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# Preferential Expression of the Mucosal Homing Receptor Integrin $\alpha_4\beta_7$ in Gastrointestinal Non-Hodgkin's Lymphomas

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***Recent studies have identified the integrin $\alpha_4\beta_7$ as a mucosal homing receptor that mediates lymphocyte migration to the intestinal mucosa by binding to MAdCAM-1, a vascular recognition molecule (addressin) selectively expressed on mucosal endothelium. In the present study, we have assessed the expression of $\alpha_4\beta_7$ on B- and T-cell non-Hodgkin's lymphomas of different primary localization and on related normal lymphocytes. Among B-lineage lymphomas, expression of $\alpha_4\beta_7$ was present in the majority of cases of malignant lymphomatous polyposis of the intestine and low-grade lymphoma of the mucosa-associated lymphoid tissue/monocytoid B-cell lymphoma and in some cases of precursor B-cell lymphoma. CLL/small lymphocytic lymphoma, (nodal) mantle cell lymphoma, follicular center cell lymphoma, Burkitt's lymphoma, and diffuse large B-cell lymphoma were virtually always $\alpha_4\beta_7$ negative, as was the case when localized in the mucosa-associated lymphoid tissue. The normal B cells of the follicle mantles and part of the B cells of the extrafollicular B-cell compartment of lymphoid tissues expressed moderate levels of $\alpha_4\beta_7$. By contrast, follicular center cells were $\alpha_4\beta_7$ negative. Among T-lineage lymphomas, expression of $\alpha_4\beta_7$ was also strongly related to the primary localization; in mucosal, nodal, and cutaneous T cell lymphomas the percentage of positive cases was 56%, 17%, and 0%, respectively. All cases of precursor T-cell lymphoma were $\alpha_4\beta_7$ negative. High expression of $\alpha_4\beta_7$ was found on a subset of peripheral blood memory T cells as well as on lymphocytes in the intestinal mucosa. We conclude that non-Hodgkin's lymphomas that are related to mucosa-associated B- and T-lymphocyte populations selectively express the mucosal homing receptor $\alpha_4\beta_7$. The presence of this receptor underscores their distinctive character and may play an important role in determining their characteristic mucosal dissemination pattern. (Am J Pathol 1997, 150:919–927)***

Maintenance of the integrity of distinct lymphoid compartments, such as mucosa or skin-associated lymphoid tissues, is critically dependent on selective recirculation and homing of lymphocytes. This homing process is carefully regulated through specialization of both endothelial cells and lymphocyte subsets in their expression and regulation of adhesion receptors and counter-receptors.[1] Evidence from several sources indicates that malignant lymphocytes may use these physiological homing pathways as a mechanism of dissemination.[2] For example, non-Hodgkin's lymphomas (NHLs) of the mucosa-associated lymphoid tissues (MALTs) and the skin tend to spread to mucosal sites and skin, respectively. In the latter tumors, this dissemination presumably is mediated by cutaneous lymphocyte antigen (CLA), a skin homing receptor, which is selectively expressed on cutaneous T-cell lymphomas[3,4] and interacts with E-selectin on skin endothelium.[5]

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Recently, important new insights have been obtained in the molecular basis of lymphocyte homing to the MALT. In mice, high endothelial venules of Peyer's patches and lamina propria venules selectively express a glycoprotein called mucosal vascular addressin (MAdCAM-1).[6] MAdCAM-1 is an immunoglobulin family member with domains that display homologies to the vascular adhesion receptors for leukocytes ICAM-1 (CD54) and VCAM-1 (CD106) as well as to another mucosa-associated Ig family member, IgA1.[7] The integrin $\alpha_4\beta_7$, which is strongly expresssed on mucosal lymphocytes, appears to represent the dominant lymphocyte receptor for MAdCAM-1 and for regulating lymphocyte homing to mucosal sites.[8,9] In man, $\alpha_4\beta_7$ may have a similar function; it was shown to be expressed on mucosal T-cell lines[10] and on a subset of peripheral blood memory T cells with gut homing properties.[11,12] Furthermore, we have recently shown that $\alpha_4\beta_7$ is expressed in malignant lymphomatous polyposis of the intestine (MLP), suggesting that it may play a role in the multifocal intestinal dissemination characteristic of this lymphoma.[13]

To further explore the relationship between $\alpha_4\beta_7$ expression and lymphoma localization/dissemination, we have now studied the expression of $\alpha_4\beta_7$ on a panel of NHLs and on related normal lymphocytes.

## Materials and Methods

### Case Selection and Classification

A panel of NHLs of different subcategories was selected from the files of the Departments of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands; the University Hospital Leiden, the Netherlands, and the University Hospital Vienna, Austria. Histological subclassification of NHLs was based on the Revised European American Lymphoma Classification.[14] Lymphomas of the MALTs were classified according to the criteria described by Isaacson.[15] Normal lymphoid tissues were retrieved from the files of the Department of Pathology, Academic Medical Center, Amsterdam.

### Immunohistochemistry

Immunoperoxidase staining was performed on acetone-fixed cryostat sections using the streptavidin-biotin-peroxidase complex method as described previously.[16] Before incubating with the secondary biotinylated anti-mouse F(ab')2 antibody (Dako Corp., Glostrup, Denmark), endogenous peroxidase was blocked by 0.3% $H_20_2$ in methanol. As an enzyme for color development, horseradish peroxidase was used, which was coupled to the biotin *via* a streptavidin-biotin-peroxidase complex (Dako). After incubation for 30 minutes, the sections were incubated with 3,3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, MO) for 5 to 10 minutes. Sections were counterstained with hematoxylin.

Double staining was performed with a two-step indirect immunotechnique using subclass-specific second-step antibodies, as described previously.[17] The second step consisted of a cocktail of goat anti-mouse $IgG_1$ (Southern Biotechnology Associates, Birmingham, AL) labeled with alkaline phosphatase and goat anti-mouse $IgG_{2a}$ (Southern Biotechnology Associates) labeled with horseradish peroxidase. For color development, 3,3-amino-9-ethylcarbazole (Sigma) and naphthol-AS-MX-P/fast blue BB was used (Sigma).

Staining intensity was scored semiquantitatively on a scale of 0 to 2 (0, no staining; 1, weak staining; 2, moderate/strong staining) by two independent observers (T. Radaszkiewicz and S. T. Pals). Discrepancies were solved by consensus. For a lymphoma to be scored positive, a minimum of 20% of the cells had to be stained. The antibody used for the detection of $\alpha_4\beta_7$ was Act-1 ($IgG_1$),[18] which has been shown to be specific for $\alpha_4\beta_7$.[11,12] The antibody against $\alpha^E\beta_7$ was Bez-Act8 (Dako). The anti-cytokeratin antibody was CAM 5.2 ($IgG_{2a}$; Becton Dickinson, San Jose, CA).

### Cell Isolation

Peripheral blood mononuclear cells (PBMCs) from normal donors were isolated by Ficoll-Isopaque density gradient centrifugation. For isolation of tonsil lymphocytes, tonsillar tissue was dissected free from surface epithelium and finely minced into a cell suspension. Mononuclear cells were isolated by Ficoll-Isopaque density gradient centrifugation. Monocytes were removed by plastic adherence (1-hour incubation at 37°C in 10-cm petri dishes (Costar, Cambridge, MA). T cells were depleted using 2-aminoethyl-isothiouronium-bromide-modified sheep red blood cells.

Normal duodenal biopsies were obtained from patients undergoing evaluation for peptic ulcer disease. For isolation of mononuclear cells, four endoscopic biopsies were taken into RPMI 1640 supplemented with fetal calf serum and 10% gentamycin. Biopsies were teased apart, added to a 14-ml tube (Falcon, Cambridge, MA) containing RPMI 1640/10% fetal calf serum and gentamycin with 50

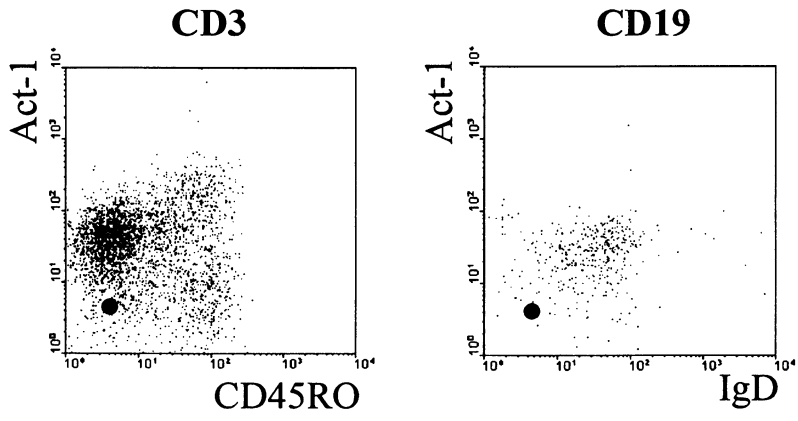


**Figure 1.** *Expression of $\alpha_4\beta_7$ (Act-1) on peripheral blood T and B cells. PBMCs were triple stained for either CD3, CD45RO, and $\alpha_4\beta_7$ or CD19, IgD, and $\alpha_4\beta_7$.* **Left:** *Expression of CD45RO* versus $\alpha_4\beta_7$ *on T cells (CD3 gated).* **Right:** *Expression of IgD* versus $\alpha_4\beta_7$ *on B cells (CD19 gated). The black dot in each histogram indicates the median fluorescence obtained when irrelevant MAbs were used as controls (representative of four samples).*

IU/ml collagenase type IV (Sigma), and placed on a mixing table (multi-purpose rotor, Scientific Industries, New York, NY) at 37°C. After 1 hour, the supernatant was pelleted and washed, and cells were resuspended in FACS buffer to a concentration of $2 \times 10^6$/ml. Cell viability was >90%. Differential isolation of intra-epithelial lymphocytes was performed by an initial incubation with 2 mmol/L EDTA and dithiothreitol (Sigma) before collagen digestion.

### *FACS Analysis*

For determining the expression of $\alpha_4\beta_7$ on lymphocyte subpopulations, triple-staining experiments were performed. Cells were preincubated with 10% human serum in phosphate-buffered saline (PBS) containing 1% bovine serum albumin and then sequentially incubated with appropriate dilutions of Act-1 and phycoerythrin-conjugated goat anti-mouse Ig (Southern Biotechnology Associates) for 30 minutes at 0°C. Free binding sites of the goat anti-mouse antibody were then blocked by incubation with 5% normal mouse serum. For detection of $\alpha_4\beta_7$ expression in peripheral blood T and B lymphocytes, the staining procedure was continued by incubating the cells with either fluorescein isothiocyanate (FITC)-labeled monoclonal antibody directed against CD45RO (UCHL-1; Dako) followed by biotin-labeled anti-CD3 (leu-4; Becton-Dickinson, Mountain View, CA) and RED613-labeled streptavidin (Gibco, Grand Island, NY) or with FITC-labeled rabbit anti-IgD (Dako) followed by biotin-labeled anti-CD19 (HD37; Dako) and RED613-labeled streptavidin. For detection of $\alpha_4\beta_7^+$ subpopulations in tonsil B cells, the 5% normal mouse serum incubation step was followed by FITC-labeled rabbit anti-IgD (Dako), biotin-labeled anti-CD38 (CALTAG Laboratories, San Francisco, CA) and RED613-labeled streptavidin. The incubations with the conjugated antibodies were performed in PBS/bovine serum albumin with 10% human serum for 30 minutes at 0°C.

## *Results*

### *Expression of the Mucosal Homing Receptor $\alpha_4\beta_7$ on Normal Lymphocytes*

Peripheral blood lymphocytes from healthy volunteers were analyzed for the simultaneous expression of CD3, CD45RO, and $\alpha_4\beta_7$ or CD19, IgD, and $\alpha_4\beta_7$ (Figure 1). In accordance with previous reports,[11,12] the $CD45RO^-$ (naive) T cells homogeneously expressed $\alpha_4\beta_7$, whereas the expression of $\alpha_4\beta_7$ on the memory T-cell subset ($CD45RO^+$) was heterogeneous with a $\alpha_4\beta_7$ low/negative subset and a subpopulation expressing high levels of $\alpha_4\beta_7$ representing gut homing T lymphocytes. The vast majority of B-cell peripheral blood lymphocytes showed a moderately strong homogeneous expression of $\alpha_4\beta_7$.

In histological sections of lymph nodes, tonsils, and small intestine, $\alpha_4\beta_7$ was weakly expressed on the cells in the mantle zones of B-cell follicles (Figure 2A) and on approximately 30% of the cells in the extrafollicular compartments of lymph nodes and tonsils. Germinal center cells were consistently $\alpha_4\beta_7$ negative (Figure 2A). In the mucosa of the small intestine, approximately 50% of the cells in the lamina propria showed expression of $\alpha_4\beta_7$ whereas intra-epithelial T lymphocytes were not stained (Figure 2B-D).

Restriction of $\alpha_4\beta_7$ expression to specific lymphocyte populations was also demonstrated on isolated tonsillar B lymphocytes (Figure 3) and duodenal T lymphocytes (Figure 4). Of the B lymphocytes, the $IgD^+$ B-cell subset, which represent naive B-cells largely derived from follicle mantle zones, were $\alpha_4\beta_7$ positive, whereas ($IgD^-/CD38^+$) germinal center B

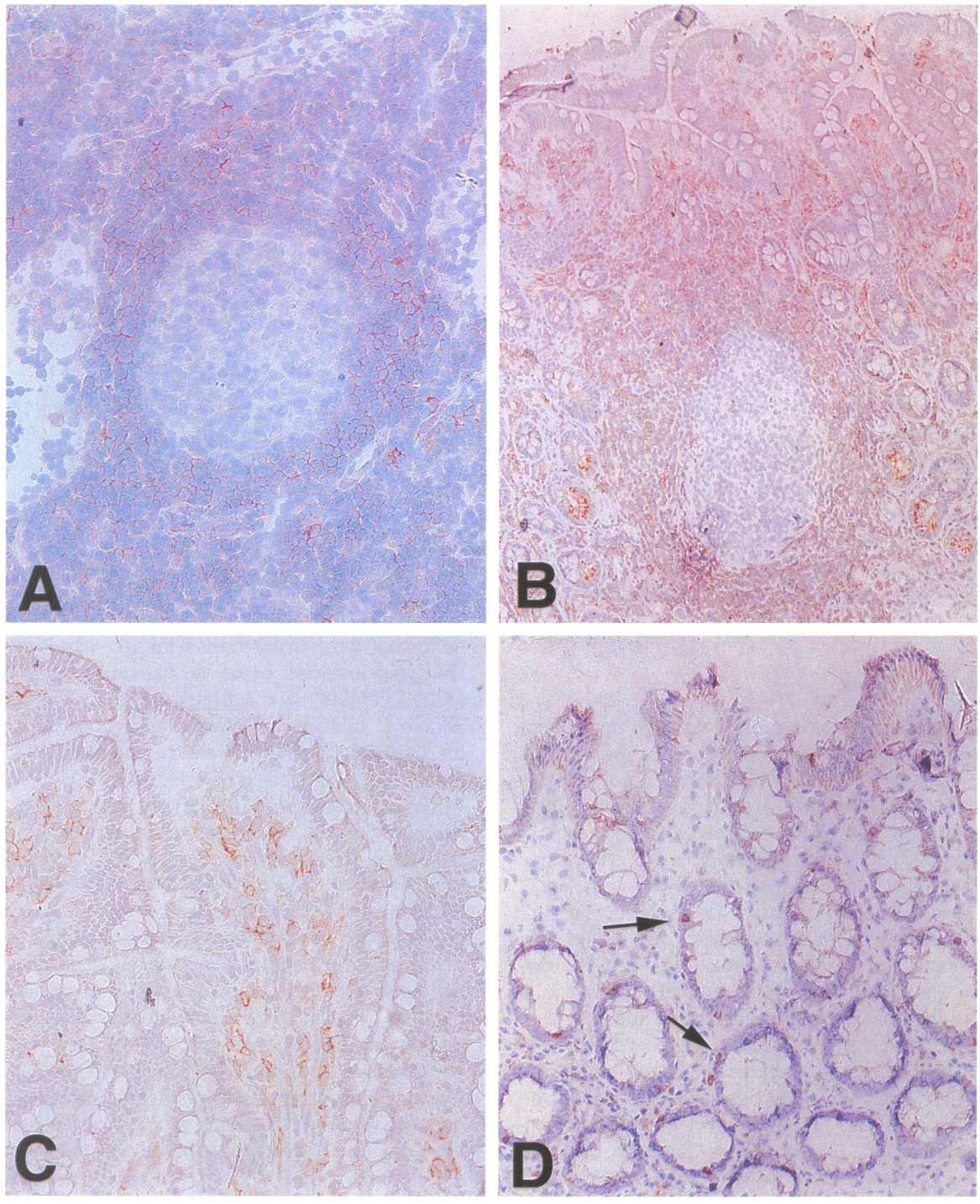


Figure 2. *Expression of* $\alpha_4\beta_7$ *in normal lymph node* (A) *and MALT* (B *and* C). D: *Expression of* $\alpha^E\beta_7$ *on the intra-epithelial lymphocytes in the normal mucosa. Magnification,* ×*230* (A *and* B), ×*460* (C), *and* ×*175* (D).

cells were negative. The third subpopulation of B cells expressing neither IgD nor CD38 was partly $\alpha_4\beta_7^+$ (Figure 3). This population represents memory B cells derived from the extrafollicular B-cell compartment of the tonsil. Of the isolated duodenal T lymphocytes, the cells derived from the lamina propria showed a relatively strong expression of $\alpha_4\beta_7$ whereas the intra-epithelial fraction was only very weakly positive (Figure 4). These findings are in line with our immunohistochemical observations (Figure

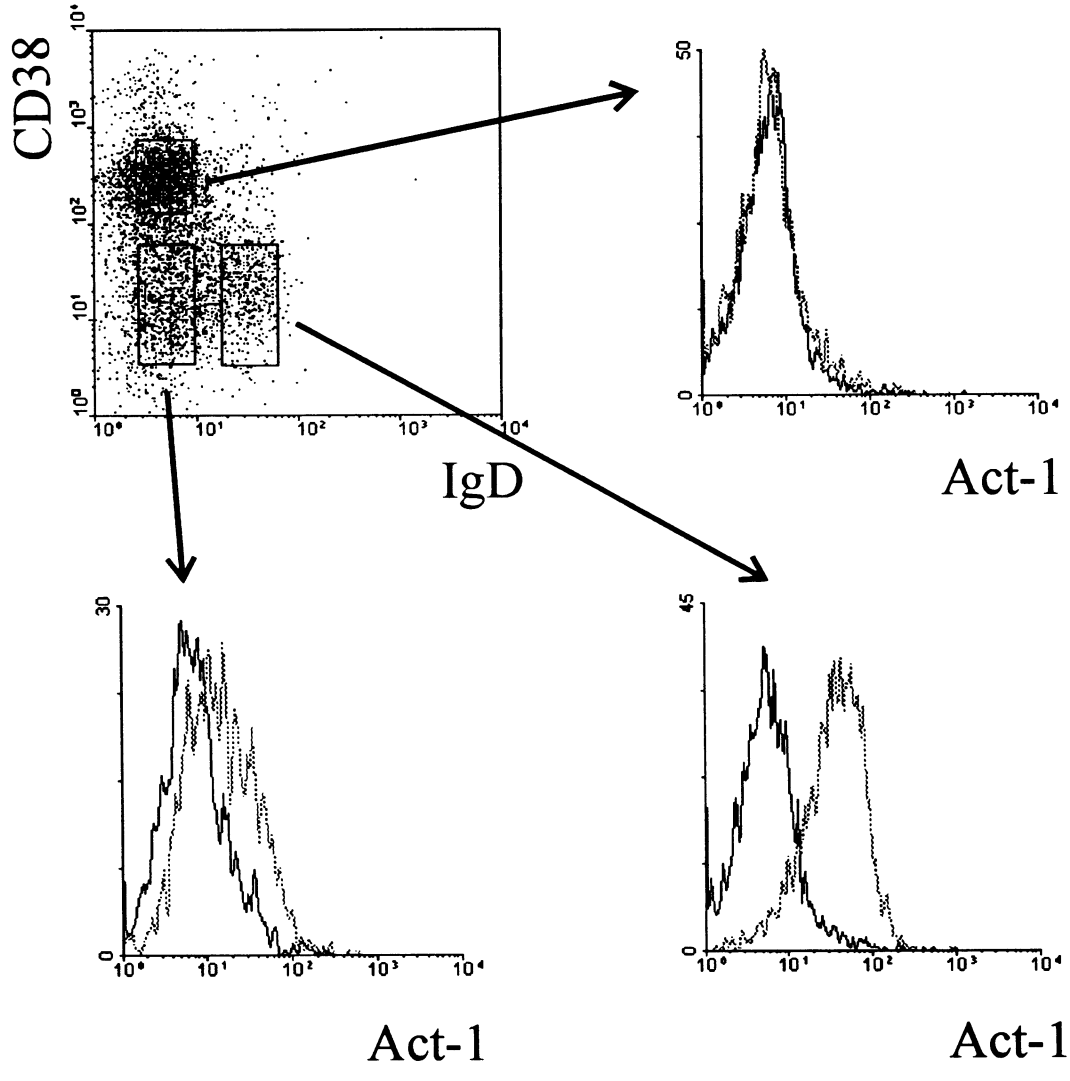


Figure 3. *Expression of $\alpha_4\beta_7$ (Act-1) on tonsil B cell subpopulations. Purified tonsil B cells (CD19 > 98%) were triple stained for IgD, CD38, and $\alpha_4\beta_7$.* Upper left: *Expression of IgD* versus *CD38 showing three distinct subpopulations, ie, IgD$^-$, CD38$^+$ (germinal center B cells); IgD$^+$, CD38$^-$ (naive B cells); and IgD$^-$, CD38$^-$ (memory B cells). Expression of $\alpha_4\beta_7$ is shown in histogram plots for IgD$^-$, CD38$^+$* (upper right), *IgD$^+$, CD38$^-$* (lower right), *and IgD$^-$, CD38$^-$* (lower left) *B cell subpopulations. ——, background; ·····, $\alpha_4\beta_7$ expression; representative of four samples.*

2) and with the observation in the mouse that $\alpha_4\beta_7$ is down-regulated upon T-lymphocyte migration to the epithelium.[19]

## *Expression of $\alpha_4\beta_7$ on B-Cell Non-Hodgkin's Lymphomas*

To assess $\alpha_4\beta_7$ expression on B-NHLs, a panel of tumors representing different pathological subtypes and with primary localization in either lymph nodes or MALT was analyzed (Table 1). Interestingly, $\alpha_4\beta_7$ expression was found to be by far the most common in two distinctive types of B-NHL characterized by primary localization in the MALTs, ie, marginal-zone lymphoma (low-grade lymphoma of MALT and monocytoid B-cell lymphoma) and MLP (Table 1 and Figure 5, A, C, and D). Positive cases of marginal-zone lymphoma were located in the gastrointestinal tract (n = 7), tonsil plus regional lymph nodes (n = 1), and salivary gland (n = 1). A detailed description of the clinical and pathological findings in the cases of MLP included in this study, which all had multiple intestinal tract lesions, has been published elsewhere.[13] Unlike low-grade B-cell lymphoma of MALT and MLP, cases of mucosa-associated diffuse large B-cell lymphoma (n = 8) or ileocecal Burkitt's lymphoma (n = 3) did not express $\alpha_4\beta_7$ (Figure 5B).

Neoplasms of precursor B cells, ie, B-lymphoblastic lymphomas, were heterogeneous with respect to $\alpha_4\beta_7$ expression, ie, two of four cases were positive.

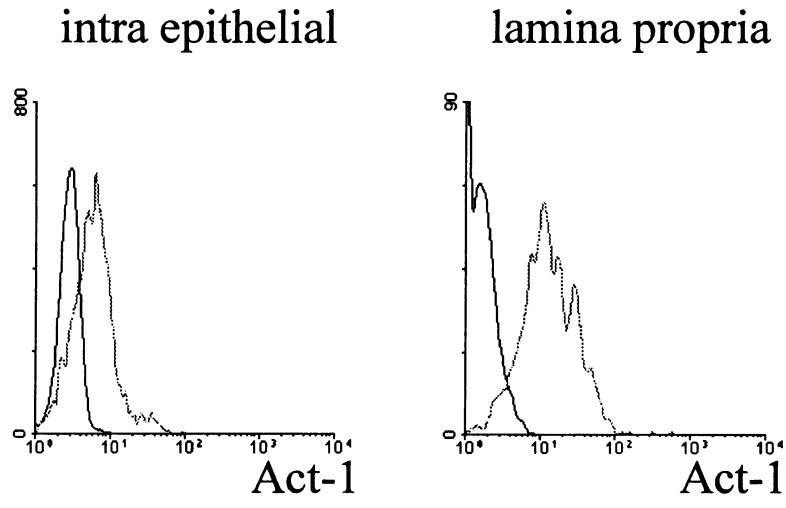


Figure 4. *Expression of $\alpha_4\beta_7$ (Act-1) on mucosal T lymphocytes.* Left: *Intra-epithelial T cells.* Right: *Lamina propria T cells.* ——, *background;* ·····, *$\alpha_4\beta_7$ expression; representative of four samples.*

Of the primary nodal peripheral (mature) B-cell neoplasms (n = 41), which included cases of CLL/small lymphocytic lymphoma, nodal mantle cell lymphoma, follicular center lymphoma, Burkitt's lymphoma, and diffuse large B-cell lymphoma, only 2 expressed $\alpha_4\beta_7$.

## *Expression of $\alpha_4\beta_7$ on T-Cell Non-Hodgkin's Lymphomas*

A panel of T-NHLs representing different pathological subtypes with primary localizations in lymph node, skin, and mucosa was analyzed for the expression of $\alpha_4\beta_7$ (Table 2). Neoplasms of precursor T cells, ie, T-lymphoblastic lymphomas, were invariably $\alpha_4\beta_7$ negative. In peripheral T-cell lymphomas, expression of $\alpha_4\beta_7$ was strongly correlated with the primary localization of the tumor. $\alpha_4\beta_7$ was found in 5 of 9 cases of primary mucosal T-cell lymphoma, in 1 of 6 cases of nodal T-cell lymphoma, and in 0 of 7 cases of primary cutaneous T-cell lymphoma (mycosis fungoides). Furthermore, $\alpha_4\beta_7$ was expressed in 2 of 17 cases of anaplastic large-cell lymphoma. Hence, like in lymphomas of the B lineage, $\alpha_4\beta_7$ expression in T-lineage lymphomas is a characteristic of primary mucosal T-cell lymphomas.

Table 1. *Expression of the Mucosal Homing Receptor $\alpha_4\beta_7$ on B-Lineage Non-Hodgkin's Lymphomas*

| Subtype | Number $\alpha_4\beta_7^+$/ Number tested (%) | Intensity |
|---|---|---|
| B-cell lymphoma | | |
| Precursor B | | |
| Lymphoblastic | 2/4 (50) | 1+ |
| Peripheral B | | |
| CLL/lymphocytic | 0/6 (0) | 0 |
| Mantle cell | 1/6 (17) | 1+ |
| MLP | 7/8 (87) | 2+ |
| Follicle center | 0/11 (0) | 0 |
| Marginal zone | | |
| Low-grade B MALT | 9/10 (90) | 1–2+ |
| Monocytoid B | 2/2 (100) | 1–2+ |
| Diffuse large B | 1/24 (4) | 2+ |
| Burkitt's | 0/5 (0) | 0 |

Lymphomas were classified according to the Revised European American Lymphoma Classification. Scoring for intensity was as follows: 0, no staining; 1+, weak staining; 2+, moderate to strong staining.

## *Discussion*

The key observation of this study is that the mucosal homing receptor $\alpha_4\beta_7$ is expressed on the vast majority of cases of low-grade B-cell lymphoma of MALT, MLP, and intestinal T-cell lymphoma but that expression of this molecule is uncommon in B- and T-cell lymphomas that are not MALT related (Tables 1 and 2). This selective expression of the mucosal homing receptor $\alpha_4\beta_7$ on MALT lymphomas strongly

Table 2. *Expression of the Mucosal Homing Receptor $\alpha_4\beta_7$ on T-Lineage Non-Hodgkin's Lymphomas*

| Subtype | Number $\alpha_4\beta_7^+$/ Number tested (%) | Intensity |
|---|---|---|
| T-cell lymphoma | | |
| Precursor T | | |
| Lymphoblastic | 0/8 (0) | 0 |
| Peripheral T | | |
| Unspecified (nodal) | 1/6 (17) | 2+ |
| Enteropathy associated | 5/9 (56) | 1–2+ |
| Mycosis fungoides/CTCL | 0/7 (0) | 0 |
| Anaplastic large cell | 2/12 (17) | 1–2+ |

Lymphomas were classified according to the Revised European American Lymphoma Classification. Scoring for intensity was as follows: 0, no staining; 1+, weak staining; 2+, moderate to strong staining. CTCL, cutaneous T-cell lymphoma.

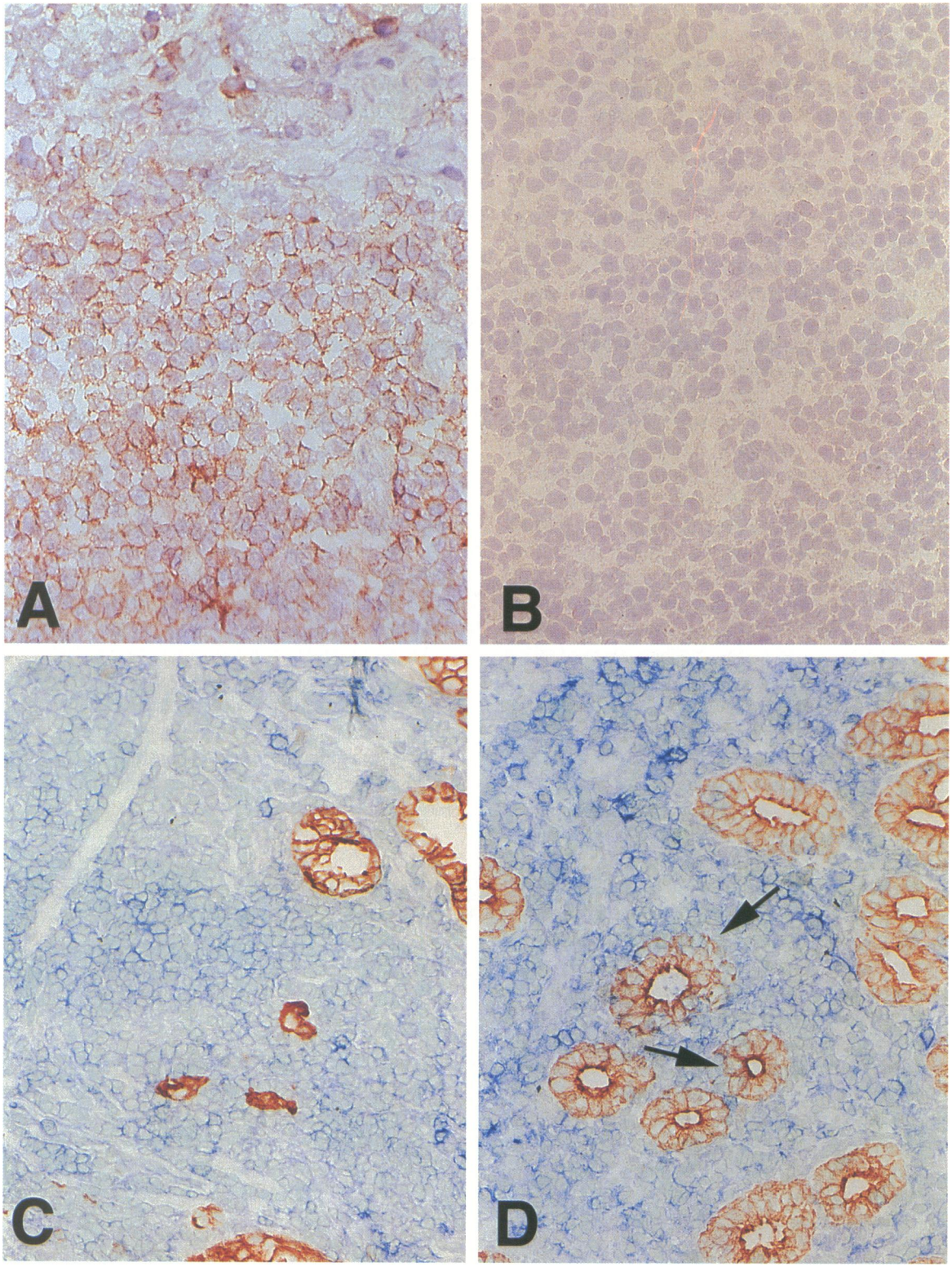


**Figure 5.** *Expression of $\alpha_4\beta_7$ on mucosal NHLs.* **A:** *Low-grade B-cell lymphoma of MALT localized in the stomach with positive staining for $\alpha_4\beta_7$.* **B:** *Diffuse large B-cell NHL localized in the stomach, with no $\alpha_4\beta_7$ expression.* **C** *and* **D:** *Low-grade B-cell lymphoma of MALT localized in the stomach, with double staining for $\alpha_4\beta_7$ (blue) and cytokeratin (red), showing extensive destruction of epithelium by $\alpha_4\beta_7$ tumor cells with lymphoepithelial lesions* (**arrows**). *Magnification, ×460* (**A** *and* **B**), *×230* (**C**), *and ×345* (**D**).

supports the concept that these lymphomas represent a biologically distinctive group of tumors.[15,20] Furthermore, the selective expression of $\alpha_4\beta_7$ may explain the propensity of mucosal lymphomas to disseminate to and relapse at distant mucosal sites.[2,15,20–22]

The existence of distinctive recirculation pathways for different lymphocyte subpopulations[1,23,24] is one of the striking features of lymphocyte homing. Whereas naive T cells recirculate preferentially through secondary lymphoid tissues such as lymph nodes, memory (and activated) T cells preferentially leave the blood in peripheral vascular beds of, eg, the skin and the mucosa.[23] Among memory T cells, there is yet further specialization; distinct subsets of memory T cells home to the skin or gut lamina propria, respectively.[1,11,24] In the human peripheral blood, $\alpha_4\beta_7$ is expressed on a subset of gut-trophic memory T lymphocytes[11,12] (Figure 1). Moreover, $\alpha_4\beta_7$ is expressed at high levels on T cells in the lamina propria of the intestine but is down-regulated on intra-epithelial lymphocytes (Figures 2C and 4). This $\alpha_4\beta_7$ memory T-cell subset is phenotypically and functionally distinct from other subsets of memory T cells[11] and, for example, is nonoverlapping with a memory T-cell subset defined by expression of CLA, a skin homing receptor.[11] The presence of $\alpha_4\beta_7$ on intestinal T-cell lymphomas strongly suggests that these tumors (which all expressed CD45RO) are directly derived from gut homing $\alpha_4\beta_7$-positive memory T cells. Like in the normal memory T-cell subsets, $\alpha_4\beta_7$ and CLA expression on T cell lymphomas was also mutually exclusive; the intestinal T-cell lymphomas in our series did not express the skin homing receptor CLA (our own unpublished observation), and *vice-versa*, we did not observe $\alpha_4\beta_7$ expression in any of the cutaneous T-cell lymphomas examined (Table 2).

Interestingly, most cases of low-grade B-cell lymphoma of MALT and monocytoid B-cell lymphoma were found to express $\alpha_4\beta_7$ (Figure 5, A, C, and D). These tumors represent closely related lymphoma subtypes, are believed to originate from memory B cells residing in the marginal zones of mucosal lymphoid tissues,[14] and hence might be related to the $\alpha_4\beta_7^+$/IgD$^-$, CD38$^-$ subset of tonsil lymphocytes identified in the present study (Figure 3). They typically arise at mucosal sites where they give rise to lympho-epithelial lesions. Although data on the molecular basis of normal B cell homing are scarce, we envision that expression of the mucosal homing receptor $\alpha_4\beta_7$ in these tumors might be instrumental in their often very typical dissemination to distant mucosal sites.[2,13,15,20–22] Also, the observation that $\alpha_4\beta_7$ was not only expressed on intestinal tumors but also on lymphomas localized in the tonsil and the salivary gland favors their relation to a common mucosal immune system involving lymphocytes committed to mucosal sites.[25] In this context, the recent report by Diss et al[21] of a single neoplastic B-cell clone in sequential biopsy specimens from a patient with primary gastric-mucosa-associated lymphoma and Sjogren's syndrome is of interest.

We observed that $\alpha_4\beta_7$ is expressed at relatively high levels in MLP. The expression of this mucosal homing receptor on the tumor cells in this uncommon but dramatic disease, characterized by multifocal gastrointestinal involvement, might be an important factor in its dissemination. MLP has been proposed to be related to follicle mantle cells,[14] which represent naive B cells. By analogy with naive T cells, they express $\alpha_4\beta_7$ (Figures 2A and 3) in concert with several other adhesion receptors including L-selectin, thus presumably providing them with a relatively broad homing specificity.[26] Interestingly, most cases of nodal mantle cell lymphoma did not express $\alpha_4\beta_7$, although they were L-selectin positive, and hence may have lost their potential to home to mucosal sites[13].

Taken together, our data indicate that NHLs that are related to mucosa-associated B- and T-lymphocyte populations selectively express the mucosal homing receptor $\alpha_4\beta_7$. The presence of this receptor underscores their distinctive character and may play an important role in determining their characteristic mucosal dissemination pattern.

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