Immunocytochemical detection of tumours of neuroectodermal origin

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SUMMARY

Immunocytochemical methods were applied to bone marrow aspirate and cerebrospinal fluid specimens to show cellular reactivity with the monoclonal antibody UJ13A, which recognises an antigen expressed by cells of neuroectodermal origin. The antigen remained stable after air drying and appropriate fixation. In five patients with various neuroectodermal tumours the diagnostic advantages of these techniques were clear; they can be performed even when only very small amounts of diagnostic material are available.

Rapid and accurate differential diagnosis of malignant disease is essential if appropriate treatment is to be given. Tumours of neuroectodermal origin often present with or develop metastatic spread to the bone marrow or cerebrospinal fluid, and these readily sampled tissues provide diagnostic cellular material. When the infiltration is heavy the morphological diagnosis of undifferentiated malignancy is straightforward, but the differential diagnosis from leukaemia, lymphoma, or other primitive tumours can be difficult even when the diagnosis is known.

UJ13A is a murine monoclonal antibody raised against human fetal brain. It recognises an antigen expressed by cells of neuroectodermal origin. Its usefulness in the diagnosis of neuroectodermal tumours using immunofluorescence techniques has been well documented. Substantial numbers of cells are required for fluorescence techniques, which may be unsuccessful if a marrow aspirate is relatively “dry”, a cerebrospinal fluid cell count low, or if a mononuclear cell separation is made from a native marrow with a low level of infiltration.

Immunocytochemical techniques using peroxidase or alkaline phosphatase labelled antibodies have been described which have the advantages of combining the immunological marker with good quality cellular morphology, and which require only small amounts of unprepared material (such as air dried native bone marrow smears or cerebrospinal fluid preparations).

We describe five cases which illustrate the usefulness of the monoclonal antibody UJ13A in conjunction with immunocytochemical techniques in the differential diagnosis of neuroectodermal tumours.

Material and methods

CASE REPORTS

Case 1
A 16 month old baby presented with stage IV neuroblastoma, with primary tumour arising from thoracic and abdominal sympathetic chain, raised urinary hydroxy methoxymandelic acid (HMMA), and homovanillic acid (HVA) concentrations and heavy bone marrow infiltration at diagnosis.

Case 2
A 2 year old girl with stage IV neuroblastoma was treated with chemotherapy. She had a low level of marrow infiltration following treatment, when urinary HMMA and HVA concentrations had returned to normal.

Case 3
A 3 year old girl had a bone marrow relapse of posterior fossa medulloblastoma.

Case 4
A 31 year old man presented with meningeal relapse of pharyngeal primitive neuroectodermal tumour (PNET) (esthesioneurotastoma). Cerebrospinal fluid contained 100 cells/μl.

Case 5
A 5 year old girl had stage IV neuroblastoma with right adrenal primary, raised urinary HMMA and HVA concentrations, and heavy bone marrow infiltration at diagnosis.

Native bone marrow aspirate smears or standard
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Figure  (a) Case 1: neuroblastoma in bone marrow aspirate (heavy infiltration). (Immunoperoxidase.) (b) Case 2: neuroblastoma in bone marrow aspirate. Particle on native bone marrow smear showing presence of several positive cells, although level of infiltration in smear trails was low. (APAAP.) (c) Case 2: bone marrow aspirate of neuroblastoma after chemotherapy. Occasional positive cells and cytoplasmic fragments still seen. (Immunoperoxidase.) (d) Case 3: bone marrow aspirate of medulloblastoma showing heavy infiltration. (Immunoalkaline phosphatase.) (e) Case 4: cerebrospinal fluid cytocentrifuge preparation of PNET. (Immunoperoxidase.) (f) Case 5: neuroblastoma. Cytocentrifuge preparation of bone marrow trephine collagenase digest, showing positive neuroblastoma cell and negative neutrophils. (Immunoalkaline phosphatase.)
cytocentrifuged cerebrospinal fluid preparations were made on glass slides. In case 5 cytocentrifuged preparations were made from cells extracted from an unfixed bone marrow trephine biopsy specimen using collagenase according to the method of Mononen and Jansson. Slides not immunostained within seven days were wrapped in polythene film and stored at −20°C.

**Antibodies**

UJ13A was supplied by the laboratory of one of the authors (JK) and may shortly be commercially available. It was diluted to 1/400 in Earle's balanced salt solution (EBSS) (Gibco Ltd, UK) with 20% fetal calf serum before use. The subsequent antibodies used in the immunolabelling techniques were obtained from Dako Ltd (High Wycombe, Buckinghamshire). These were either rabbit antimouse peroxidase (Dako No P260) diluted 1/25 in EBSS, or rabbit antimouse conjugated with calf alkaline phosphatase diluted 1/25 (Dako No D314), or unconjugated rabbit antimouse (Dako No Z259) followed by APAAP complexes (Dako No D651) diluted 1/20.

**Immunocytochemical Labelling**

Slides stored at −20°C were thawed at room temperature and then unwrapped. A 1 cm diameter circle was marked with a diamond pencil around the cytocentrifuged cells or the tail of the bone marrow smear. After fixation this area was kept moist throughout the procedure.

Slides were fixed in a methanol: acetone (1:1) mixture for 90 seconds at room temperature and then transferred to washing buffer (phosphate buffered saline (pH 7.4) for the immunoperoxidase method, Tris-buffered saline (pH 7.6) for the immunoalkaline phosphatase methods). Other standard immunocytochemical fixatives which proved satisfactory included dry acetone alone and glutaraldehyde, but fixatives containing formalin caused loss of reactivity presumably due to antigen damage.

 Diluted UJ13A (100 µl) was then applied after drying round the marked area, and the slides were incubated at room temperature in a humid box for 30 minutes before a further five minute wash in buffer. An equal quantity of a second antibody was then applied for 30 minutes. One of three standard immunocytochemical methods was used:
1. Immunoperoxidase, using a peroxidase conjugated second antibody.
2. Immunoalkaline phosphatase, using an alkaline phosphatase conjugated second antibody, or
3. The APAAP sandwich technique using an unconjugated second antibody followed by APAAP complexes.

After development of the colour reactions slides were washed in buffer and then distilled water before being counterstained with Carazzi's haematoxylin and mounted using an aqueous mounting medium.

For each slide a negative control slide of the same material was processed using buffer in place of the primary antibody to ensure that no false positive labelling occurred due to non-specific binding of the conjugated antibodies.

**Results**

The neoplastic cells from all five of these cases showed membrane and cytoplasmic reactivity with UJ13A. The antibody worked well with all three immunocytochemical techniques. In these and other UJ13A positive cases examined there was sufficient cytoplasmic antigen to render good positivity with the direct labelled second antibody techniques, conferring less advantage on the added sensitivity of the more complicated APAAP sandwich method.

A prominent morphological feature of bone marrow aspirates in neuroblastoma is the ease with which the neoplastic cells are disrupted, leaving a scattering of cytoplasmic fragments in the background of the smears. Positive immunostaining of these fragments is a useful diagnostic feature even when the proportion of intact infiltrating cells is low.

Examples of the immunocytochemical staining reactions are shown in the figure.

**Discussion**

The usefulness of UJ13A in the diagnosis of tumours of neuroectodermal origin has already been described, but this report shows that the antigen detected withstands air drying and fixation, and it illustrates the particular advantages of using immunocytochemical methods to show cellular reactivity.

Many haematological laboratories have immunoperoxidase or immunoalkaline phosphatase methods in routine use, and either technique works well with this antibody. In the cases we studied positivity was readily shown with the simple conjugated second antibody methods. As endogenous alkaline phosphatase is more readily inactivated than peroxidase, the immunoalkaline phosphatase method with a directly conjugated second antibody is our preferred technique. This is quicker and considerably more economical than the APAAP sandwich method, which has certain advantages with more weakly expressed antigens.

These slide techniques allowed reactivity with UJ13A to be shown on tiny amounts of material. The ability to detect low levels of infiltration by examining bone marrow particles or observing positivity in cytoplasmic fragments, and to digest cell suspensions
from trephine biopsy specimens with collagenase without loss of antigenicity has important implications for the detection of minimal residual disease.

Immunological methods for confirmation of the diagnosis of neuroectodermal tumours and screening for residual disease are not widely used. Transport of suspect cell suspensions from one institute to another is fraught with delay and loss of viability. Air dried slides are easily transferred by post and can be batched for convenient testing. Immunocytochemical investigation offers several advantages over fluorescence techniques and increases our ability to make immunological diagnoses in these rare tumours.

We thank Dr P W G Saunders and Mr G Buckle for providing the collagenase digest specimens from case 5.

References

Requests for reprints to: Dr P J Carey, Department of Haematology, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP, England.
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P J Carey, A J Malcolm, A D Pearson, J T Kemshead and M M Reid

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tion of intracellular localisation of steroid hormone receptors in a series of articles which critically evaluate all of the available methodology including both biochemical and histochemical techniques. As such it is a valuable and timely addition to the literature.

Despite the fact that the steroid hormone receptors have been actively studied over a period of some 30 years, there is still a good deal of confusion about the intracellular compartments in which they exist.

In an introductory chapter EV Jensen puts a brave face on this confusion by pointing out that if all receptors, both native and activated, had remained bound in the nuclear fraction of tissue homogenates, it would have been much more difficult to have recognised the different forms of the protein and their interrelation, so there may have been considerable delay in discovering the phenomenon of hormone induced receptor activation. Nevertheless, it still remains possible that other sites exist for receptors, and a later chapter in the book deals with the question of membrane associated steroid hormone receptors, although this is still an area of controversy.

There is thus a considerable amount of information available in this book which is timely and useful to a wide readership. Regrettably, extracting the information when required is a good deal more difficult. The index is very thin and hardly amounts to much more than a list of the different types of hormones, while the contents page lists the chapter titles all right but didn’t give page numbers. These amateurish errors in subediting regrettably distract from what is otherwise an extremely useful publication.

GP VINSON

Correction

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There was an error in the Laboratory technique by P J Carey et al (May 1988;41:586–589). The last two sentences of the first paragraph on page 586 should have read:

When the infiltration is heavy the morphological diagnosis of undifferentiated malignancy is straightforward, but the differential diagnosis from leukaemia, lymphoma, or other primitive tumours can be difficult on the basis of appearance alone. When the level of infiltration is low, morphological detection can be difficult even when the diagnosis is known.

Notices

Postgraduate Course in Gynaecological and Obstetric Pathology with clinical correlation

April 10–14, 1989

The departments of Pathology, Massachusetts General Hospital and Brigham and Women’s Hospital, Harvard Medical School, will present a postgraduate course in gynaecological and obstetric pathology under the direction of Drs Robert E Scully, Robert H Young, and Shirley G Driscoll.

This course is designed for pathologists and obstetrician-gynaecologists at resident and practitioner levels. It will provide an in depth review of gynaecological and obstetric pathology with emphasis on morphological diagnostic features and clinicopathological correlation, including management. Special attention will be paid to recent advances and newly recognised entities. Instruction will be primarily by lecture, but will also include case presentations and discussion periods. Each participant will receive a comprehensive course syllabus.

Fee: $595.00 (residents and fellows $395.00).

For further information contact: Department of Continuing Education, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA (Telephone: 617-732-1525.)

Cytology tutorial workshop 1989

Friday March 31 and Saturday April 1

Postgraduate Medical Centre, Brighton General Hospital

Lectures and microscopy on gynaecological, breast, genitourinary, lymph node, thyroid and lung cytology.

Speakers: Elizabeth Hudson, David Melcher, Russell Smith, John Webb and Jennifer Young.

Enquiries to: Mrs Jean Crisp, Department of Clinical Cytology, Brighton General Hospital, Elm Grove, Brighton BN2 3EW.

Dermatopathology self-assessment workshop

A dermatopathology self-assessment workshop will be held at Guy’s Hospital, London, Tuesday 18 and Wednesday 19 April 1989.

The course is for both pathologists and dermatologists and will cover a broad range of subjects. Participants will have an opportunity to study 100 unknown slides during two microscope sessions, followed by a formal discussion.

Fee: £75.00 for all participants.

For further information please contact: Marion Ellis, Secretary to Dr M MacDonald, Dermatology Department, Guy’s Hospital, St Thomas Street, London SE1 9RT, United Kingdom.

Journal of Clinical Pathology

Symposium

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Speakers will include Dr Stephen Lock, Editor, British Medical Journal, Dr John Lilleyman and Dr David Lowe, Editor and Deputy Editors, Journal of Clinical Pathology, Caroline White, Technical Editor, and Bill Friend, Head Reader at Eyre and Spottiswoode printers.

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