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

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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-β1. 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-β1

**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. Therefore, integrins, such as very late antigen-1 (VLA-1), VLA-2, and VLA-6, receptors for laminin and/or collagen, 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. 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. 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 (αLβ2) expressed at high levels

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

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Kemperman, et al.7 found that TA3/St cells, derived from a murine mammary carcinoma cell line, bind to hepatocytes and express VLA-α5β1. Attachment of TA3/St cells to both FN and hepatocytes is inhibited by the RGD peptide, a minimal crucial sequence for cell attachment8,9, 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 α4-integrin gene showed that the expression of α4-integrins inhibits lymphoma metastasis without affecting migration10.

RL-β1 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 vitronectin5. Because RL-β1 cells, in contrast to other lymphoma cells11,12, have no Fcγ receptor, it is easy to determine the expression of integrins without preparation of F(ab′)2 reagents.

RL-β1 cells probably express a novel FN receptor recognized by LAD-4 mAb because this mAb inhibits binding of RL-β1 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 previously4. The cDNA sequence of this molecule is under investigation. RL-β1 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 peptides13.

Approximately 35% of 51Cr-labeled RL-β1 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 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-β1 cells, a post-homing event. RL-β1 cells (105 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 results14 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 Ebs lymphoma cells transfected with the lacZ 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-CAF1 cells from infiltrating lymphocytes and the data obtained by this method showed that LAD-4 mAb significantly inhibits liver metastasis by RL-CAF1 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 Roossier15 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 behavior16. Other studies6,7 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 endothelium18–20. In most tissues, the expression of ICAM-1 is regulated by the presence of inflammatory cytokines. However, it is present in normal human liver20 and on the dorsal surface of rat hepatocytes21, suggesting that it may be one of the factors involved in liver metastasis. In contrast, Hamann and Thiele22 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 al23 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 potential24,25. Furthermore, Aoudjit, et al26 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 block-

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ing mAb (M17/4), that is specific for LFA-1, had no effect on migration of RL-CAF1 cells to the liver within 24 hours injection2, but partially inhibited liver metastasis with the same efficacy as LAD-4 mAb 3 weeks after inoculation of lymphoma cells24. 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.

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