Expression of Ia-like antigen molecules on human granulocytes during early phases of differentiation. *Proc. Natl. Acad. Sci. USA.* **74**, 4012.

YATA, J., KLEIN, G., KOBAYASHI, N., FURUKAWA, T. & YANAGISAWA, M. (1970) Human thymus-lymphoid tissue antigen and its presence in leukaemia and lymphoma. *Clin. exp. Immunol.* **7**, 781.