

Intestinal Intraepithelial Lymphocytes Bind to Colon Cancer Cells by HML-1 and CD11a¹

Arthur I. Roberts, Sean M. O'Connell, and Ellen C. Ebert²

Departments of Medicine and Surgery, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903

ABSTRACT

Human intraepithelial lymphocytes (IEL), predominantly CD8⁺ T-lymphocytes located between intestinal epithelial cells (EC), may represent the first-line immune defense against colon cancer. The mechanism by which IEL bind to the colon cancer line, DLD-1, was evaluated.

A larger fraction of IEL than peripheral blood mononuclear cells bound to DLD-1 monolayers (25 ± 16 versus $8 \pm 4\%$ binding, $P < 0.05$). Binding increased when DLD-1 monolayers were incubated with interferon- γ but not with tumor necrosis factor- α . Similar numbers of IEL adhered to EC tumors, HT-29 and 5637, and the non-EC tumor, A375, but fewer bound to nonmalignant smooth muscle (HISM) and fibroblast (KD) lines ($P < 0.01$). Binding of IEL to DLD-1 was reduced by monoclonal antibodies to HML-1 and CD11a (47 ± 9 and $26 \pm 13\%$ inhibition, respectively) and was completely eliminated by both combined ($93 \pm 4\%$ inhibition). Anti-HML-1 also inhibited the binding of IEL to other EC tumors but did not affect binding to non-EC tumors or fibroblasts. To conclude, the binding of IEL to EC tumors is mediated by HML-1 and CD11a [A. I. Roberts, S. M. O'Connell, and E. C. Ebert. Binding of intraepithelial lymphocytes to colon cancer cells is mediated by HML-1 and LFA-1 (abstract). *Gastroenterology*, 102: A685, 1992].

INTRODUCTION

The early host immune responses to dysplastic intestinal EC³ and colonic adenocarcinomas are unknown. The first components of the immune system to encounter such abnormal cells are the IEL, predominantly CD8⁺ T-cells dispersed between EC. Several lines of evidence suggest that this compartment of lymphocytes may play an important role in the destruction of colon cancer: (a) the CD8⁺ T-cell subset of human IEL displays spontaneous cytotoxicity directed selectively against EC tumors but not against K-562 cells, whereas the natural killer cells in peripheral blood lyse both tumor types (1); (b) colon tumor-infiltrating lymphocytes are predominantly positive for HML-1, a surface marker found on intraepithelial, but not circulating, lymphocytes (2); and (c) anti-HML-1 antibody inhibits lymphokine-activated killer activity by IEL against colon cancer target cells but not K-562 cells (3).

Direct contact of lymphocytes with colon cancer cells is required for most cell-mediated antitumor immune events. Several antigen-independent binding mechanisms have been described in other systems. One is the attachment of the CD11a molecule of circulating lymphocytes to CD54 on epidermal keratinocytes (4), vascular endothelial cells (5), and colonic adenocarcinoma cell lines (6). Certain lymphokines, such as IFN- γ , TNF- α , or interleukin 1, enhance this interaction. Another is the binding of the CD2 structure on thymocytes to CD58 on thymic EC, which results in the activation of the thymocytes (7). IEL, too, are activated through the CD2 receptor (8).

Received 8/19/92; accepted 1/21/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This project was funded by a grant from the NIH (DK 42166).

² To whom requests for reprints should be addressed, at Department of Medicine, University of Medicine & Dentistry of New Jersey, Robert Wood Johnson Medical School, One Robert Wood Johnson Place-CN 19, New Brunswick, NJ 08903.

³ The abbreviations used are: EC, epithelial cell(s); PBMC, peripheral blood mononuclear cell(s); IFN, interferon; TNF, tumor necrosis factor; IEL, intraepithelial lymphocyte(s); VLA, very late activation antigen.

In this study, we examined the mechanism by which IEL bind to colon cancer cells. Both pathways described above were evaluated in detail.

MATERIALS AND METHODS

Isolation of Lymphocytes. PBMC were separated from freshly drawn whole blood using a Ficoll density gradient (Organon Teknica, Durham, NC). IEL were separated from jejunal mucosa obtained from healthy individuals undergoing gastric bypass operations for morbid obesity. In brief, the minced mucosa was treated for 25 min at 37°C with 1 mM dithiothreitol (Sigma Chemical Co., St. Louis, MO), followed by three 45-min incubations in a shaking water bath with 0.75 mM EDTA (Sigma) in calcium- and magnesium-free Hanks' buffered salt solution (GIBCO, Grand Island, NY). The cells in the supernatants were collected and kept at 4°C overnight. IEL were separated by a Percoll (Pharmacia, Piscataway, NJ) density gradient as described previously (1, 8), with the exception that cells were resuspended in the 40% Percoll layer, not the 100% layer, before centrifugation. Purified IEL from above the 60% Percoll layer contained >90% lymphocytes that were $94 \pm 5\%$ CD2⁺ and $89 \pm 2\%$ CD8⁺, consistent with the phenotype of IEL shown by immunohistochemistry (9).

Magnetic Sorting. For some experiments, PBMC were enriched for CD8⁺ T-cells by negative selection using magnetic sorting. PBMC (10×10^6) in 0.1 ml of complete medium were incubated on ice for 30 min with 5 μ g of azide-free monoclonal antibody to CD4 (AMAC, Inc., Westbrook, ME) and then washed three times. Cells were resuspended to 4×10^6 /ml, and 0.5 ml of prewashed magnetic goat anti-mouse IgG suspension (Collaborative Biomedical Products, Bedford, MA) was added. After a 20-min incubation on ice, magnetic particle-cell complexes were removed by application of an Alnico V magnet to the side of the tube for 10 min at 4°C. After separation, PBMC preparations were <1% CD4⁺ by immunofluorescence.

Binding Assay. Various cell lines (American Type Culture Collection, Rockville, MD) were seeded into flat-bottom microwells in complete medium: RPMI-1640 containing 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), 1% antibiotic-antimycotic solution and glutamine (GIBCO), and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma). Some cultures were supplemented with IFN- γ , TNF- α , or both lymphokines combined (each at 100 units/ml; Amgen, Thousand Oaks, CA). All cultures formed confluent monolayers after an overnight incubation. The supernatant was aspirated, and 4×10^5 lymphocytes were labeled with [⁵¹Cr]sodium chromate (New England Nuclear, Boston, MA) were added to each well. After incubating 2 h at 37°C, nonadherent cells were removed by gentle washes accomplished by repeatedly adding and decanting warm complete medium. A 2% cetrimide solution (Sigma) was then added to lyse the adherent cells, the contents of each well were collected, and the radioactivity was counted. After the spontaneous release of radiolabel was adjusted for, the fraction of lymphocytes bound to the monolayer was calculated as a percentage of the total added.

In some experiments, monoclonal antibodies to the following surface markers were added to the cell cultures 10 min before adding labeled lymphocytes and left in for the duration of the assay: CD2 (T11; Coulter Immunology, Hialeah, FL); CD11a (LFA-1), CD54 (ICAM1), CD58 (LFA-3), HML-1 (AMAC, Inc.), and VLA-1 (T Cell Sciences, Cambridge, MA), with or without a 300-fold molar excess of human IgG-Fc fragment (Accurate Chemical, Westbury, NY).

Flow Cytometrics Analysis. Lymphocytes were stained by indirect immunofluorescence with various monoclonal IgG antibodies, followed by fluorescein-conjugated goat anti-mouse IgG (Coulter). Fluorescence was analyzed using a Coulter Profile analytical flow cytometer with a 25-mW argon laser. Cell size was measured by forward angle light scatter, and intracellular granularity differences were measured by right angle light scatter. These parameters were used to identify and gate the major cell subpopulations for

fluorescence analysis. For each group, $1-2 \times 10^4$ events (cells) were collected. Data were analyzed using Overton's Cumulative Subtraction program in the Cytologic Flow Cytometry Analysis Software (Coulter; 10).

Statistical Analysis. For each set of data, an arithmetic mean, SD, and SEM were calculated. Pairs of data sets were compared with Student's *t* test for paired or independent variables.

RESULTS

To determine the kinetics of binding, radiolabeled lymphocytes were added to DLD-1 monolayers, incubated for various times up to 6 h, and the percentage of lymphocytes bound was measured (Fig. 1). More IEL than PBMC remained attached to the monolayer for all incubation times tested. This was confirmed by counting the number of nonadherent lymphocytes with a hemacytometer.

Next, DLD-1 monolayers were grown with IFN- γ , TNF- α , or both for 18 h before assaying the binding of IEL or PBMC (Table 1). IFN- γ treatment of DLD-1 cells increased binding by IEL only slightly but tripled PBMC binding to a level equivalent to that of IEL binding. TNF- α treatment had no effect on binding, and there was no synergy with combined lymphokines. Extending to 48 h the incubation of DLD-1 cells with IFN- γ did not further increase binding by either lymphocyte type.

Since IEL are almost exclusively CD8⁺ T-lymphocytes and are subject to a unique isolation procedure, the possibility that the differences in binding between IEL and PBMC could be due to phenotypic differences alone, or to the isolation procedure itself, was examined. PBMC were enriched for CD8⁺ T-cells by removing CD4⁺ cells with magnetic sorting. Then, half were treated by the same method used to isolate IEL. Binding to DLD-1 monolayers by peripheral blood CD8⁺ T-cells was the same before ($36.0 \pm 11.0\%$) and after ($37.0 \pm 19.8\%$) treatment and was equivalent to PBMC binding ($34.5 \pm 7.8\%$, $n = 2$), excluding this explanation. The possibility that RBCs in the IEL preparation contributed to binding was ruled out since radiolabeled erythrocytes did not bind at all to DLD-1 monolayers.

To determine the generality of this binding event, other human cell types grown as adherent monolayers were used: HT-29 (colonic adenocarcinoma), 5637 (bladder epidermoid carcinoma), A375 (malig-

Table 1 Effect of lymphokine treatment on binding of IEL or PBMC to DLD-1 monolayers

DLD-1 cells (10^5) were cultured in microwells with or without IFN- γ (100 units/ml), TNF- α (100 units/ml), or both combined and grown to confluence for 18 h. Chromium-51-labeled lymphocytes (4×10^5) were added and incubated for 2 h at 37° and nonadherent cells were washed off. Adherent cells were lysed, and binding was calculated as a percentage of total lymphocytes added. Mean \pm SD of the number of experiments is given in parentheses. Student's *t* test (independent variables): a versus c, $P < 0.05$. Student's *t* test (paired variables): a versus b, $P = 0.01$; c versus d, $P = 0.001$.

Lymphocyte type	Treatment of DLD-1 cells			
	Control	IFN- γ	TNF- α	IFN- γ + TNF- α
IEL	25 \pm 16 (8)a	29 \pm 15 (8)b	25 \pm 15 (6)	31 \pm 18 (6)
PBMC	8 \pm 4 (6)c	22 \pm 9 (6)d	13 \pm 7 (3)	24 \pm 9 (3)

Table 2 Binding of IEL to various cell types

The various cell lines were grown to confluence in microwells. Binding of IEL to each cell line was performed as described in the "Materials and Methods" and expressed as a proportion of the binding to DLD-1 cells (mean \pm SD; number of experiments in parentheses). Student's *t* test (paired variables): a versus DLD-1 control of 1.0, $P < 0.01$; b versus DLD-1 control of 1.0, $p < 0.001$.

Cell types	Binding
Malignant	
HT-29	0.9 \pm 0.0 (3)
5637	1.4 \pm 0.3 (3)
A375	1.0 \pm 0.0 (2)
Nonmalignant	
HISM	0.5 \pm 0.2 (4)a
KD	0.3 \pm 0.1 (4)b

nant melanoma), HISM (intestinal smooth muscle), and KD (lip fibroblast). The percentages of IEL binding to tumors of EC origin (HT-29 and 5637) or non-EC origin (A375) were the same as that binding to DLD-1 cells (Table 2). IEL bound to nonmalignant monolayers (HISM and KD) to a much lesser extent. IEL did not bind to plastic culture surfaces lacking cell monolayers. When the assay was carried out at 4°C, no IEL bound, suggesting that active metabolism is necessary. Also, IEL did not bind to paraformaldehyde-fixed DLD-1 cells.

To determine which surface structures mediate the binding of IEL to DLD-1 cells, various monoclonal antibodies (2.5 μ g/ml) were added to the binding assay and their effects measured. Adhesion was significantly reduced by antibodies to CD11a, HML-1, and VLA-1 but not by antibodies to CD54, CD58, or CD2 (Table 3). When all pairs of antibodies were tested in combination, only anti-HML-1 synergized with either anti-CD11a or anti-CD54 to maximally inhibit IEL binding to DLD-1. Antibody to HML-1 also inhibited binding to HT-29 ($63.6 \pm 11.1\%$, $n = 2$) and 5637 cells but did not affect binding to A375. Antibodies to CD11a and CD54 had greater inhibitory effects on IEL binding to A375 than to the other malignant monolayers. Anti-CD11a, but not anti-HML-1, inhibited binding to KD fibroblasts, and the combination was not synergistic. To eliminate the possible confounding effect of Fc receptor-mediated cell binding, a high concentration of human IgG Fc fragments was added to the antibody inhibition experiments to saturate any Fc receptors. The results were the same with or without Fc fragments.

Binding by PBMC to DLD-1 monolayers was unaffected by anti-HML-1 and inhibited by anti-CD11a and anti-CD54 by only 27.6 ± 5.0 and $9.2 \pm 7.3\%$ ($n = 2$), respectively. The large increase in binding after IFN- γ treatment of DLD-1 cells was not due to increased CD54 expression since, again, anti-CD54 antibody inhibited binding by only $12.3 \pm 2.5\%$ ($n = 2$). In fact, various concentrations of anti-CD54 antibody from 0.1 to 10 μ g/ml had the same inhibitory effects on binding by IEL or PBMC whether or not the DLD-1 monolayers were pretreated with IFN- γ .

IEL were evaluated by flow cytometry for those surface markers that appeared to be involved in binding. Morphological characteristics

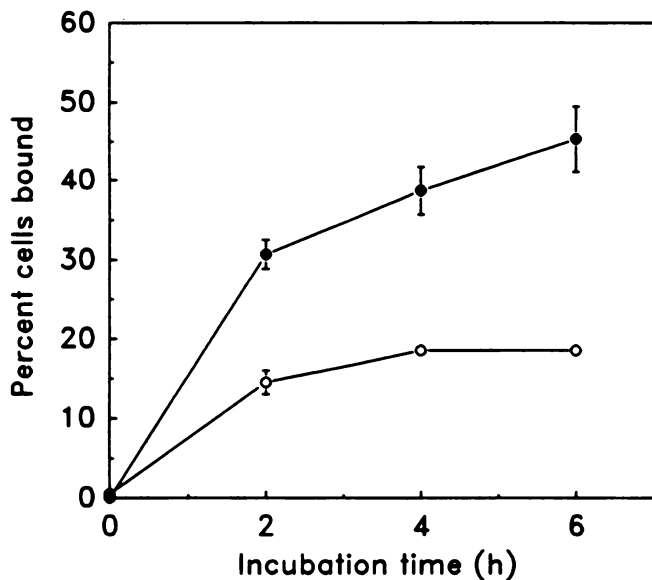


Fig. 1. Kinetics of binding of IEL (●) and PBMC (○) to DLD-1 monolayers. Chromium-51-labeled lymphocytes (4×10^5) were applied to newly confluent monolayers of DLD-1 cells in microwells and incubated at 37°C. Nonadherent cells were washed off after the time indicated. Adherent cells were lysed, and binding was calculated as a percentage of total lymphocytes added. Data points, means of 4 experiments; error bars, SEM. At 4 and 6 h, SEM values for PBMC were too small to include.

alone allowed the identification of two distinct subpopulations of IEL: the majority of a similar size and granularity as PBMC (Fig. 2A, map 1) and a minority (10–25% of IEL) that are larger and more granular (Fig. 2A, map 2). IEL from both subpopulations bound equally well, as indicated by an unchanged distribution before and after depletion by DLD-1 monolayer adherence. Fluorescence data show that the majority of IEL in both maps express HML-1 and VLA-1, the former at very high density. Both markers are almost absent on PBMC (Table 4). In contrast, CD11a is expressed by both lymphocyte types, although it is found on a larger percentage of PBMC than IEL. DLD-1 and KD monolayers were also evaluated by fluorescence microscopy for those surface markers that may be involved in binding. Both cell lines were negative for CD2, CD11a, and CD58 but weakly positive for CD54 (not shown).

DISCUSSION

A role of IEL in the early host immune defense against colon cancer may be to identify and kill dysplastic or malignantly transformed EC. Interspersed between EC, IEL are well positioned to carry out these functions. In addition, IEL may be a major source of tumor-infiltrating lymphocytes because these two cell populations are predominantly HML-1⁺. Most important, IEL demonstrate spontaneous cytotoxicity *in vitro* that is directed specifically against EC tumors rather than the natural killer-sensitive K-562 cells.

In order to lyse its intended target, a cytotoxic cell must first identify and bind to the target cell through complementary cell surface receptors. One well-studied example is the CD11a-CD54 interaction, known to mediate the aggregation of activated lymphocytes and the coupling of cytotoxic lymphocytes to their targets. This study examined the involvement of several cell surface receptors in the adhesion of human IEL to colon cancer cells. The binding of IEL to DLD-1 and other EC tumors involved the CD11a-CD54 interaction and the liga-

Table 4 Expression of HML-1, CD11a, and VLA-1 on IEL and PBMC
 IEL and PBMC were stained by indirect immunofluorescence and analyzed by flow cytometry for % positive cells and relative intensity of fluorescence compared to a negative control stained with only fluorescein-conjugated goat anti-mouse IgG. The number of experiments performed is in parentheses. Student's *t* test (independent variables): a versus b: *P* < 0.001; c versus d, e versus f, g versus h: *P* < 0.05.

Lymphocyte type and surface marker	Map 1		Map 2	
	% positive	Relative intensity of fluorescence	% positive	Relative intensity of fluorescence
IEL/HML-1 (4)	87 ± 8a	24 ± 5	68 ± 23c	16 ± 8
PBMC/HML-1 (3)	4 ± 1b	6 ± 2	13 ± 3d	2 ± 0
IEL/CD11a (5)	73 ± 10c	9 ± 6	64 ± 20g	8 ± 8
PBMC/CD11a (3)	91 ± 5f	24 ± 16	90 ± 6h	15 ± 3
IEL/VLA-1 (2)	71 ± 31	7 ± 3	74 ± 20	8 ± 3

tion of HML-1 on IEL to an unknown receptor on tumor cells. In contrast, IEL binding to a non-EC tumor and to mucosal fibroblasts involved CD11a-CD54 but not HML-1.

CD54, a glycoprotein of 80–110 kDa, is a ligand for CD11a. It is found on many hematopoietic cells, such as B-lymphocytes, macrophages, and dendritic cells (11), as well as such nonhematopoietic cells as EC in the intestine, skin, thymus, and thyroid, and endothelial cells (4, 6, 12). On endothelial cells, CD54 expression is strongly upregulated by TNF-α, lipopolysaccharide, and interleukin 1 and only weakly by IFN-γ, while CD54 expression on fibroblasts, keratinocytes, and colonic adenocarcinoma lines is markedly enhanced by IFN-γ (5, 6, 13, 14). CD11a is found on a majority of PBMC and at high density on natural killer cells (15). Since CD11a is also expressed by IEL, its involvement in IEL-colon cancer cell binding is not unexpected. Binding of mitogen-stimulated PBMC to another colonic adenocarcinoma line, Caco-2, is inhibited by 60% with anti-CD54 antibody (6). In the present study we found only 20% inhibition using unstimulated PBMC and DLD-1 monolayers. This difference may be due to the mitogen activation of PBMC and to the low-density expression of CD54 on DLD-1 cells (16). IFN-γ has been found to increase CD54 expression on DLD-1 cells (16), and in this study incubation of the monolayer with IFN-γ greatly increased binding by PBMC but not IEL. Yet, antibody to this marker had no greater inhibitory effect on PBMC binding after IFN-γ treatment, suggesting that the binding of PBMC to colon cancer cells must involve other interactions as well.

The novel finding is the role of HML-1 in the binding of IEL to colon cancer cells. This result was recently confirmed using the binding of an IEL cell line to T84 colonic adenocarcinoma cells (17). HML-1 is found on 95% of IEL, 40% of lamina propria lymphocytes, and most lymphocytes from other EC compartments. It is not expressed by gastrointestinal B-cell lymphomas, splenocytes, or lymphocytes in peripheral blood or lymph nodes (2, 18–20). Thus, this marker is associated preferentially with epithelium. However, its expression can be induced on PBMC, by prolonged stimulation with mitogen, phorbol ester, antigen, or IL 2, implicating it as a marker of late activation (19). A cell surface heterodimer of the integrin supergene family, HML-1 is composed of a novel 150-kDa α chain and a 120-kDa β chain identical with β-7. It is different structurally from the VLA antigens (20). Any ligands for HML-1 remain unidentified.

Antibody to HML-1 has been shown to inhibit the lymphokine-activated killing of DLD-1 but not K-562 target cells by IEL (3). Also, IEL effectors cannot bind and spontaneously lyse K-562 target cells. This specificity could be explained if K-562 cells lack a ligand for HML-1.

Since HML-1 is found almost exclusively on epithelial lymphocytes, this novel binding mechanism may be important in lymphocyte-EC interactions. Possibly, HML-1 may enhance the adhesion of

Table 3 Antibody inhibition of IEL binding to DLD-1 or KD monolayers
 Binding of IEL to cell monolayers was performed in the presence or absence of monoclonal antibodies (2.5 μg/ml). The results are expressed as a percentage of inhibition of binding (mean ± SD; number of experiments in parentheses). Student's *t* test (paired variables): a, c, d versus control of 1.0, *P* < 0.001; b versus control of 1.0, *P* < 0.01.

Antibodies against	Monolayer type			
	DLD-1	5637	A375	KD
HML-1	47.2 ± 9.0 (6)a	39.0 ± 0.8 (2)	-2.1 ± 9.1 (2)	2.0 ± 15.1 (2)
CD11a	25.5 ± 13.4 (13)b	16.9 ± 10.4 (2)	35.5 ± 9.1 (2)	66.5 ± 10.6 (2)
CD54	15.8 ± 14.6 (8)	15.3 ± 10.5 (2)	45.2 ± 4.5 (2)	
CD2	8.6 ± 8.5 (8)	0.1 ± 10.3 (2)	9.8 ± 7.5 (2)	
CD58	10.8 ± 9.0 (5)	4.2 ± 7.1 (2)	8.8 ± 6.3 (2)	
VLA-1	32.0 ± 8.2 (4)c			
CD11a + HML-1	92.8 ± 3.8 (4)d	49.3 ± 6.5 (2)	36.5 ± 10.6 (2)	65.0 ± 4.2 (2)

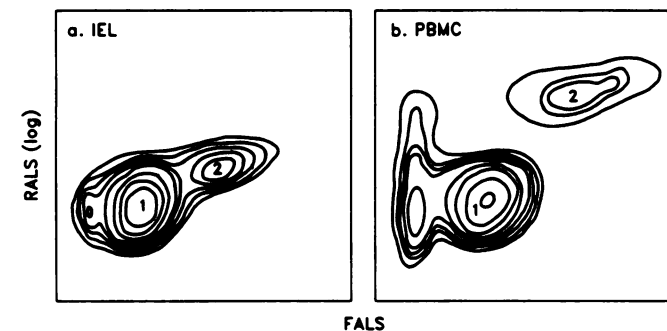


Fig. 2. Flow cytometer contour plots depicting subpopulations of IEL (a) and PBMC (b) according to relative size [forward angle light scatter (FALS)] and granularity [right angle light scatter (RALS)]. There are two predominant lymphocyte populations: one smaller and less granular (map 1) and the other larger and more granular (map 2). The minor group, leftmost in each plot, is composed of erythrocytes and debris.

IEL to intestinal EC, promoting an immune response to processed antigen by EC. It may also stabilize the adherence of IEL to dysplastic or malignant EC, allowing cell-mediated lysis of recognized EC. CD2 was shown to be insignificant in the adhesion of IEL to DLD-1. This was surprising since it is through CD2 that IEL are maximally stimulated to proliferate and produce lymphokines (8), and thymocytes are activated through this pathway after binding to thymic EC (7). Neither of these events involves cytotoxicity, however. Apparently, ligands that bind to CD2 on IEL promote proliferation and lymphokine production but not cytotoxicity. Thus, there may be a division of functions by IEL that correlates with the ligation of certain receptors.

HML-1 is likely to participate in the selective functions of epithelial lymphocytes. The isolation of its ligand and the development of monoclonal antibodies to it are essential to determine the precise function and mechanism of this molecule and will aid in discovering whether normal, inflamed, or only malignant EC bind IEL.

ACKNOWLEDGMENTS

The authors would like to thank Robert E. Brolin, M.D., for providing jejunal tissue.

REFERENCES

1. Taunk, J., Roberts, A. I., and Ebert, E. C. Spontaneous cytotoxicity of human intraepithelial lymphocytes against epithelial cell tumors. *Gastroenterology*, *102*: 69-75, 1992.
2. Jarry, A., Cerf-Bensussan, N., Brousse, N., Guy-Grand, D., Muzeau, F., and Potet, F. Same peculiar subset of HML-1⁺ lymphocytes present within normal intestinal epithelium is associated with tumoral epithelium of gastrointestinal carcinomas. *Gut*, *29*: 1632-1638, 1988.
3. Ebert, E. C., and Roberts, A. I. Lymphokine-activated killing by human intestinal lymphocytes. *Cell. Immunol.*, *146*: 107-116, 1993.
4. Dustin, M. L., Singer, K. H., Tuck, D. T., and Springer, T. A. Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by IFN-gamma and is mediated by intercellular adhesion molecule 1 (ICAM1). *J. Exp. Med.*, *167*: 1323-1339, 1988.
5. Lo, S. K., VanSeventer, G. A., Levin, S. M., and Wright, S. D. Two leukocyte receptors (CD11a/CD18 and CD11b/CD18) mediate transient adhesion to endothelium by binding to different ligands. *J. Immunol.*, *143*: 3325-3329, 1989.
6. Kaiserlian, D., Rigal, D., Abello, J., and Revillard, J. Expression, function and regulation of the intercellular adhesion molecule-1 (ICAM-1) on human intestinal epithelial cell lines. *Eur. J. Immunol.*, *21*: 2415-2421, 1991.
7. Vollger, L. W., Tuck, D. T., Springer, T. A., Haynes, B. F., and Singer, K. H. Thymocyte binding to human thymic epithelial cells is inhibited by monoclonal antibodies to CD 2 and LFA-3 antigens. *J. Immunol.*, *138*: 358-363, 1987.
8. Ebert, E. C. Proliferative responses of human intraepithelial lymphocytes to various T cell stimuli. *Gastroenterology*, *98*: 1372-1381, 1989.
9. Brandtzaeg, P., Sollid, L. M., Thrane, P. S., Kvale, D., Bjerke, K., Scott, H., Kett, K., and Rognum, T. O. Lymphoepithelial interactions in the mucosal immune system. *Gut*, *29*: 1116-1130, 1988.
10. Ebert, E. C., Roberts, A. I., O'Connell, S. M., Robertson, F. M., and Nagase, H. Characterization of an immunosuppressive factor derived from colon cancer cells. *J. Immunol.*, *138*: 2161-2168, 1987.
11. Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A., and Springer, T. A. Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J. Immunol.*, *137*: 245-254, 1986.
12. Lisby, S., Ralfkiaer, E., Rothlein, R., and Vejlsgaard, G. L. Intercellular adhesion molecule-1 (ICAM-1) expression correlated to inflammation. *Br. J. Dermatol.*, *120*: 479-484, 1989.
13. Cavender, D., Saegusa, Y., and Ziff, M. Stimulation of endothelial cell binding of lymphocytes by tumor necrosis factor. *J. Immunol.*, *139*: 1855-1860, 1987.
14. Cavender, D. E., Haskard, D. O., Maliakkal, D., and Ziff, M. Separation and characterization of human T lymphocytes with varying adhesiveness for endothelial cells. *Cell. Immunol.*, *117*: 111-126, 1988.
15. Robertson, M. J., Caligiuri, M. A., Manley, T. J., Levine, H., and Ritz, J. Human natural killer cell adhesion molecules. *J. Immunol.*, *145*: 3194-3201, 1990.
16. Das, K. M., Squillante, L., and Robertson, F. M. Amplified expression of intercellular adhesion molecule-1 (ICAM-1) and Mr 40K protein by DLD-1 colon tumor cells by gamma interferon. *Cell. Immunol.*, *147*: 215-221, 1993.
17. Cepek, K. L., Parker, C. M., Madara, J. L. and Brenner, M. B. Integrin $\alpha^E \beta_7$ mediates adhesion of T lymphocytes to epithelial cells. *J. Immunol.*, in press, 1993.
18. Cerf-Bensussan, N., Jarry, A., Brousse, N., Lisowska-Grosppierre, B., Guy-Grand, D., and Griscelli, C. A monoclonal antibody (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *Eur. J. Immunol.*, *17*: 1279-1285, 1987.
19. Schieferdecker, H. L., Ullrich, R., Weiss-Breckwoldt, A. N., Schwarting, R., Stein, H., Riecken, E., and Zeitz, M. The HML-1 antigen of intestinal lymphocytes is an activation antigen. *J. Immunol.*, *144*: 2541-2549, 1990.
20. Cerf-Bensussan, N., Begue, B., Gagnon, J., and Meo, T. The human intraepithelial lymphocyte marker HML-1 is an integrin consisting of a beta 7 subunit associated with a distinctive alpha chain. *Eur. J. Immunol.*, *22*: 273-277, 1992.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Intestinal Intraepithelial Lymphocytes Bind to Colon Cancer Cells by HML-1 and CD11a

Arthur I. Roberts, Sean M. O'Connell and Ellen C. Ebert

Cancer Res 1993;53:1608-1611.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/53/7/1608>

- E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.
- Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
- Permissions** To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.