Density of T4 and T8 lymphocytes in inflammatory foci and three superficial layers of the intervening dermis (cells/mm²)

Regions of dermis	Section treatment	Set of sections	T8 density	T4 density	T4:T8 ratio
Focal infiltrate	None	A	1025	3601	3·51
		B	804	2687	3·34
		C	790	2368	3·00
	Acetone/glycerol	D	944	3502	3·71
		E	983	3369	3·42
		F	944	4044	4·28
Most superficial 240 μm layer of dermis (zone A)	None	A	314	972	3·10
		B	282	789	2·80
		C	319	1164	3·65
	Acetone/glycerol	D	420	1701	4·05
		E	500	1980	3·96
		F	402	1884	4·69
Next 240 μm layer of dermis (zone B)	None	A	407	1403	3·45
		B	399	1239	3·23
		C	296	1878	6·34
	Acetone/glycerol	D	433	1267	2·93
		E	309	1574	5·09
		F	221	1191	5·41
Next 240 μm layer of dermis (zone C)	None	A	198	484	2·44
		B	154	579	3·76
		C	172	1191	6·92
	Acetone/glycerol	D	274	709	2·59
		E	223	812	3·64
		F	93	611	6·57

results of the histometric studies. Although there are differences in the density of the cells of the two main subsets in the inflammatory foci and the three superficial layers of the dermis, the overall variation between the staining of sections from the two blocks was no more than would be expected from a knowledge of the histopathology of the human Mantoux reaction.[3]

The distributions of staining of the pan-T markers (Leu 1 and Leu 4), the Il2 and transferrin receptors (OKT9, Ortho) and OKT10 (an activation marker of T cells), M1 (Leu M3) (macrophage marker), and of OKT6 (Langerhans' cell marker) were similar in sections from the two blocks. Interestingly the staining of Il2 receptors with the Tac monoclonal antibody was much more intense in sections from the acetone and cryopreservant treated block than in sections from the uncontaminated block (Figure). In all studies the sections of the treated blocks were much easier to interpret because of the increased refractility of the dermal collagen and the presence of faint outlines of the skin appendages. Moreover, the sections showed many fewer folds, tears, or other distortions than those from unfixed blocks.

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Versatile field's stain

Field's stain is widely used as a rapid staining technique for thick blood films for diagnosing malaria.[1] Thin films are usually stained with Giemsa stain (Gurr's R66, BDH, Poole, Dorset), which requires staining for up to 30 minutes. Dacie and Lewis described the use of a modified Field's stain for rapid staining of thin blood films.[2] Here we describe its application to the staining of other protozoa and also microfilariae.

The modification to the Field stain (BDH, Poole, Dorset) consists of diluting one volume of solution B with four volumes of buffered distilled water at pH 7·2; solution A is used in its prepared form without dilution. Thin blood films are fixed in methanol for 30 seconds, the alcohol tipped off, and the slide covered with 1 ml of the diluted Field stain B. An equal volume of undiluted Field stain A is added immediately and the two parts mixed well on the slide with a Pasteur pipette. After one minute the slide is rinsed in tap water and blotted or air dried.

All malaria films stained by this method showed adequate staining of all stages of *Plasmodium* including the Schuffner's and James's dots of *P vivax* and *P ovale*. Trypomastigote forms of *Trypanosoma* stained well in thin blood films and also in lysed, fixed, and thick blood films. *Leishmania* amastigotes in slit skin smears and bone marrow aspirations stained in a similar manner gave excellent diagnostic features. Microfilariae were well stained but

may need five seconds' differentiation in tap water to give discrete nuclear patterns. The sheaths were not stained by this method. The stain is also useful for permanent faecal smear preparations of trophozoites of protozoa, such as *Giardia, Trichomonas,* and amoebae, but it did not give satisfactory staining of all forms of cyst.

We think that this staining method has great potential for rapid diagnosis in the laboratory and is a useful "one stain" method for field work.

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Expression of leucocyte common antigen and epithelial membrane antigen in plasmacytic malignancies

Immunoperoxidase staining of paraffin sections with monoclonal antibodies is being used increasingly as a diagnostic aid in histopathology. Monoclonal antibodies against the leucocyte common antigen[1] and epithelial membrane antigen[2 3] are of potential value in distinguishing between lymphoid and epithelial neoplasms. Some plasma cells react with antibodies against epithelial membrane antigen in paraffin sections, however, and a recent report by Delsol *et al*[4] has shown that epithelial membrane antigen is also expressed in some non-Hodgkin's lymphomas. The leucocyte common antigen shows variable expression in non-Hodgkin's lymphoma, being reduced or absent in lymphocytic differentiation towards plasma cells.[5] It is therefore theoretically possible that a lymphoma showing plasmacytoid differentiation may express both, either, or neither of these antigens. We have compared results of staining with antibodies to epithelial membrane antigen and leucocyte common (Dako LC and F8-11-13) in eight cases: five cases of non-Hodgkin's lymphoma with plasmacytoid differentiation, as shown by strong cytoplasmic immunoglobulin detected by immunoperoxidase staining; two cases of plasma cell leukaemia; and one case of myeloma. The Table shows the results.

In four of the eight cases cells reacted with antibody to epithelial membrane antigen. Most of the cells in three of these cases showed strong membrane staining; variable results were seen with antibodies to the leucocyte common antigen. In one case (no 5), in which an immunoblastic lymphoma had developed in a patient with multiple myeloma, there was no staining with either monoclonal antibodies against the leucocyte common antigen but strong staining was seen with antibody to epithelial membrane antigen. Dako leucocyte common reacted with cells in seven of eight cases, staining the majority of the neoplastic cells in five cases and a proportion of the cells in two others. F8-11-13, which recognises a high molecular weight form of the leucocyte common antigen[6] did not stain cells in three of the eight cases and stained only a minority of the cells in the remainder.

Our observations confirm that antibodies against epithelial membrane antigen react with a proportion of lymphomas and plasma cell leukaemias and agree with recent reports.[4 7 8] The failure of antibodies against the leucocyte common antigen to stain a number of our cases and to react with only a proportion of the cells in others is consistent with the fact that the leucocyte common antigen may be lost during the differentiation of B cells; the high molecular weight form recognised by F8-11-13 is lost earlier.[5 9]

These findings have obvious diagnostic implications. Immunoreactivity with epithelial membrane antigen in the absence of reactivity with antibodies to leucocyte common antigen suggests an epithelial histogenesis of a neoplasm. In some cases of non-Hodgkin's lymphoma, showing plasmacytoid differentiation, the typical staining reactions may be reversed, however; the lymphoid cells reacting with antibodies to epithelial membrane antigen and not with antibodies to leucocyte common antigen. Pizzolo *et al*[10] have reported a case in which the morphological appearance was that of a lymphoma but cells failed to react with antibodies to leucocyte common antigen, immunoglobulin, T and B cell markers but did react with antibody to epithelial membrane antigen. In most cases plasmacytic malignancies may be identified by their characteristic morphological features, but in some cases, especially those of immunoblastic lymphoma, differentiation between lymphoma and anaplastic carcinoma may be difficult. It is in these cases that monoclonal antibodies such as those against epithelial membrane antigen and leucocyte common antigen should be of value to the diagnostic histopathologist.

The loss of leucocyte common antigen and the expression of epithelial membrane antigen in lymphoid neoplasms which are differentiating towards plasma cells should, however, be kept in mind when evaluating the results of staining with these antibodies. In cases where doubt persists as to the diagnosis we agree with recent comments by Heyderman and MacCartney[7]

Immunoperoxidase staining of plasmacytic malignancies with monoclonal antibodies to leucocyte common antigen and epithelial membrane antigen

Case no	Diagnosis	Monoclonal antibodies		
		F8-11-13	Dako LC	Epithelial membrane antigen
1	Malignant lymphoma, plasmacytic	+	+++	Neg
2	Malignant lymphoma, diffuse centroblastic	Neg	++	Neg
3	Malignant lymphoma, diffuse centroblastic	Neg	+++	Neg
4	Malignant lymphoma, immunoblastic/plasmacytoma	+	+	+
5	Malignant lymphoma, immunoblastic/plasmacytoma	Neg	Neg	+++
6	Multiple myeloma	+	+	Neg
7	Plasma cell leukaemia	+	++	+++
8	Plasma cell leukaemia	++	++	+++

+ = 2–20%; ++ = 20–70%; +++ = >70% positive staining cells.
Samples from cases 1–6 were fixed in buffered formaldehyde and processed for paraffin embedding and section; in cases 7 and 8 cytospins of blood mononuclear cell suspensions were made and fixed in acetone. In all cases staining for immunoglobulins showed the neoplastic cells to be strongly positive for intracytoplasmic immunoglobulin.
An indirect immunoperoxidase staining technique was used as described by Salter *et al*.[5]