

Detection of a Tyrosine Phosphatase LAR on Intestinal Epithelial Cells and Intraepithelial Lymphocytes in the Human Duodenum

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Studies of tyrosine phosphorylation in the human duodenum have indicated that proliferating cells in the middle portion of the duodenal crypt were devoid of this feature, suggesting that tyrosine kinase activation is not a dominant factor in crypt cell proliferation, and that consequently tyrosine phosphatase activity may be a more critical factor in crypt cell development. We investigated the expression of the leukocyte common antigen-related receptor (LAR) family of tyrosine phosphatases. A flow cytometry system was used to examine cells from the surface, mid-portion, and lower part of the crypt. Individual cell populations were immunostained with anti-LAR antibodies using phycoerythrin-conjugated anti-CD3 to discriminate between epithelial cells (CD3⁻) and intraepithelial lymphocytes (CD3⁺). Epithelial cells expressed LAR throughout the crypt. Expression of LAR was maximal in the mid-portion of the crypt with lower expression at the top of the villi. Intraepithelial lymphocytes expressed low levels of LAR at the tips of the villi with stronger expression extending towards the base of the crypt. These findings were confirmed by immunohistochemistry on paraffin-fixed sections. Of note, peripheral blood lymphocytes expressed less LAR than IEL. These observations suggest the possibility that tyrosine phosphatase LAR may be of importance in the regulation of crypt cell proliferation. Moreover, as the extracellular domain of LAR has homology with adhesion molecules, the finding of this molecule on IEL could suggest a possible functional role in homing of this unique lymphocyte.

INTRODUCTION

Protein tyrosine phosphatases (PTPases) comprise a structurally diverse family of transmembrane and cytoplasmic-type enzymes that, together with protein tyrosine kinases (PTKases) control protein tyrosine phosphorylation [1]. Tyrosine phosphorylation is known to regulate such physiological events as cell-cell, and cell-matrix interactions and is associated with many cellular processes including activation, proliferation, differentiation, and migration [2, 3, 4]. The human transmembrane molecule leukocyte common antigen-related receptor (LAR) is part of a family of receptor protein tyrosine phosphatases, of which there are approximately 20 known members. It has structural features similar to the extracellular receptor region of the immunoglobulin

superfamily of adhesion molecules, and the fibronectin type-III like repeats in the same region of the neural cell adhesion molecule 1 (N-CAM-1) [5, 6]. The cytoplasmic segment of LAR consists of either one or two tandem phosphatase domains with a single transmembrane region [7]. Thus, LAR which is part of a distinct class of proteins might be involved in cell adhesive interactions as well as having tyrosine phosphorylation function [8].

The cytoplasmic domain of LAR is also similar to the leukocyte common antigen (LCA), CD45 (also known as T200 B220, and Ly-5), the prototype and first discovered receptor-linked protein tyrosine phosphatase [9]. CD45 is a high-molecular-weight glycoprotein, exclusively restricted to leukocytes, including intraepithelial lymphocytes (IEL) [1, 10]. CD45 tyrosine phosphatase appears to be required for the coupling of T-cell antigen receptor signals to the phosphoinositol second messenger signal and may also participate in the rapid increase in tyrosine phosphorylation induced by activation of the T cell receptor [11, 12]. It has been shown that both LCA and LAR have phosphatase activity *in vitro* [13, 14].

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The diversity in the extracellular region of LAR presumably reflects a diversity of ligands to which this enzyme responds. The identity of a ligand for CD45 is still not clear. It has been shown that heparin sulphate on bone marrow stromal cells serves as a ligand for CD45, while CD22 has been shown to be the molecule on B cells [15, 16]. To date, little is known about the ligand for LAR although homotypic interactions have been reported. LAR may associate with the actin cytoskeleton through cadherins or catenin molecules [17]. It is also possible that LAR which is expressed in many tissues and cell types may act by modulating phosphorylation of the components of the signal pathways that regulate cell adhesion [18].

Until recently, protein phosphatases have played a low profile in the protein phosphorylation field. However, it is clear that protein phosphatases do not simply constitutively reverse the effects of protein kinases but rather play a specific role in cellular biology [19]. Studies using knock-out mice have demonstrated that intestinal crypts can develop in the absence of the epidermal growth factor (EGF), a tyrosine kinase receptor [20].

Furthermore, studies of tyrosine phosphorylation in the human duodenum have indicated that proliferating cells in the middle and lower portions of the duodenal crypt were devoid of tyrosine phosphorylation [21]. These data indicate that tyrosine kinase activation mediated by EGF is not necessarily a dominant factor in crypt cell proliferation and suggest that tyrosine phosphatase activity may be a more critical factor in crypt development. Lastly, it has been reported that cellular levels of tyrosine kinase activity is downregulated in colon cancer suggesting a possible correlation with colonic tumor growth, while structures similar to the extracellular domain of LAR have been reported in the colon-cancer-associated gene DCC [22, 23].

Antibodies to the LAR receptor protein tyrosine phosphatase (RTP) have made it possible to study the expression of LAR in different tissues and in single-cell suspensions [5, 6]. We therefore investigated the expression of this family of molecules in the human duodenal crypt. A system was devised using two-colour flow cytometry to assay the sequential elution of intraepithelial lymphocytes (CD3⁺) and epithelial cells (CD3⁻) from the upper, middle, and lower regions of the human duodenal crypt. We determined the level of expression of LAR in IEL and epithelial cells in the duodenal crypt. In addition, we confirmed the specific location of LAR within the duodenal crypt by immunohistochemical staining on paraffin-fixed section.

METHODS

Patients

Duodenal biopsy specimens were obtained endoscopically from patients giving informed consent. Four biopsy fragments were taken from each patient. These patients

were receiving treatment for dyspeptic symptoms and were all found to have a normal small intestine on histological examination. The Ethics Committee of the Federated Dublin Voluntary Hospital approved protocols for the use of duodenal biopsy tissue.

Flow cytometric analysis of enterocyte LAR expression

LAR expression was measured by indirect immunofluorescence and flow cytometry. A mixed population of IEL and epithelial cells was removed from biopsy tissue by sequential elution over periods of 30, 60, 90, and 120 minutes, using a method previously described [24]. Briefly, specimens were incubated in a solution of 1 mM EDTA and 1 mM DTT in Ca²⁺ and Mg²⁺ free Hanks buffered saline solution. Cells were harvested, counted and adjusted to 10⁶ cells/mL. They were incubated for 15 minutes in 2% (v/v) paraformaldehyde and washed twice in a solution of phosphated buffered saline containing 0.01% (v/v) bovine serum albumin, and 0.01% (v/v) sodium azide (PBS/BSA/NaN₃), henceforth used in all subsequent washes. Cells were incubated with an affinity purified polyclonal antibody to the E-subunit of the LAR molecule, rabbit anti-LAR (Transduction Laboratories, Lexington, Ky), for 30 minutes at a concentration of 0.1 μ g/mL. The antibody, though not tested for cross-reactivity with other isoforms of LAR, should recognise any LAR proteins containing the E-subunit. A control polyclonal antibody, anti-keyhole limpet haemocyanin (KLH), (Sigma, St Louis, Mo), was also used and cells were incubated for 30 minutes with a control antibody at concentration 0.1 μ g/mL. The cells were then washed twice with PBS solution. Samples were reincubated with a fluorescein-conjugated goat anti-rabbit secondary antibody (Dakopatts, Glostrup, Denmark) for a further 30 minutes, and again washed twice with PBS solution. Normal rabbit serum was used to block nonspecific binding sites, for 10 minutes. Samples were again washed twice with PBS and finally incubated for 10 minutes with an anti-CD3 monoclonal antibody directly conjugated to phycoerythrin (Becton Dickinson, Mountain View, Calif). Two-colour flow cytometric analysis was performed using the Becton-Dickinson FACScan with Lysis 11 software. The CD3⁺ and CD3⁻ cell populations were identified by gating on forward scatter (FSC) against FL2/PE, (CD3⁺) showing histograms of FL1 positivity for LAR and KLH.

Immunohistochemistry

Paraffin-embedded sections were cut from duodenal biopsy tissue, showing clearly the upper, middle, and lower parts of the duodenal crypts. Pellets were prepared from a human umbilical vein endothelial cell (HUVEC) line, and paraffin-embedded sections cut to use as a positive control for LAR staining. Sections were then mounted on APS (3-aminopropyltriethoxysilane) slides and left overnight at 55°C. Sections were fixed in

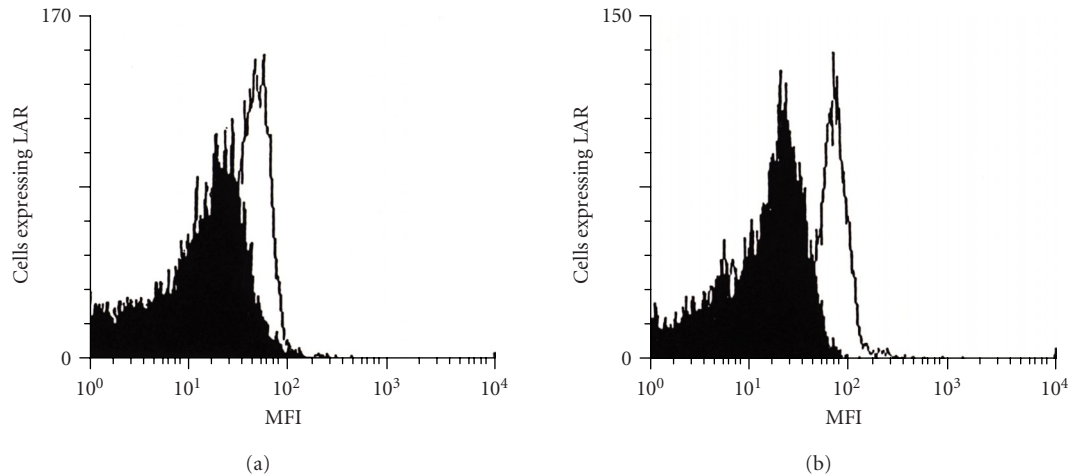


FIGURE 1. Flow cytometric histogram profiles of LAR expression in (a) unfixed human umbilical vein endothelial cells (HUVEC) and (b) fixed HUVEC. The shaded profile represents the expression obtained with the isotype control antibody, anti-KLH, and the open profile represents the expression with an anti-LAR antibody. These results are representative of three experiments. MFI denotes mean fluorescence intensity.

methanol/acetone for 10 minutes followed by dewaxing in xylene and alcohol. Endogenous peroxidase was blocked by incubating sections in a solution of 0.3% H_2O_2 in methanol for 30 minutes. After washing in H_2O followed by a Tris buffered saline (TBS) wash, the samples were microwaved at 800 W in 10 mM citrate buffer, pH 6, for 20 minutes as previously described [25]. Sections were blocked with 1.0% bovine serum albumin for 10 minutes. The LAR antibody was applied and incubated at 4°C overnight, followed by two TBS washes. Sections were incubated in a biotinylated antirabbit second antibody (Amersham, Buckingham, UK) for 60 minutes at room temperature and, following two TBS washes, were incubated in ABC Elite solution (Vectastatin, Vector Laboratories, Burlingon, Calif) for 30 minutes in a humidified chamber. Finally, sections were stained with diaminobenzidine tetrahydrochloride (DAB) solution for 7 minutes counterstained in haematoxylin (5 seconds), and mounted in DPX (distyrene tricresylephosphate xylene mountant for Microscopy, 360294H), BDH, Poole, England. Photographs were taken with a Nikon camera mounted on a Polyvar 2 Reichart Jung light microscope.

RESULTS

FACScan profiles show LAR expression in unfixed and fixed HUVECs (Figures 1a and 1b). There is less LAR expression detected in unfixed cells in comparison to that detected in fixed cells suggesting that the LAR molecule may be shed in unfixed cells. This is consistent with previous findings that LAR is shed [26, 27]. Histograms of IEL $CD3^+$ (positive) and epithelial $CD3^-$ (negative) enterocyte cell populations, eluted after 30, 60, 90, and 120 minutes intervals following EDTA/DTT incubation

show differential expression of LAR in IEL $CD3^+$ cells (Figures 2a, 2c, 2e, and 2g) and in epithelial $CD3^-$ cells (Figures 2b, 2d, 2f, and 2h). The 30-minute incubation with EDTA/DTT removes surface enterocytes from the tips of villi. This is an area where the cells are more differentiated and fewer IEL are extracted [21]. It is evident from Figure 2c that the phosphatase molecule LAR is significantly expressed in $CD3^+$ intraepithelial lymphocytes, isolated after 60 minutes. This corresponds to the upper and middle portions of the crypt [21]. Cells in the middle and lower portions of the crypt are more likely to undergo proliferation. LAR is expressed in the $CD3^-$ cell population at higher levels in the upper, middle, and lower portions of the crypts where cellular proliferation is more likely to occur (Figures 2b, 2d, 2f, and 2h) [28, 29]. LAR is also expressed in peripheral blood lymphocytes (Figure 3a), but expression is low in comparison with IEL (Figure 3b).

Intraepithelial lymphocytes intercalate between the epithelial cells of the villi and crypts. Under high power ($\times 400$) they can be detected in the upper, middle and lower areas of the villi. LAR staining is present in IEL at the tips of the villi (Figures 4a and 4b), while there was strong staining evident in IEL at the base of the crypt (Figures 4a and 4c). A black arrow indicates the IEL. LAR expression was also detected within the duodenal lamina propria lymphocytes. Closer examination of the pattern of expression of LAR staining within the cells suggests a predominantly surface pattern. This is confirmed by the fact that LAR was detected by surface staining and flow cytometry in non-permeabilized cells. Finally, similar surface expression is seen in control HUVECs (Figure 4d). The negative control KLH staining is seen in duodenal biopsy tissue (Figure 4e).

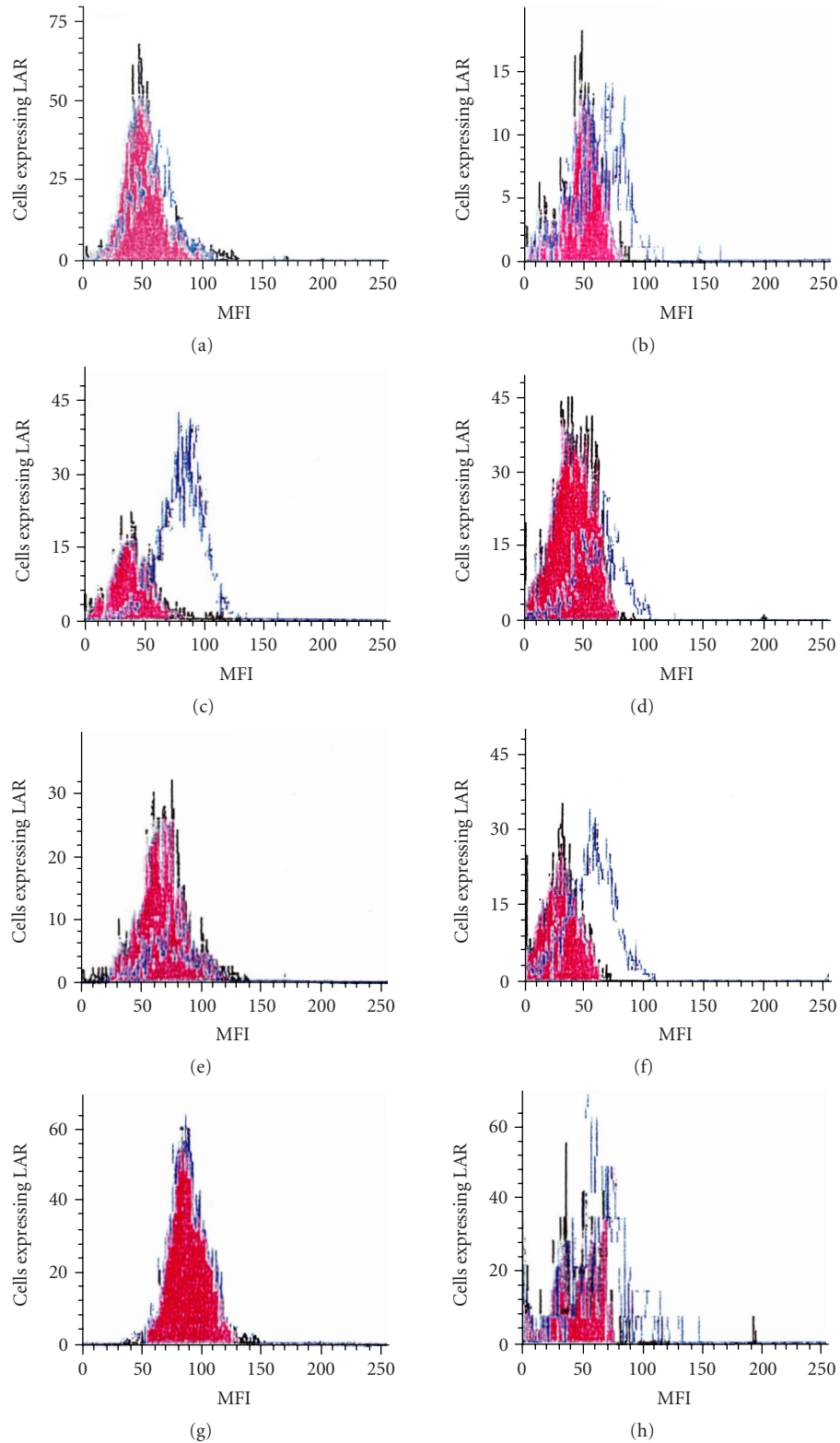


FIGURE 2. Flow cytometric histogram profiles of LAR expression in (a), (c), (e), (g) CD3⁺ (positive) cells and (b), (d), (f), (h) CD3⁻ (negative) cells, reflecting isolation periods, 30, 60, 90, and 120 minutes. The shaded histograms on CD3⁺ and CD3⁻ cell populations represent the expression of the anti-KLH antibody, and the open histograms in the CD3⁺ and CD3⁻ cell populations represent the expression obtained with the anti-LAR antibody. Note: anti-KLH and anti-LAR expressions in the CD3⁺ cells have moved from 0 to 50 on the forward scatter axis (g). Nonspecific uptake of the antibody due to the length of incubation, or a population of lymphocytes with different phenotypic characteristics may explain this result. These results are representative of three experiments. MFI denotes mean fluorescence identity.

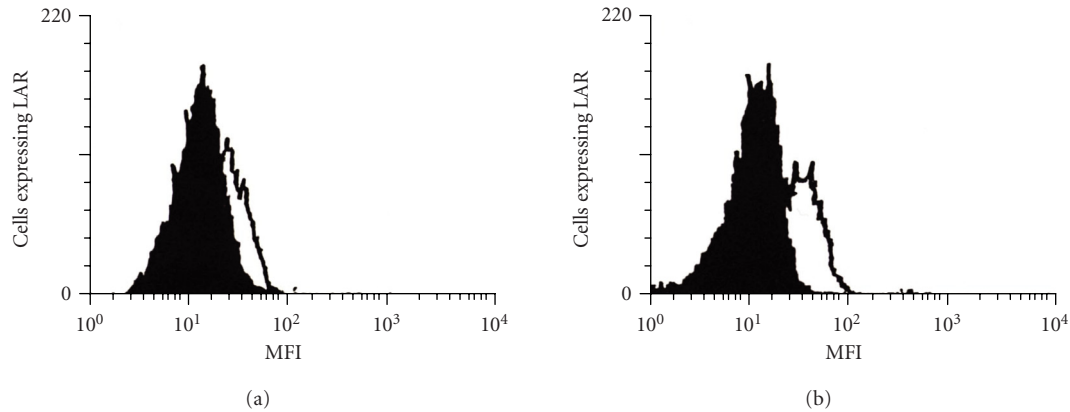


FIGURE 3. Flow cytometric histogram profiles showing LAR expression in (a) peripheral blood lymphocytes (PBL), and (b) intraepithelial lymphocytes (IEL). The shaded histograms represent the expression obtained with the isotype control antibody, anti-KLH, and the open histograms represent the expression obtained with the anti-LAR antibody. These results are representative of three experiments. MFI denotes mean fluorescence identity.

DISCUSSION

In this study we have developed a two-colour flow cytometric method for the detection of the tyrosine phosphatase LAR on epithelial cells and IEL eluted from endoscopically obtained duodenal biopsy tissue. We have shown that LAR is expressed in CD3 negative epithelial cells in the upper, middle, and lower regions of the duodenal crypts. It has been shown that the middle and lower regions of the crypts are areas of cellular proliferation and that low levels of tyrosine phosphorylation have been detected in the epithelial cells in these areas [21, 28]. Although there is no doubt that EGF can cause intestinal epithelial cellular proliferation, there is emerging evidence that this is not its only function within the gastrointestinal tract [30]. It is also reported that crypt villi can grow in the absence of EGF receptor, a tyrosine kinase receptor molecule [20]. In addition, LAR gene products were detected to be abundantly expressed in rat oesophagus, skin, and small intestine, all of which are areas of continuously renewable epithelia [31]. These findings suggest that molecules other than tyrosine kinases may have a greater role to play in crypt cell proliferation. Tyrosine phosphatases, which cleave phosphate groups from tyrosine residues, may be the relevant molecules involved in crypt epithelial proliferation. The LAR family of tyrosine phosphatases may play an active role [32].

We have also shown LAR to be expressed in CD3 positive IEL eluted at 60 minutes in EDTA/DTT. These are a highly specialised subpopulation of T lymphocytes restricted to the mucosal epithelia. They differ from T cells in other lymphoid organs in that they are predominantly CD3+CD8+. The integrin $\alpha\text{E}\beta\text{7}$ is highly expressed in these cells and it has been hypothesised that IEL possess specific receptors enabling interaction with local ligands and controlling homing to and migration through basement membrane [33]. It is thought that adhesion molecules may play a major role, but the mechanism by which IEL rapidly migrate to sites of inflammation is not

fully understood. The expression of LAR in a significant subpopulation of IEL suggests a role in the homing of this unique population to distinct sites in the gut mucosa. The extracellular domain of LAR has homology with adhesion molecules, for example N-CAM, suggesting a possible role in IEL enterocyte interactions [17]. As LAR interacts homotypically, LAR-LAR interactions between IEL and epithelial cells could represent one mechanism by which IEL epithelial cell interactions occur. Further definition of the phenotype of LAR positive IEL subpopulations should provide important insight into the mechanisms of IEL migration.

It has been shown that LAR is expressed in the cell surface as a complex of two noncovalently associated subunits. LAR E-subunit contain the cell adhesion molecule receptor region, and the LAR P-subunit contain a short segment of extracellular, transmembrane, and cytoplasmic domains. The LAR cell adhesion molecule E-subunit is shed during cell growth, suggesting that receptor shedding may be an important mechanism for regulating PTPase function [26, 27]. The apparent loss of LAR expression in the CD3 positive cell population at the tips of the villi could reflect simply loss of the E-subunit. Hence, the shedding of LAR E-subunit might reduce the responsiveness of LAR to a cognate ligand. The LAR (PTPase) and LAR interacting protein 1 (LIP.1) have been shown to colocalise at focal adhesions suggesting that LAR and LIP.1 may regulate the disassembly of focal adhesions and thus regulate cell-cell, cell matrix interactions and migration, by facilitating the recruitment of LAR to cytoskeletal targets. They may also regulate LAR extracellular matrix interactions, retaining LAR at the cell surface [8].

These findings suggest the possibility that tyrosine phosphatase activity may be of significant importance in the regulation of crypt cell proliferation. Secondly, as the extra cellular domain of LAR has homology with adhesion molecules, the finding of this molecule on IEL could suggest a possible functional role in migration and homing of this unique lymphocyte subpopulation.

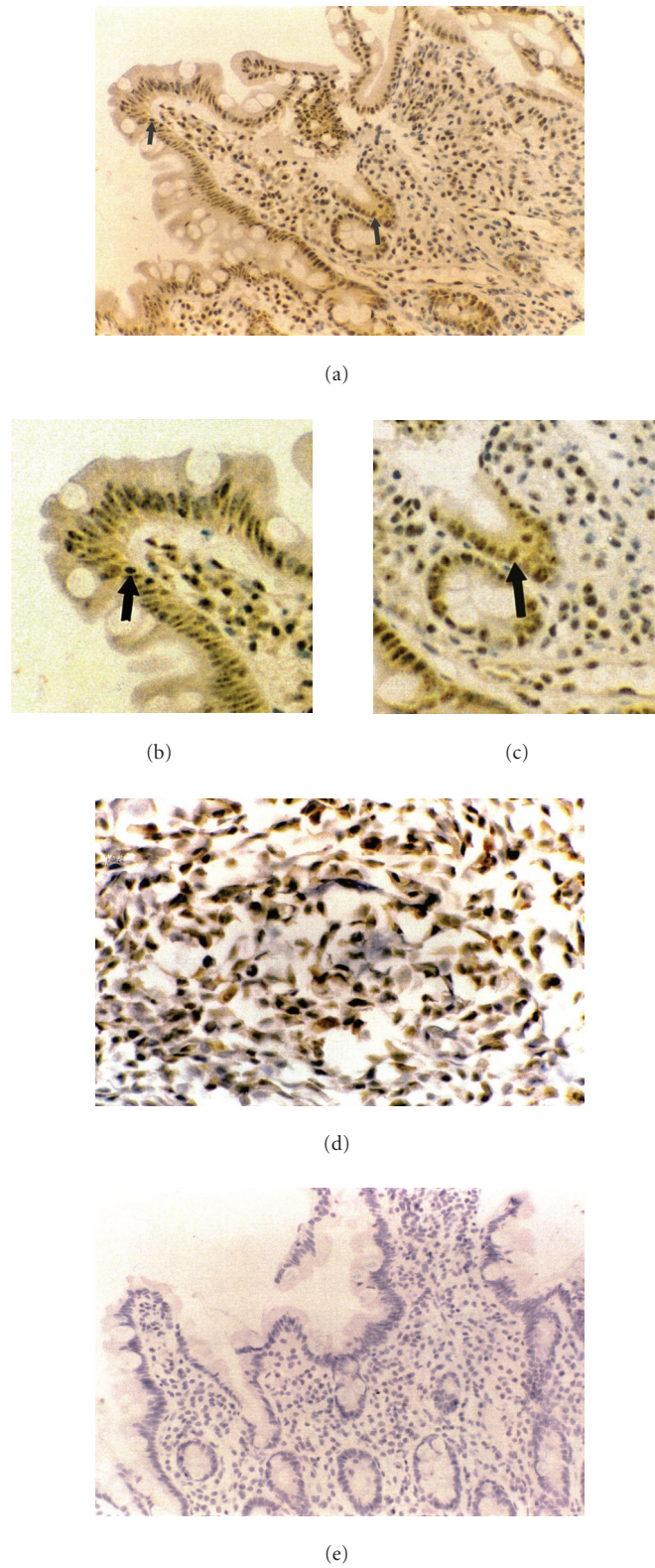


FIGURE 4. Immunohistochemistry of LAR staining on duodenal biopsy tissue. (a) Duodenal biopsy tissue stained with anti-LAR at magnification of $\times 200$. The positive staining is brown and the presence of IEL with weak staining is indicated by black arrows. (b) Note: LAR expression in intraepithelial lymphocytes at the tips of the villi is very low (indicated by a black arrow). (c) Staining is stronger in the IEL within the lower third of the crypt (indicated by a black arrow). (d) Staining of positive control HUVECs with anti-LAR antibody. (e) Staining of duodenal biopsy tissue with anti-KLH, negative control.

REFERENCES

- [1] Fischer EH, Charbonneau H, Tonks NK. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science*. 1991;253(5018):401–406.
- [2] Burridge K, Turner CE, Romer LH. Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *J Cell Biol*. 1992;119(4):893–903.
- [3] Schlessinger J, Ullrich A. Growth factor signaling by receptor tyrosine kinases. *Neuron*. 1992;9(3):383–391.
- [4] Clark EA, Brugge JS. Integrins and signal transduction pathways: the road taken. *Science*. 1995;268(5208):233–239.
- [5] Longo FM, Martignetti JA, Le Beau JM, Zhang JS, Barnes JP, Brosius J. Leukocyte common antigen-related receptor-linked tyrosine phosphatase. Regulation of mRNA expression. *J Biol Chem*. 1993;268(35):26503–26511.
- [6] Zhang JS, Longo FM. LAR tyrosine phosphatase receptor: alternative splicing is preferential to the nervous system, coordinated with cell growth and generates novel isoforms containing extensive CAG repeats. *J Cell Biol*. 1995;128(3):415–431.
- [7] Saito H. Structural diversity of eukaryotic protein tyrosine phosphatases: functional and evolutionary implications. *Semin Cell Biol*. 1993;4(6):379–387.
- [8] Pulido R, Serra-Pages C, Tang M, Streuli M. The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP1. *Proc Natl Acad Sci U S A*. 1995;92(25):11686–11690.
- [9] Thomas ML. The leukocyte common antigen family. *Annu Rev Immunol*. 1989;7:339–369.
- [10] Trowbridge IS, Thomas ML. CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. *Annu Rev Immunol*. 1994;12:85–116.
- [11] Koretzky GA, Kohmetscher MA, Kadleck T, Weiss A. Restoration of T cell receptor-mediated signal transduction by transfection of CD45 cDNA into a CD45-deficient variant of the Jurkat T cell line. *J Immunol*. 1992;149(4):1138–1142.
- [12] Peyron JF, Verma S, de Waal Malefyt R, Sancho J, Terhorst C, Spits H. The CD45 protein tyrosine phosphatase is required for the completion of the activation program leading to lymphokine production in the Jurkat human T cell line. *Int Immunol*. 1991;3(12):1357–1366.
- [13] Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA. Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. *Biochemistry*. 1988;27(24):8695–8701.
- [14] Streuli M, Krueger NX, Tsai AY, Saito H. A family of receptor-linked protein tyrosine phosphatases in humans and Drosophila. *Proc Natl Acad Sci U S A*. 1989;86(22):8698–8702.
- [15] Sgroi D, Varki A, Braesch-Andersen S, Stamenkovic I. CD22, a B cell-specific immunoglobulin superfamily member, is a sialic acid-binding lectin. *J Biol Chem*. 1993;268(10):7011–7018.
- [16] Coombe DR, Watt SM, Parish CR. Mac-1 (CD11b/CD18) and CD45 mediate the adhesion of hematopoietic progenitor cells to stromal cell elements via recognition of stromal heparan sulfate. *Blood*. 1994;84(3):739–752.
- [17] Brady-Kalnay SM, Rimm DL, Tonks NK. Receptor protein tyrosine phosphatase PTPmu associates with cadherins and catenins in vivo. *J Cell Biol*. 1995;130(4):977–986.
- [18] Streuli M, Krueger NX, Hall LR, Schlossman SF, Saito H. A new member of the immunoglobulin superfamily that has a cytoplasmic region homologous to the leukocyte common antigen. *J Exp Med*. 1988;168(5):1523–1530.
- [19] Cyert MS, Thorner J. Putting it on and taking it off: phosphoprotein phosphatase involvement in cell cycle regulation. *Cell*. 1989;57(6):891–893.
- [20] Threadgill DW, Dlugosz AA, Hansen LA, et al. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science*. 1995;269(5221):230–234.
- [21] Kelleher D, Murphy A, Sheils O, Long A, McDevitt J. Tyrosine phosphorylation in the human duodenum. *Gut*. 1995;36(1):34–38.
- [22] Sakanoue Y, Kusunoki M, Hatada T, Sakiyama T, Yamamura T, Utsunomiya J. Altered protein tyrosine kinase levels in human colon carcinoma. *Cancer*. 1991;67(3):590–596.
- [23] Fearon ER, Cho KR, Nigro JM, et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science*. 1990;247(4938):49–56.
- [24] Madrigal L, Lynch S, Feighery C, Weir D, Kelleher D, O'Farrelly C. Flow cytometric analysis of surface major histocompatibility complex class II expression on human epithelial cells prepared from small intestinal biopsies. *J Immunol Methods*. 1993;158(2):207–214.
- [25] Cattoretti G, Pileri S, Parravicini C, et al. Antigen unmasking on formalin-fixed, paraffin-embedded tissue sections. *J Pathol*. 1993;171(2):83–98.
- [26] Serra-Pages C, Saito H, Streuli M. Mutational analysis of proprotein processing, subunit association, and shedding of the LAR transmembrane protein tyrosine phosphatase. *J Biol Chem*. 1994;269(38):23632–23641.
- [27] Streuli M, Krueger NX, Ariniello PD, et al. Expression of the receptor-linked protein tyrosine phosphatase LAR: proteolytic cleavage and shedding of the CAM-like extracellular region. *EMBO J*. 1992;11(3):897–907.
- [28] Wright N, Watson A, Morley A, Appleton D, Marks J. Cell kinetics in flat (avillous) mucosa of the human

- small intestine. *Gut*. 1973;14(9):701–710.
- [29] Podolsky DK. Regulation of intestinal epithelial proliferation: a few answers, many questions. *Am J Physiol*. 1993;264(pt 1):G179–G186.
- [30] Uribe JM, Barrett KE. Nonmitogenic actions of growth factors: an integrated view of their role in intestinal physiology and pathophysiology. *Gastroenterology*. 1997;112(1):255–268.
- [31] Kim H, Yeger H, Han R, Wallace M, Goldstein B, Rotin D. Expression of LAR-PTP2 in rat lung is confined to proliferating epithelia lining the airways and air sacs. *Am J Physiol*. 1996;270(pt 1):L566–L576.
- [32] Hunter T. Protein-tyrosine phosphatases: the other side of the coin. *Cell*. 1989;58(6):1013–1016.
- [33] Cerf-Bensussan N, Jarry A, Brousse N, Lisowska-Grospierre B, Guy-Grand D, Griscelli C. A monoclonal antibody (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *Eur J Immunol*. 1987;17(9):1279–1285.