Combined immunological and histochemical analysis of skin and lymph node lesions in histiocytosis X

JA THOMAS, G JANOSSY, M CHILOSI, J PRITCHARD, JR PINCOTT

From the *Department of Immunology, Royal Free Hospital School of Medicine, London NW3, the †Institute of Histopathology, University of Padua, Verona, Italy, and the Departments of §Haematology and Oncology §Pathology, Hospital for Sick Children, Great Ormond Street, London WC1

SUMMARY The immunological phenotype of the cells involved in skin and lymph node lesions from two cases of histiocytosis X (H-X) were analysed by immunofluorescence techniques using combinations of heterologous and monoclonal antisera to Ia-like antigen and human cortical thymocyte (HTA-1) determinant. These cells were also characterised by a new technique using simultaneous immunofluorescence and enzyme histochemistry for acid phosphatase (ACPase). The major cell type in the lesions was found to express the same Ia+, HTA-1+ phenotype as normal epidermal Langerhans' cells (LC) and was unreactive for ACPase. Additional cell types included Ia-, HTA-1- multinucleate giant cells and residual lymphoid populations. These findings endorse previous concepts that H-X is a proliferation of abnormal LC and emphasise the heterogeneous nature of the cells involved in the disease.

Histiocytosis X (H-X) is a generic term encompassing a group of rare clinical disorders (Hand-Schüller-Christian syndrome, Letterer-Siwe disease and eosinophilic granuloma) which show similar histological features. The lesions in these diseases contain granulomatous infiltrates of histiocytic cells with abundant eosinophilic cytoplasm. There is now extensive evidence to show that these cells are morphologically and ultrastructurally similar to epidermal Langerhans' cells (LC). Both cell types contain ultrastructurally distinct cytoplasmic bodies of Birbeck granules and are histochemically active for adenosine triphosphatase (ATPase) and α-analpyl acetate esterase (ANAE). In addition, LC weakly express surface Fc IgG and C3 receptors and contain abundant amounts of Ia-like antigen. Recent immunological studies have revealed surface receptors for HTA-1 (human thymocyte antigen) on LC defined by monoclonal antibodies NA1/34 and OKT6. The expression of HTA-1 and Ia-like antigen on LC provides an important new phenotype (HTA-1+, Ia+) by which these cells may be identified in immunohistological analyses of normal and pathological tissues. It is possible to identify the phenotypic expression of different immunological markers on the same cell type using two colour immunofluorescence techniques.

In addition to routine histological staining procedures, enzyme histochemistry has been used for many years to identify further various cell types in histological preparations. Hitherto, immunological and histochemical techniques have been confined to separate tissue sections as the optimal tissue processing for each method was considered to be different and incompatible. In this study, combined immunological and histochemical markers have been used to identify the proliferating cells in cutaneous and lymph node lesions from two cases of histiocytosis X. The aims of the study were threefold. Combinations of heterologous and monoclonal antibodies to lymphoid and non-lymphoid cells were used in double-labelled immunofluorescence (IF) studies on frozen sections (i) to establish whether there is a phenotypic relation between HTA-1+, Ia+ LC and H-X cells, (ii) to characterise the relation between H-X cells and other cell types and (iii) to compare the histochemical characteristics of different cells with their immunological phenotype.

Material and methods

Tissue preparation

A portion of each tissue biopsy (skin from case 1; lymph node from case 2) was processed for routine histology (haematoxylin and eosin), by 10% formalin...
fixation, and paraffin wax embedding. The other portions to be used in the immunological and histochemical studies were snap-frozen in isopentane (2-methyl-butane: BDH) and liquid nitrogen and stored at −70°C. Cryostat sections (6 μm and 10 μm) were mounted on to clean glass slides, air-dried for 12 h at room temperature (20°C) before fixation in cold ethanol (4°C) for 3 min. Unfixed cryostat sections were also successfully stored at −20°C before fixation.

**ANTISERA**
The panel of heterologous and monoclonal antisera are shown in Table 1. The specificities of these reagents have been previously characterised in cell suspension and frozen sections of normal12,13 and pathological12 tissues. Combinations of indirectly labelled heterologous antisera to Ia-like antigen (chicken (C) anti-Ia), human T leucocyte antigen (rabbit (R) anti-HuTLA) and directly labelled antisera to human immunoglobulin subclasses were used to identify B (Ia+, Ig+) and T (HuTLA+, Ig−) lymphocytes. Cells expressing receptors for HTA-1 determinant and Ia-like antigen were demonstrated by monoclonal antibodies OKT6 or NA1/34 in combination with C antiserum to Ia-like antigen. The histochemical staining reaction for acid phosphatase (ACPase) was used simultaneously with fluorescent labelled antisera to HTA-1 or Ia-like antigen.

**IMMUNOFLUORESCENT TECHNIQUES**
Direct and indirect IF techniques were used on 6 μm cryostat sections. Applications of 5-10 μl of appropriately diluted primary antisera were made to moist sections then incubated in a damp chamber at 20°C for 30 min. Excess antibody was removed by washing in phosphate-buffered saline (PBS) pH 7-6 at 20°C for 10 min. In the indirect IF test, species-specific rhodamine (tetramethyl rhodamine-isothiocyanate: TRITC) or fluorescein (fluorescein-isothiocyanate: FITC) labelled second layer reagents were applied to the sections for a further 30 min. After final rinsing in PBS pH 7-6, the sections were mounted in 1% formal glycerol under a coverslip and examined with a ×40 oil objective on a Zeiss 14 epifluorescence IV/Z microscope. Two distinctly labelled cell populations could be examined simultaneously using a selective (red/green) filter attachment.

**HISTOCHEMICAL TECHNIQUES**
Enzyme histochemistry was performed on 10 μm cryostat sections prefixed in cold (4°C) calcium formalin for 3 min. Modifications of standard histochemical methods described by Chilosi et al15 were used to demonstrate activity for ACPase, ANAE, ATPase and tartrate-resistant acid phosphatase (TRAP).

**COMBINED IMMUNOFLUORESCENCE AND HISTOCHEMICAL TECHNIQUES**
Ethanol-fixed (4°C) 6 μm cryostat sections were initially stained in the indirect IF test with TRITC-labelled C-anti-Ia-like antigen or monoclonal antibody to HTA-1 determinant (OKT6 or NA1/34). After the final rinse in PBS pH 7-6, the sections were immediately processed for the ACPase reaction using the modified Gomori metal salt procedure.15,16 The IF sections were briefly (60 s) postfixed in 10% formalin, rinsed in distilled water, then immersed in freshly filtered incubation medium* at 37°C for 60 min. After two short washes in distilled water, 0.1 M acetate buffer pH 5; 0.24% lead nitrate (Sigma); 13% sodium β glycerophosphate (Sigma). The medium was incubated at 37°C until complete precipitation of lead phosphate (6-24 h), cooled and filtered before use.

### Table 1 Immunological reagents used in the direct and indirect IF analysis of cryostat sections

<table>
<thead>
<tr>
<th>First layer antibody*</th>
<th>Second layer antibody*</th>
<th>Reactivity pattern</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken (C) anti-Ia-like antigen (1/80)</td>
<td>Sheep anti-C-IgG (Fab2)</td>
<td>+ + IDR cells, LC “veiled”+ + B cells; ± myeloblasts</td>
<td>12, 13</td>
</tr>
<tr>
<td>Rabbit (R) antihuman T leucocyte antigen (HuTLA) (1/20)</td>
<td>TRITC (1/20)</td>
<td>cells; + B cells; ± myeloblasts</td>
<td>12, 14</td>
</tr>
<tr>
<td>FITC/TRITC conjugated antisera to whole human Ig, IgM, IgD, IgG, k, λ raised in goat and burro</td>
<td>Goat (G) anti-R-IgG (Fab2)</td>
<td>+ thymocytes; peripheral T cells, Thy-ALL blasts</td>
<td>12</td>
</tr>
<tr>
<td>Mouse (M) monoclonal antibodies to human thymocyte antigen HTA-1 NA1/34</td>
<td>FITC (1/10)</td>
<td>+ B cells, plasma cells</td>
<td>9</td>
</tr>
<tr>
<td>OKT6 (1/10)</td>
<td>G-anti-M-IgG-FITC (1/10)</td>
<td>+ cortical thymocytes (90%)</td>
<td>9, 10, 11</td>
</tr>
<tr>
<td>G-anti-M-IgG-FITC (1/10)</td>
<td>- medullary thymocytes (5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Dilutions of antisera shown in brackets.
† Culture supernatant.
‡ Ascitic fluid.
the enzyme reaction was developed in 0.5% yellow ammonium sulphide (Sigma) for 2 min. The sections were rinsed finally in distilled water and mounted under a coverslip in 1% formol glycerol. Both the brown enzyme reaction product and TRITC-labelled antibodies could be easily examined under the fluorescence microscope using phase contrast and the appropriate (green) fluorescence filter channel.

Precautions were taken to ensure that the same enzyme reaction patterns were obtained in the combined IF/enzyme tests as were demonstrated on sections which had been treated separately for IF or ACPase activity. Separate sections were stained for IF followed by ACPase localisation using either the pararosaniline16 or lead salt methods. Photographs of marked areas on the slide were taken after each procedure which were later superimposed to show identical areas of ACPase activity.

PATIENTS

Case 1
An 8-year-old boy presented in April 1980 with polydipsia and polyuria following a short influenza-like illness. Diabetes insipidus was diagnosed and he was treated with 1-diamino-8-D-arginine vasopressin (DDAVP). Five months later in September 1980, he developed severe breathlessness, weight loss and general lassitude. He was readmitted to hospital and on examination he was noted to have generalised areas of depigmentation (face, axillae, trunk and knees) as well as a papular eruption over the back. There was no lymphadenopathy or hepatosplenomegaly. Chest x-ray revealed a right pneumothorax and reticular changes in both lungs. No bone lesions were apparent on skeletal survey. The diagnosis of histiocytosis X was confirmed by skin biopsy and a right pleurectomy was performed. Postoperatively the child was treated with vinblastine and prednisolone and his condition markedly improved. At the latest follow-up (July 1981) the patient was well and continues on a modified regimen of vinblastine, and DDAVP to control the diabetes insipidus.

Case 2
A 6-month-old baby boy of Turkish Cypriot origin presented in March 1980 with failure to thrive, swellings behind the ears and apparent bone pain. On examination he had marked bilateral lymphadenopathy (cervical, parotid, submandibular, postauricular) and hepatosplenomegaly. In addition there was a petechial lesion on the left foot and papular lesions over the scalp. An x-ray examination showed multiple lytic areas throughout the skeleton (skull, femora, tibiae, humeri, lumbar vertebrae, ribs) as well as a large anteromedial shadow. There were no radiological lung changes. Excision biopsy of a left upper cervical lymph node showed the histological features consistent with histiocytosis X. Treatment with vinblastine, prednisolone and antibiotics was followed by a rapid and marked clinical improvement. The lymphadenopathy, mediastinal mass and skeletal lesions resolved within three months of starting therapy. At 12 month follow-up, having continued on an intermittent regimen of vinblastine and steroids, the patient was well and developing normally.

Results

HISTOLOGICAL PREPARATIONS
(HAEMATOXYLIN AND EOSIN)
In case 1, the skin (Fig. 1a) showed marked narrowing of the epidermis with focal areas of large, round cells containing eccentric nuclei infiltrating the dermis and epidermis. These cells were accompanied by scanty numbers of small lymphocytes. In case 2, the normal lymph node (Fig. 1b) architecture was virtually replaced by a profuse, mixed cell infiltrate consisting of irregular histiocytes with abundant eosinophilic cytoplasm, moderate numbers of plasma cells, lymphocytes and occasional eosinophils. The histiocytic cells showed wide morphological variation from bizarre multinucleate giant cells (containing from 6 to 12 nuclei) to smaller, irregular mononuclear forms with vesicular and folded nuclei. Small aggregates of residual lymphocytes were clearly present in the tissue apparently compressed by the abnormally proliferating cell population. Mitotic figures were occasionally recognised in both biopsies but these were too infrequent to substantiate a malignant process.

DEMONSTRATION OF IF AND HISTOCHEMICAL MARKERS ON CELL POPULATIONS IN H-X SKIN (CASE 1)

Immunological analysis
The dermal infiltrate of large, round cells showed strong reactivity for Ia-like antigen (Fig. 2a). These cells were situated in the upper dermis particularly along the dermoeipidermal border. Scanty numbers of smaller Ia+ round cells were present in the epidermis. In addition to the Ia+ round cell population, there were occasional large, Ia+ irregular cells in the dermis.

Using monoclonal antibodies OKT6 or NA1/34 (both detecting HTA/1 antigen) in combination with C-anti-Ia-like antigen, all the Ia+ epidermal cells and the majority (> 70%) of the Ia+ dermal round cells expressed the HTA-1 determinant (Fig. 2b). Conspicuous numbers of HTA-1+, Ia+ round cells were
Fig. 1 Histiocytosis X (Haematoxylin and eosin paraffin wax sections). (a) Skin case 1: focal infiltrate of large round cells in the dermis and epidermis (arrows) × 480. (b) Lymph node case 2: diffuse infiltrate of mixed cell types replaces the normal lymph nodes architecture. The main cell type consists of large cells with eosinophilic cytoplasm (arrows) and multinucleate giant cells (asterisks). Other cells include residual lymphoid aggregates (L) × 480.

Present at the dermoepidermal border. Not all the cells in the infiltrate, however, expressed the HTA-1⁰, Ia⁰ phenotype. The large, dermal irregular cells were Ia⁰ but HTA-1⁻. Small numbers of HTA-1⁰, Ia⁻ round mononuclear cells were also observed. These cells were smaller than either of the HTA-1⁺, Ia⁺ and Ia⁺, HTA-1⁻ cell types but were not morphologically or immunologically identifiable as T lymphoblasts. None of the cells in the skin reacted with anti-immunoglobulin reagents and virtually no T
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Fig. 2 Histiocytosis X (cryostat section). Skin (case 1) stained with (a) C-anti-Ia-like antigen (TRITC) in combination with (b) monoclonal antibody to HTA-1 determinant (OKT6-FITC). Numerous Ia+, HTA-I+ round type I cells (arrows) are present in the dermis, along the dermoepidermal border and in the epidermis. Ia+, HTA-I- irregular type II cells (big arrows) are situated in the dermis as well as occasional Ia-, HTA-I+ mononuclear cells (star).

(HuTLA+) lymphocytes were identified in the frozen sections.

Histochemical analysis
The round cell infiltrate in the dermis and epidermis showed distinct membrane localisation of ATPase (Fig. 3a) but demonstrated weak cytoplasmic reactivity for ANAE (Fig. 3b) and ACPase. These ATPase+, ANAE+, ACPase+ round cells were unreactive with TRAP. By contrast, the infrequent Ia+ irregular cells showed absent or weak localisation of ATPase but contained diffuse, cytoplasmic ANAE (Fig. 3b), ACPase and granular, cytoplasmic TRAP+ activity.

Demonstration of IF and histochemical markers on cell populations in H-X lymph node (case 2)

Immunological analysis
A wide variety of cells expressed variable amounts of Ia-like antigen. Four main cell types could be identified on the basis of Ia-like antigen expression (Fig. 4a-d and Table 2).
(i) The majority of the infiltrating cell population consisted of round or asymmetrical large cells expressing moderate amounts of Ia-like antigen which appeared as a fine, granular stained pattern (Fig. 4a). On phase contrast microscopy, these cells were agranular and sometimes contained one or two characteristically folded nuclei.

(ii) Diffuse Ia-like antigen was present on a smaller population of cells with distinct irregular or stellate outlines produced by long processes (Fig. 4a).

(iii) In contrast to these cell types, the bizarre multinucleate giant cells were conspicuous by their total absence or very weak expression of Ia-like antigen. The morphology of these cells could be identified on phase contrast microscopy and were distinguished as “black holes” in the fluorescent tissue preparations (Fig. 4c).

(iv) Collections of membrane Ia+ small mononuclear lymphocytes constituted the fourth main cell type. These were surface membrane immunoglobulin (SMIg+) B cells (Fig. 4a).

When the sections were stained for HTA-1 determinant (Fig. 4b, d) prominent clusters of large Ia+, HTA-1+ round or asymmetrical cells (type I) were observed throughout the tissue. These Ia+, HTA-1+ cells contrasted with large irregular Ia+, HTA-1- cells (type II) and with confluent and scattered areas of Ia-, HTA-1- giant cells (type III). Cells with the Ia-, HTA-1+ phenotype were occasionally observed but these were much less conspicuous in the lymph node than in the skin biopsy. Very few T (HuTLA+) lymphocytes were identified. These were scattered throughout the tissue and constituted < 2% of the total cell population.

As immunofluorescence for Ia-like and HTA-1 antigens could be combined with ACPase, the foregoing main cell types were also classified by enzyme histochemistry (Fig. 5a, b and Table 2). None of the round or asymmetrical Ia+, HTA-1+ cells (type I) exhibited strong ACPase activity. The irregular Ia+, HTA-1+ cells (type II) showed focal areas of moderate ACPase deposition (Fig. 5a, b insets) and the majority of Ia-, HTA-1- multinucleate giant cells (type III) were strongly ACPase+. A few ACPase+ giant cells however showed weak expression of Ia-like antigen (HTA-1-; Fig. 5a, b insets).

A further cell type was revealed by combined IF and histochemical analysis. Low numbers of small round Ia+, HTA-1- mononuclear cells contained prominent cytoplasmic ACPase+ “dots” or granules. These cells were morphologically different from the Ia+, HTA-1- (type II) cells which were much more prominent in the tissue.

**Histochemical analysis**

Multinucleate giant cells exhibited strong or moderate ACPase and ANAE activity. The large round cells with characteristically folded nuclei were...
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**Fig. 4** Histiocytosis X (cryostat section). Lymph node (case 2) stained with combinations of (a)-(c) C-anti-Ia-like antigen (TRITC) and (b)-(d) monoclonal antibody to HTA-1 determinant (OKT6-FITC). (a), (b). In most areas the cell infiltrate consists of dense clusters of Ia⁺, HTA-1⁺ round type I cells (arrows) with a few Ia⁺, HTA-1⁻ irregular type II cells (big arrows) and Ia⁻, HTA-1⁻ multinucleate giant type III cells (asterisk). Residual lymphoid areas (L) contain membrane Ia⁺ (HTA-1⁻) B cells. (c), (d). Other areas of the same tissue contain prominent numbers of Ia⁻, HTA-1⁻ multinucleate giant type III cells (asterisk) and scanty Ia⁺, HTA-1⁺ type I cells (arrows).

Generally unreactive or weakly reactive for ANAE, ACPase and ATPase. Additional ANAE⁺, ACPase⁺ cell types included irregular and round mononuclear cells with either cytoplasmic granules or diffuse deposition of enzyme reaction products. Only a small proportion of multinucleate giant cells (30%) showed ATPase and TRAP activity.

**Discussion**

The two cases presented in this study showed typical (though different) clinical features associated with histiocytosis X. Histologically characteristic lesions in the skin and lymph node biopsies confirmed the diagnosis in both patients. In the skin, the...
Table 2  Immunological and histochemical characteristics of the major cell types in H-X lymph node (case 2)

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Ia-like antigen</th>
<th>HTA-I antigen</th>
<th>SMi*</th>
<th>ACPase</th>
<th>ATPase</th>
<th>ANAE</th>
<th>TRAP</th>
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</thead>
<tbody>
<tr>
<td>Round/asymmetrical cells</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>(type I)</td>
<td>(granular)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irregular/stellate cells</td>
<td>+ (-)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>(type II)</td>
<td>(diffuse)</td>
<td></td>
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<td></td>
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<tr>
<td>Multinucleate giant cells</td>
<td>- ±</td>
<td>-</td>
<td>+++</td>
<td>± +</td>
<td>+</td>
<td>±</td>
<td>+</td>
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<tr>
<td>(type III)</td>
<td></td>
<td></td>
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<tr>
<td>Residual B lymphocytes</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>± +</td>
<td>+</td>
<td>±</td>
<td>+</td>
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<tr>
<td>(type IV)</td>
<td></td>
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</table>

*Surface membrane immunoglobulin.
†ATPase and TRAP demonstrated in 30% multinucleate giant cells.

Fig. 5 Histioctyosis X (cryostat section). Lymph node case 2: combined histochemical and immunofluorescence analysis with (a) monoclonal antibody to HTA-I antigen (NAI/34-TRITC), (inset a) C-anti-Ia-like antigen (TRITC) and (b) ACPase. A cluster of HTA-I+ (Ia+) round (type I) cells do not exhibit ACPase activity (arrows). Intense ACPase reactions are present in large and medium sized HTA-I- (Ia-) giant type III cells (asterisk). Insets (a), (b) show a single Ia+, (HTA-I-) irregular type II cell containing localised ACPase* reaction products (asterisk). A giant cell with weak expression of Ia-like antigen and moderate ACPase activity is also identified (big asterisk).

relatively homogeneous population of large round cells with kidney shaped nuclei were similar in morphology and distribution to previous descriptions of H-X cell infiltrates.\textsuperscript{5,17,18}

The main feature of this study is the demonstration that many of the cells in the skin and lymph node express the same Ia\textsuperscript{+}, HTA-I\textsuperscript{+} phenotype as normal epidermal LC. This finding demonstrated by com-
Combined IF analysis confirms previous evidence that H-X cells may be related to LC. Abundant amounts of Ia-like antigens have been demonstrated on normal LC by IF and immuno-electronmicroscopic studies. Recently, cortical thymocyte antigen HTA-1 has also been demonstrated on LC defined by monoclonal antibody OKT6. It has been shown that both OKT6 and NA1/34 monoclonal antibodies react with HTA-1 antigen although with different epitopes on the same molecule. The fact that these two independently produced monoclonal antibodies react with LC indicates that this is not a chance cross-reaction between single epitopes on two unrelated molecules but rather that these cells carry at least part of the same HTA-1 molecule. Thus the Ia+, HTA-1+ phenotype provides an important new marker for LC in addition to the well-established morphological criteria of ultrastructurally defined Birbeck granules and histochemical demonstration of ATPase.

Most of the proliferating cells in the skin expressed the Ia+, HTA-1+ phenotype although a few irregular forms exhibited Ia-like antigen alone. It was interesting to observe the presence of many Ia+, HTA-1+ cells in the dermis. This contrasted with a variety of dermatological conditions such as mycosis fungoides (MF), lichen planus (LP) and graft-versus-host disease (GVHD) in which the dermis is infiltrated by numerous strongly Ia+ but HTA-1− irregular cells. The Ia+, HTA-1+ cells in these disorders (MF, LP, GVHD) are primarily restricted to the epidermis and Ia+, HTA-1+ cell types constitute only a small proportion (5-20%) of the dermal Ia+ (HTA-1−) populations.

In contrast to the skin, the lymph node showed a more heterogeneous infiltrate. Again, there were numerous Ia+, HTA-1+ cells throughout the tissue which is an uncommon finding in normal or stimulated lymph nodes. Likewise, lymph nodes from patients with MF or Sézary syndrome contain less than 10-15% of Ia+, HTA-1+ cell types within the cleat giant cells, often described in H-X lesions. Ia+ non-lymphoid population. The bizarre multinucleate cells were Ia−, HTA-1−. Only a weak expression of Ia-like antigen was observed in the peripheral cyttoplasm of some giant cells. Characteristically the nodal infiltrates in H-X efface the normal architecture and are accompanied by a variety of normal cell types. Although typical eosinophil granulomas were not present in this lymph node, numerous plasma cells and moderate numbers of eosinophils were observed. Residual areas of B lymphocytes maintained the same phenotype (Ia+, IgM+, IgD+, mixed λ+, λ+ light chains) as immunocompetent B cells in primary nodules and follicle mantles of normal lymphoid tissue. There was, however, a striking lack of HuTLA+ T cells.

The second feature of this study is the demonstration that IF reagents can be successfully combined with ACPase enzyme histochemistry on frozen tissue sections without loss of reactivity in either system. Using these techniques separately on adjacent tissue sections, the enzyme reactions and immunological findings were strikingly variable in both skin and lymph node biopsies. However, these features could be closely related to each other using the combined IF and histochemical technology. Demonstration of Ia+, HTA-1+ cells in the skin with weak ACPase and ANAE activity but moderate amounts of ATPase supports previous histochemical studies. Similarly in the lymph node, the Ia+, HTA-1+ cells were unreactive or weakly reactive for ACPase, ATPase and ANAE. In addition, the multinucleate giant cells were Ia−, HTA-1− but were strikingly ACPase+ and showed variable amounts of ANAE and ATPase. Other minor cell populations including Ia+, HTA-1+, ACPase+ (type II) irregular cells and small numbers of Ia+, HTA-1−, ACPase+ round cells were similar to interdigitating reticulum cells and tissue macrophages. These various immunological and enzyme patterns more clearly define the heterogeneous nature of the cells involved in H-X. It is not known whether the predominating Ia+, HTA-1+, ACPase+ cells and the other cell types including Ia−, HTA-1−, ACPase+ giant cells are derived from the same cell lineage or whether the latter cell type represents a reactive element stimulated by the aberrant Ia+, HTA-1+ population.

The phenotypic similarity between Ia+, HTA-1+ H-X cells and normal Ia+, HTA-1+ LC confirms previous observations that H-X cells contain ultrastructural bodies which are indistinguishable from the Birbeck granules in LC. These findings support the concept that H-X represents an abnormal proliferation of LC. There is much evidence to show that normal epidermal LC function as specialised immunological accessory cells to initiate immune responses in allergen-stimulated states. These bone marrow-derived cells are capable of trapping antigen and migrating through dermal lymphatics to regional lymph nodes to present antigen to effector T cells. LC also resembles a variety of other Ia+ bone marrow derived macrophages including interdigitating reticulum cells (IDR) in T-dependent areas of peripheral lymphoid tissue and “veiled” cells of the dermal afferent lymphatics. Clearly a spectrum of these types as well as tissue macrophages are involved in H-X lesions.

It was not surprising to find that only scanty numbers of T lymphocytes were present in the skin and lymph node lesions. Immunological abnormalities such as combined immunodeficiency and
impaired cell mediated immune responses have been associated in some cases of H-X. Recently, abnormal immune function has been demonstrated in a series of H-X patients who lacked T cell H2 receptors. In normal tonsil, inducer T cells (OKT4+, OKT8−) form close contact with Ia+ IDR cells. Similar T cell-Ia+ cell contacts are demonstrated in MF where Ia-like antigens are strongly expressed on the proliferating non-lymphoid elements. Despite increased numbers of Ia+ cells in H-X lesions, the lack of T cells suggest either a loss of normal T lymphocyte control or a reactive local T cell deficiency. It will be interesting to see if the reported beneficial effects of thymic hormonal extracts show any influence on the T cell deficiency in the lesions.

It is clear from this study that the Ia+, HTA-1+ cells in H-X express the usual immunohistochemical characteristics of Ia+, HTA-1+LC but have abnormal migratory patterns in the skin, lymph node and bone marrow (unpublished observations). The reduction of intracellular enzymes, particularly ACPlase, suggests that these cells are non-functional types derived from the LC lineage. In addition the aberrant features of these cells might be influenced by the T lymphoid system.

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References


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Requests for reprints to: Dr JA Thomas, Department of Histopathology, Royal Marsden Hospital, Sutton, Surrey, England.

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