J. Clin. Exp. Hematopathol
Vol. 41, No. 2, Oct 2001

The role of a new fibronectin receptor p55 in liver metastasis by mouse lymphoma

Masafumi Ito
Department of Pathology, Yamagata University School of Medicine

The liver is a target organ for lymphoma and other types of tumor cells. Because there is no basement membrane beneath the sinusoidal endothelium, metastasis of lymphomas to the liver may involve interaction of fibronectin on hepatocytes with a fibronectin receptor on lymphoma cells, although no such fibronectin receptors have yet been demonstrated. Recently, I developed a new monoclonal antibody, LAD-4, that recognizes a novel FN receptor in the mouse lymphoma cell line RL-♂1. LAD-4 partially, but significantly, inhibited both migration and formation of metastasis by lymphoma cells in the liver, as determined by an in-vivo migration assay using radioisotopes and by a metastasis assay involving histological examination. There was a functional difference between LAD-4 and the antibody specific for lymphocyte-function-associated antigen 1. The latter only inhibited metastasis formation by lymphoma cells in the liver without affecting migration.
Key words lymphocyte-function-associated antigen 1, monoclonal antibody, RL-♂1

INTRODUCTION

For many tumor cell types, the liver is a major site of metastasis. The liver differs from other organs in that no basement membrane is present beneath the endothelium of the microvessels[1]. Therefore, integrins, such as very late antigen-1 (VLA-1), VLA-2, and VLA-6, receptors for laminin and/or collagen[2], are unlikely to play a role in liver metastasis. In contrast, fibronectin (FN) is present in abundance on the hepatocyte surface; FN receptors, therefore, may be important for liver metastasis (see Fig. 1).

Although expression of classic FN receptors, VLA-4 and VLA-5, on lymphoma cells has not been demonstrated, some lymphomas invade liver tissue and spread diffusely without forming nodules[3–5]. Recently, I have developed a monoclonal antibody, (mAb) LAD-4, that inhibits the binding of lymphoma cells to FN in vitro and partially inhibits liver infiltration by isotope-labeled lymphoma cells in vivo[6]. The role of this novel receptor for FN in liver metastasis is of particular interest. In addition, the role of lymphocyte-function-associated antigen 1 (LFA 1), only integrin ($\alpha L\beta 2$) expressed at high levels

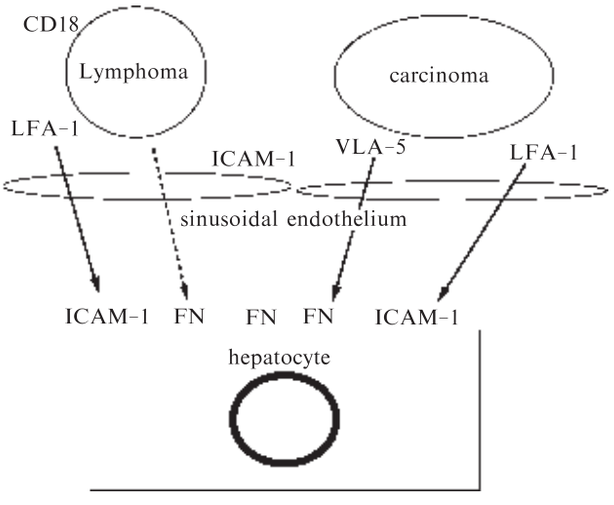

Fig. 1. Illustration of adhesion mechanisms mediating liver metastasis by tumor cells. There is no basement membrane beneath the sinusoidal endothelium of the liver. The blood-born tumor cells directly invade liver parenchyma after extravasation. A novel fibronectin receptor and $\beta 2$ integrin (LFA-1 and CD18) are involved in liver metastasis by lymphoma cells (RL-♂1), while mammary carcinoma cells (TA3/St) use a classic FN receptor (VLA-5) for liver metastasis (reference 7).

Received: July 11, 2001
Revised: August 17, 2001
Accepted: August 24, 2001

on murine lymphoma cells, in liver metastasis formation is discussed.

The role of the fibronectin receptor in liver metastasis

Kemperman, et al.[7] found that TA3/St cells, derived from a murine mammary carcinoma cell line, bind to hepatocytes and express VLA-$\alpha 5\beta 1$. Attachment of TA3/St cells to both FN and hepatocytes is inhibited by the RGD peptide, a minimal crucial sequence for cell attachment[8,9], and by anti-FN polyclonal antibodies. These findings strongly suggest that FN receptor/FN interactions are critical for lymphoma cell invasion, migration and tumor formation in liver, assuming that lymphoma cells do, indeed, express FN receptors (Fig. 1). In contrast, another study using lymphomas transfected with the $\alpha 4$-integrin gene showed that the expression of $\alpha 4$-integrins inhibits lymphoma metastasis without affecting migration[10].

RL-♂1 cells, a murine T cell lymphoma cell line, bind to FN in vitro even though they do not express FN receptors such as VLA-4, VLA-5, VLA-3 and vitronectin[6]. Because RL-♂1 cells, in contrast to other lymphoma cells[11,12], have no Fcγ receptor, it is easy to determine the expression of integrins without preparation of F (ab′)^2 reagents.

RL-♂1 cells probably express a novel FN receptor recognized by LAD-4 mAb because this mAb inhibits binding of RL-♂1 cells to FN in vitro, as described above. The molecular weight of the antigen is approximately 55,000 - several times smaller than those of known FN receptors - and its NH2-terminal amino acid sequence differs also, as reported previously[6]. The cDNA sequence of this molecule is under investigation. RL-♂1 cells express the CD44 molecule (Ito M, unpublished data). The LAD-4 antigen is different from CD44 because the latter's molecular weight is 85,000 to 90,000 and the binding of CD44 protein to the COOH-terminal heparin-binding fragment of FN is inhibited by chondroitin sulfate but not by RGDS peptides[13].

Approximately 35% of ^{51}Cr-labeled RL♂1 cells injected intravenously into mice accumulated in the liver within 24 hours, whereas less than 1% of the total accumulated in other organs, such as lung, spleen, and intestine (Fig. 2). Migration to the liver can be inhibited by RGDS peptides (Fig. 2), which strongly suggests the presence of

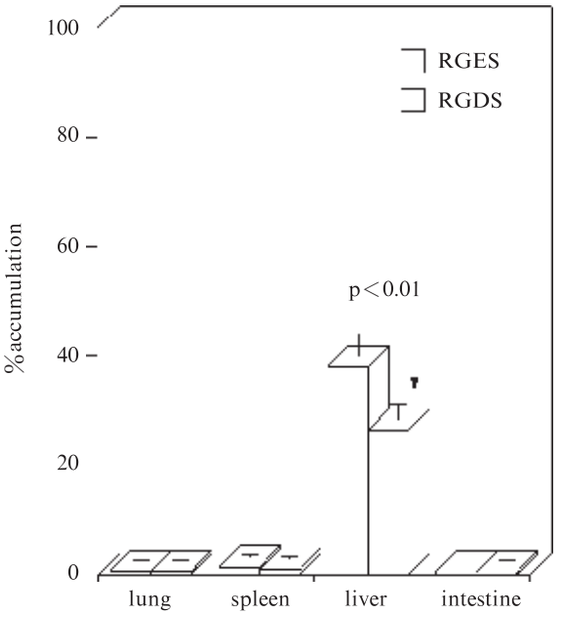

Fig. 2. In vivo migration assay of RL-♂1 lymphoma cells. 51Cr-labeled cells were injected i. v. in the presence of peptides (RGES or RGDS). Radioactivity was counted in the organs after 24 hours and expressed as the percent of total radioactivity injected. Data represents the mean ± SD (n=5).

the FN receptor on the tumor cells. Because the expression of cell adhesion molecules does not necessarily correlate with the homing and metastatic potential of tumor cells, I tested whether LAD-4 mAb inhibits liver metastasis by RL-♂1 cells, a post-homing event. RL-♂1 cells (10^5 per mouse) were injected subcutaneously into Balb/c mice, followed by LAD-4 mAb or anti-LFA-1 mAb (M17/4) injected intraperitoneally. After 3 weeks, the livers were evaluated for metastasis by histological examination.

Preliminary results[14] suggest that LAD-4 mAb partially inhibits liver metastasis similar in extent to anti-LFA-1 mAb (M17/4). Diffuse metastasis in the liver is difficult to quantify by histological examination. Kruger, et al[4] used a fluorescein-activated cell sorter (FACS) analysis to quantify Esb lymphoma cells transfected with the lac Z gene. Recently, I have collaborated with Drs. Kato and Hayashi in trying to quantify metastasized lymphoma cells in liver homogenates by FACS analysis using LAD-4 mAb. Our data showed that gating with forward and side scatter in FACS analysis is sufficient to

differentiate RL-♂1 cells from infiltrating lymphocytes and the data obtained by this method showed that LAD-4 mAb significantly inhibits liver metastasis by RL-♂1 cells (Kato Y, Hayashi T, Ito M, Manuscript in preparation). Taken together, these data indicate that FN receptor/FN interaction is involved in both migration, an initial event, and metastasis formation, a post-homing event.

The role of LFA-1

LFA-1 is a known integrin expressed on lymphoma cells, and its role in liver metastasis has been analyzed thoroughly. Roos and Roossien[15] showed that anti-LFA-1 antibodies strongly inhibited invasion of MB6A lymphoma cells into both hepatocyte and fibroblast monolayers. The study used LFA-1-deficient mutants of T cell hybridoma to demonstrate that LFA-1 expression at the surface of tumor cells correlates with metastatic behavior[16]. Other studies[5,17] showed that the metastatic potential of lymphoma cells (EL-4 or EsbL-lacZ) toward the liver was reduced partially, but significantly, by blocking mAb specific for LFA-1.

Two of the three ligands interacting with LFA-1, intercellular adhesion molecule-1 (ICAM-1) and ICAM-2, are expressed on the vascular endothelium[18–20]. In most tissues, the expression of ICAM-1 is regulated by the presence of inflammatory cytokines. However, it is present in normal human liver[20] and on the dorsal surface of rat hepatocytes[21], suggesting that it may be one of the factors involved in liver metastasis. In contrast, Hamann and Thiele[22] showed that blocking mAb, that are capable of inhibiting the function of LFA-1, could not prevent the entry of Moloney-transformed lymphoma cells into the spleen. Zahalka, et al[23] found that anti-β2 integrin (CD18) mAb could not block the infiltration of LB lymphoma cells into the lymph nodes. Other studies using intravital video microscopy showed that most tumor cells entering the circulation extravasate efficiently into tissues regardless of their metastatic potential[24,25]. Furthermore, Aoudjit, et al[26] have shown that 164T2 lymphoma cells migrated with the same efficiency to the liver in both normal and ICAM-1 deficient mice, but do not form liver metastasis in the latter. In agreement with these results, our experiments with intravenous injection of blocking mAb (M17/4), that is specific for LFA-1, had no effect on migration of RL-♂1 cells to the liver within 24 hours injection[6], but partially inhibited liver metastasis with the same efficacy as LAD-4 mAb 3 weeks after inoculation of lymphoma cells[14]. These results suggest that the LFA-1/ICAM-1 interaction may mediate metastasis formation, a post-homing event, instead of migration, an initial event.

Conclusion

Some lymphoma cell lines use a novel FN receptor recognized by mAb LAD-4 for both migration and metastasis formation in the liver, although they lack known FN receptors. LFA-1, a known integrin expressed in many lymphoma cell lines, may mediate metastasis formation as a post-homing event in the liver, but not tumor cell migration.

Acknowledgment

The in vivo metastasis assay was performed in collaboration with Drs. Y. Kato and T. Hayashi of the Third Department of Internal Medicine, Yamagata University School of Medicine.

REFERENCES

1 Roos E, Dingemans KP, Van de Pavert IV, Van den Bergh, Weerman MA : Invasion of lymphosarcoma cells into the perfused mouse liver. J Natl Cancer Inst 58 : 399–407, 1977.
2 Hemler, M. E : VLA proteins in the integrin family : Structures, function, and their role on leukocytes. Ann Rev Immunol 8 : 365–400, 1990.
3 Stauder R., Grei R., Schulz T. F., Gattringer C, Radaskiwicz T, Dierich M. P, Huber H : Expression of leukocyte function-associated antigen-1 and 7F7 antigen, an adhesion molecule related to intercellular adhesion molecules-1 (ICAM-1) in non-Hodgkin lymphomas and leukemias : possible influence on growth pattern and leukaemic behaviour. Clin Exp Immunol 77 : 234–238, 1989.
4 Krüger A., Schirrmacher V., and von Hoegen P : Scattered micrometastasis visualized at the single-cell level : detection and re-isolation of lac Z-labeled metastasized lymphoma cells. Int. J. Cancer 58 : 275–284, 1994.
5 Rocha M, Kruger A, Umansky V, von Hoegen P, Naor D, Schirrmacher V : Dynamic expression changes in vivo of adhesion and costimulatory

molecules determines load and pattern of lymphoma liver metastasis. Clin Cancer Res 2 : 811–820, 1996.
6 Gazi MH, Ito M : Use of a novel fibronectin receptor for liver infiltration by a mouse lymphoma cell line RL-♂1. Cancer Res 59 : 1115–1119, 1999.
7 Kemperman H, Wijnands Y, Meijne AML, Roos E : TA3/St, but not TA3/Ha mammary carcinoma cell adhesion to hepatocytes is mediated by $\alpha 5\beta 1$ interacting with surface-associated fibronectin. Cell Adhesion and Communication 2 : 45–48, 1994.
8 Ruoslahti, E, and Pierschbacher, MD : New perspectives in cell adhesion : RGD and integrins : Science 238 : 491–497, 1987.
9 Springer, TA : Adhesion receptors of the immune system : Nature (London) 346 : 425–433, 1990.
10 Gosslar U, Jonas P, Luz A, Lifka A, Naor D, Hamann A, Holzmann B : Predominant role of a4-integrins for distinct steps of lymphoma metastasis. Proc Natl Acad Sci, USA 93 : 4821–4826, 1996.
11 Unkeless JC, Scigliano E, Freedman V : Structure and function of human and murine receptors for IgG. Ann Rev Immunol 6 : 251–281, 1988.
12 Ito M, Usuba O, Unkeless JC, Schreiber R, Celada F, Bona CA, Moran T : Sideway killing : the cytolysis of Fc receptor-bearing cells through bridging to cytolytic T lymphocytes by antibodies specific for the T-cell receptor-T3 complex. Scand J Immunol 29 : 659–669, 1989.
13 Jalkanen S, Jalkanen M : Lymphocyte CD44 binds the COOH- terminal heparin-binding domain of fibronectin. J Cell Biol 116 : 817–825, 1992.
14 Kato Y, Ito M, Hayashi T : Effect of LAD-4 mAb on liver metastasis by mouse lymphoma cells and induction of apoptosis in vitro by LAD-4 mAb. Proc Japn Soc Immunol 30 : 2C180, 2000 (in Japanese).
15 Roos E, Roossien FF : Involvement of leukocyte function-associated antigen-1 (LFA-1) in the invasion of hepatocyte culture by lymphoma and T-cell hybridoma cells. J Cell Biol 105 : 553–559, 1987.
16 Roossien FF, Bikker A, De Rijk D, Roos E : Involvement of LFA-1 in lymphoma invasion and metastasis demonstrated with LFA-1 deficient mutants. J Cell Biol 108 : 1979–1985, 1989.
17 Harning R, Myers C, Merluzzi VJ : Monoclonal antibodies to lymphocyte function-associated antigen-1 inhibit invasion of human lymphoma and metastasis of murine lymphoma. Clin Exp Metastasis 11 : 337–342, 1993.
18 Makgoba MW, Sanders ME, Ginter Luce GE, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S : ICAM-1 is a ligand for LFA-1 dependent adhesion of B, T, and myeloid cells. Nature (London) 331 : 86–88, 1988.
19 Staunton ED, Dustin ML, Springer TA : Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. Nature (London) 339 : 61–64, 1989.
20 De Fougerolles AR, Stacker SA, Schwarting R, Springer TA : Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. J Exp Med 174 : 253–267, 1991.
21 Meijne AM, Driessens MH, La Riviere G, Casey D, Feltkamp CA, Roos E : LFA-1 integrin redistribution during T-cell hybridoma invasion of hepatocyte cultures and manganese-induced adhesion to ICAM-1. J Cell Sci 107 : 2557–2566, 1994.
22 Hamann A, Thiele HG : molecules and regulation in lymphocyte migration. Immunol Rev 108 : 19–44, 1989.
23 Zahalka MA, Okon E, Naor D : Blocking lymphoma invasiveness with a monoclonal antibody directed against the β-chain of the leukocyte adhesion molecule (CD18). J Immunol 150 : 4466–4477, 1993.
24 Morris VL, Koop S, MacDonald IC, Schmidt EE, Grattan M, Percy D, Chambers AF, Groom AC : mammary carcinoma cell lines of high and low metastatic potential differ not in extravasation but in subsequent migration and growth. Clin Exp Metastasis 12 : 357–367, 1994
25 Koop S, MacDonald IC, Luzzi K, Schmidt EE, Morris VL, Grattan M, Khokha R, Chambers AF, Groom AC : Fate of melanoma cells entering the microcirculation : over 80% survive and extravasate : Cancer Res 55 : 2520–2523, 1995.
26 Audjit F, Potworowski EF, Springer TA, St-Piere Y : Protection from lymphoma cell metastasis in ICAM-1 mutant mice : a post homing event. J Immunol 161 : 2333–2338, 1998.