system of Jervis [7] is used here.) Previous electrophoretic examination of the LDH of most other plants was carried out with extracts from leaves [1, 2]. Such a study of potato leaves would not have revealed the existence of isoenzyme forms in this species. We have, therefore, examined extracts from various regions of a number of plants: roots, stem-base, upper stems, leaves and any special tissue, such as the tubers of the potato.

The plants used were grown under normal horticultural conditions, harvested before flowering, and immediately extracted. They were first washed thoroughly, but quickly, with ice-cold water, and representative portions were finely chopped and ground in a pestle and mortar with acid-washed sand and 50 mM-potassium phosphate buffer, pH 7.4, containing an 8% (w/v) suspension of polyvinylpyrrolidone (1–4 volumes of this buffer were used, depending on the consistency of the homogenate). The homogenates were then centrifuged at 8000 g for 30 min and the supernatants were subjected to ammonium sulphate fractionation (largely to concentrate the LDH activity, which is orders of magnitude lower in plants than in animals and bacteria). The fraction precipitating between 30 and 70% saturation of ammonium sulphate was redissolved in a minimal volume of 50 mM-Tris/glycine buffer, pH 8.6, in 7.5% (w/v) polyacrylamide gels either by the 'disc-gel' method of Davis [9] or using the 'Mighty Small' SE 250 slab gel apparatus (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.). The LDH activity was visualized using the zymogram-staining 'cocktail' of Epstein et al. [10].

We have experienced problems with many plants due, apparently, to interfering substances that may adsorb the LDH. These might otherwise interfere with its extraction or electrophoresis. With special extraction procedures we have been able to demonstrate LDH activity in extracts from all higher plants [11], even those previously thought to lack this enzyme (see, e.g. [1]). However, we have been less successful in overcoming interference with electrophoresis. The LDH activity in extracts from many plants did not penetrate properly into the polyacrylamide gels, remaining as a diffuse streak near the point of application and this limited the number of species we could usefully include in this study.

Of these, the majority showed only a single electrophoretically identical form in all tissues. Such species include onion (Allium cepa), leek (Allium porrum) and turnip (Brassica rapa). But multiple forms were found in potato (Solanum tuberosum) and in the related tomato (Lyco-persicum esculentum). Other members of the Solanaceae, Capsicum annuum and Solanum dulcamara, were also examined, but although extracts from the tissues of these species have LDH activity, we were unable to 'find' it on electrophorograms, only diffuse activity near the origin being detectable, as described above. The same problem, probably attributable to interfering factor(s) in the extracts, was encountered with extracts from leaves of the two tomato cultivars studied, 'Moneymaker' and 'Hildares', but the other tissues gave clear electrophoretic patterns with up to four LDH bands, the number and intensity of the bands varying with tissue and with cultivar.

In addition to the leaves and tubers mentioned above, other regions of potato plants showed variations of isoenzyme pattern. With the cultivar 'Kerr's Pink', the tubers showed all five isoenzymes, with isoenzymes 2 and 3 greatly predominating. In the stems and roots, there was a predominance of isoenzymes 1 and 2, but the others were also detectable, while only isoenzyme-1 could be detected in extracts from the leaves. In another potato cultivar, 'British Queen', the leaf extracts again had predominantly isoenzyme-1, but also a trace of isoenzyme-2, while isoenzyme-5 appeared missing from the tubers and other tissues whose LDH patterns were otherwise similar to those found in the 'Kerr's Pink' cultivar. Only three isoenzymes could be detected in tubers of the cultivar 'Homeguard'. Such variation among cultivars seems odd, if the isoenzyme forms fulfil any important differential function.

A recent survey by Hoffman et al. [12] of LDH isoenzymes in barley seedlings shows somewhat similar variation among cultivars. More significantly, these authors describe one strain that seemed to have only a single isoenzymic form of LDH without suffering any apparent disadvantage. This seems to cast doubt on any important physiological role for the isoenzymic multiplicity in barley.

We have considered the possibility that the variation of isoenzyme pattern among potato tissues and cultivars might be an artifact produced by the interfering substances so evident in our experiments with other plants. However, we have been unable to find any evidence for this. Co-extraction of tissues having different isoenzyme patterns (e.g. a half-and-half mixture of leaf and tuber tissue) gave seemingly additive isoenzyme patterns.

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Tissue distribution of mammalian lactate dehydrogenase isoenzymes

PADRAIG O'CARRA and PATRICIA MULCAHY
Department of Biochemistry, University College, Galway, Republic of Ireland

Although the mammalian lactate dehydrogenase (LDH) isoenzymes were among the first isoenzyme phenomena to be characterized, and have been the subjects of a very extensive literature, the functional significance of this isoenzymic differentiation remains obscure. Excluding the more recently discovered isoenzyme, LDH-X, which seems to be confined to spermatozoa, the 'classic' electrophoretically separable isoenzymes 1–5 are tetramers composed of a random association of two different types of kinetically non-interacting polypeptide subunits, variously distinguished as A and B or


Abbreviation used: LDH, lactate dehydrogenase (EC 1.1.1.27).
as M and H. The latter designation seems to have regained general currency and is used here. This M/H nomenclature was originally introduced by Dawson et al. [1] to designate (skeletal) muscle and heart (muscle), respectively, and, while the terminology is clearly very untidy, this ‘basic’ tissue distribution has been fully validated — heart muscle of all mammalian species seems to express predominantly H-type LDH and the skeletal muscle of rapid motion (leg and arm muscles) expresses predominantly the M-type. On this basis, Kaplan and co-workers [1–4] propounded the best-known and most widely accepted theory concerning the differential physiological functions of the LDH isoenzymes — the so-called ‘aerobic-anaerobic’ theory, which postulates that H-type LDH is suited to aerobic metabolism (e.g. in heart muscle), while the M-type is more suited to tissues subject to periods of oxygen shortage (e.g. skeletal muscle). This theory constantly reappears (particularly in textbooks), but it has been strongly criticized on various grounds, not least the fact that human liver, another ‘aerobic’ organ, expresses the M form of LDH, while erythrocytes, which perform only anaerobic glycolysis, express the H form. These two tissues have more recently been added to the heart/muscle pair to constitute what might be termed the revised classical distribution pattern (Fig. 1, left-hand side) and this has formed the basis of attempts (including our own) to find a more convincing rationale for the differential tissue distribution and functions of the isoenzymes. (It should be noted, however, that some textbooks have erroneously ‘corrected’ the liver/erythrocyte distribution pattern to conform to the aerobic–anaerobic theory.) What has been generally overlooked is the fact that, while the heart/skeletal muscle distribution seems to apply to all mammalian species, the liver/erythrocyte pattern applies to man, but varies widely among other mammalian species, as can be seen in the middle two rows of Fig. 1. We had been unaware of the extent of this variation until we re-discovered it experimentally. In fact, similar observations were made in the 1960s [7], but seem to have been largely ignored or overlooked since.

This species-variable tissue distribution seems to cast doubt on most of the alternative ‘functional hypotheses’; for example, the idea that the differing $K_v$ values of the LDH isoenzymes are specialized to deal with high (M) or low (H) fluxes of pyruvate or lactate. While the distribution in liver and erythrocyte is highly species variable, we find that some other tissues, unexpectedly, display a conserved differential expression of the isoenzymic forms approaching that of heart and skeletal muscle. Thus, kidney tissue is usually represented as expressing both forms approximately equally, but such results (presumably obtained with whole kidney) hide the fact that the cortex and medulla differentially express predominately the H and M forms, respectively (Fig. 1, bottom two rows). Similarly, extracts from whole brain show about equal activities of H and M, but different regions of the brain express different ratios of the two forms. Therefore, the distribution pattern that must be explained in any theory of LDH isoenzyme function is a dauntingly complex one comprising the original ‘heart/muscle’ pattern considered by Kaplan and co-workers. Further, it should be noted that there are reports of a human who seemed to be devoid of H-type LDH without apparent ill effects [8, 9]. If this be true, interpreting the tissue distribution of the LDH isoenzymes may be on a par with trying to devise a biochemical function for the distribution patterns of body hair.

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Monoclonal antibody UJ127.11 recognizes the human homologue of mouse L1 cell adhesion molecule

K. PATEL,* F. KIELY,* F. RATHJEN† and J. KEMSHEAD*†

*Imperial Cancer Research Fund, Oncology Laboratory, Institute of Child Health, 30 Guilford Street, London WC1N IEH, U.K. and †Centre for Molecular Neurobiology, Pavillon 22, Martinistrabe 52, D-200 Hamburg 20, F.R.G.

Monoclonal antibodies have been widely employed in the search for tumour-specific antigens. Many of these antibodies appear to recognize differentiation-specific antigens which become expressed on tumour cells. Antibody UJ127.11, raised against 16-week human foetal brain, has found use in the diagnosis of embryonic tumours, e.g. neuroblastoma and schwannomas. Data are described here to show that the antigen recognized by UJ127.11 is the human homologue of the mouse L1 cell adhesion molecule.

Mouse L1 has been shown to be immunologically and structurally similar to NgCAM described in chicken and nerve growth factor inducible large external glycoprotein described in rat. Three proteins of 200, 140 and 80 kDa have been described. They are coded for by one gene, LI 1 is a member of the immunoglobulin supergene family [2].

Recently, monoclonal antibody 5G3 has been used to isolate a protein from human brain whose N-terminal amino acid sequence shows strong similarity to that of mouse L1, suggesting that this protein is the human homologue of L1 [3]. We have compared the binding profiles of UJ127.11 and 5G3 on a variety of tumour tissues by indirect immunofluorescence. In general, concordance between the binding profiles of the two reagents was observed on the tissues tested. In addition, cross-blocking studies were conducted to show whether binding of UJ127.11 was affected by the presence of anti-human L1 antiserum. Radiolabelled UJ127.11 was incubated with neuroblastoma cells in the absence or presence of anti-human L1 antiserum for 30 min.

Excess antibody was washed off and the amount of radio-labelled antibody bound was assayed. The binding of radio-labelled UJ127.11 was inhibited by anti-human L1 antiserum in a dose-dependent manner, indicating that the two reagents react with the same or similar protein.

Finally, sequential immunoprecipitation and immunoblotting were performed to show that depletion of the antigen by one reagent abolished the reactivity of the second on subsequent immunoblotting. Aliquots of cell extracts were depleted of the antigen using Sepharose-conjugated UJ127.11. The extracts were then electrophoresed on SDS-gels and separated proteins were transferred to nitrocellulose. Immunoblotting with UJ127.11 showed that the 220–240 kDa protein was absent as a result of preclearence. A similar result was obtained with anti-human L1 antiserum. However, if the depletion was performed with an irrelevant antibody not binding to the UJ127.11 antigen, then both UJ127.11 and anti-human L1 antiserum detected the 220–240 kDa protein.

The data described above indicate that UJ127.11 recognizes human LI and that LI is present on a variety of embryonic and primary brain tumours. Since L1 has now been described in a number of species, it presumably is one of the key factors that is involved in development of the nervous system. Therefore, it is important to assess its role in human neurological disorders. To this end, we are currently investigating the expression of human L1 in a variety of tumours and cell lines.

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References


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Characterization of a monoclonal antibody recognizing a novel leucocyte adhesion molecule

LYNDA J. PARTRIDGE,* IAN DRANSFIELD† and DENNIS BURTON*

*Biochemistry Section, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, and †Imperial Cancer Research Fund, Lincoln’s Inn Fields, London WC2A 3PX, U.K.

Introduction

Cell–cell interactions are important at various stages of the immune response. A number of the adhesion molecules on the cell surface which mediate or contribute to these contacts have recently been identified, e.g. LFA-1 and ICAM-1 [1], ICAM-2 [2], LFA-3 and CD2 [3]. Here we describe the properties of a monoclonal antibody, JW7, which recognizes an apparently novel leucocyte adhesion molecule.

Results and discussion

The JW7 antibody (IgGl λ) is produced by a Balb/C histocompatible hybridoma obtained by fusing NS1 myeloma and spleen cells from a Balb/C mouse immunized with adherent human monocytes.

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