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Laboratory Investigation

Expression of LFA-1/ICAM-1 in CNS lymphomas: possible mechanism for lymphoma homing into the brain

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Abstract

We examined a possible role for the adhesion molecules LFA-1 and ICAM-1 in localizing central nervous system non-Hodgkin's lymphomas (CNS-NHLs) to the brain. Fresh frozen sections from 12 monoclonal CNS NHLs (11 primary, one secondary) were stained with monoclonal antibodies to LFA-1 α chain (CD11a), β chain (CD18) and, ICAM-1 (CD54). Additional staining made use of rat monoclonal antibodies to the human and mouse high endothelial venule antigens HECA 452 and MECA 79 and mouse ICAM-1. The expression of these same molecules was also studied in mice with severe combined immunodeficiency (SCID) mice, bearing intracranial human lymphoblastoid cells.

Eleven of the CNS-NHL tumors expressed LFA-1α (one strongly, one intermediate, nine weakly). Nine of the tumors weakly expressed LFA-1β.. Nine of twelve tumors weakly expressed ICAM-1. In six of seven tumors definite blood vessels stained for ICAM-1. Non-tumor brain from two patients and non-tumor cerebral blood vessels showed no staining with CD11a, CD18 or CD54 antibodies. Strong expression of LFA-α and LFA-β as well as ICAM-1 was noted in human lymphoblastoid cells (LCLs)/SCID mouse CNS lymphomas. Tumor blood vessels in these mice stained for mouse ICAM-1. Normal SCID mouse brains showed no staining with CD11a, CD18, CD54 or mouse ICAM-1 antibodies. Human, human/mouse CNS lymphomas, normal human, and mouse brains showed no staining with either HECA 452 or MECA 79.

Our data suggests that CNS lymphomas express LFA-1α, LFA-1β and to a lesser degree ICAM-1 antigen, while tumor blood vessel endothelium expresses ICAM-1. LFA-1α-LFA-1β/ICAM-1 interaction may play a role in large cell lymphoma homing and persistence in the brain.

Introduction

Primary central nervous system non-Hodgkin's lymphoma (CNS-NHL) is a B cell tumor that selectively involves the brain [1–4]. A dramatic rise in incidence of this tumor over the past two decades has been attributed to a wider use of immunosuppressants and the AIDS epidemic [4–9]. Unexplained is the increasing incidence in immunocompetent subjects [4, 9]. CNS lymphomas, restricted to the brain, usually recur within the neuraxis after treatment [2, 4]. This localization has been attributed to the immune privileged nature of the CNS [4, 10]. Recent studies on lymphocyte trafficking have suggested that localization of lymphocytes to specific sites is not a random event [11, 12]. Lymphocytes express a number of adhesion molecules at different stages of maturation. These molecules influence organ-specific lymphoid binding [13, 15].

A family of adhesion molecules, the addressins, expressed by high endothelial venules in man and mouse [16–20]. HECA 452 expressed by human endothelial venules in both normal lymphoid tissue and inflamed blood vessels interacts with Hermes-1 receptors on the surface of lymphocytes [15, 17]. This interaction allows localization of lymphocytes to these venules in normal lymphoid tissue. MECA-79, expressed by mouse high endothelial venules, may play a role in lymphocyte localization to peripheral and mesenteric mouse lymph nodes [19]. Another family of adhesion molecules, the integrins, is expressed by lymphocytes and play an important role in their attachment to other white cells or to activated blood vessels [12–15]. LFA-1 is made up of two chains, LFA-1α and LFA-1β, which are expressed by B and T lymphocytes [13–15]. ICAM-1, the ligand for LFA-1, is expressed by activated lymphocytes as well as cerebral blood vessels after stimulation with Tumor Necrosis Factor (TNF) α and gamma interferon [20, 21]. LFA-1α and LFA-1β are also produced by large cell non-Burkitt lymphomas [13]. Interaction of LFA-1/ICAM-1 is necessary for invasion of lymphoma into cultured liver cells *in vitro* [22].

Mice with inherited severe combined immunodeficiency (SCID/SCID CB17) lack functional T and B cells and have been used to study the properties of transplanted human B cells *in vivo* [23–25]. B cells immortalized with the Epstein-Barr virus (EBV) produce highly infiltrative tumors when injected intracerebrally in nude as well as SCID mice [25–27]. These tumors resemble human CNS lymphomas morphologically and express high levels of LFA-1 and ICAM-1 [25, 28–30].

To investigate a possible role for adhesion molecules in lymphoma homing to brain, we examined their expression in a group of large cell human CNS lymphomas. We compared these tumors with normal human and SCID mouse brain as well as human lymphoblastoid cells/SCID mouse chimeras.

Materials and methods

Human CNS lymphomas

Fresh frozen tumor tissues from 12 patients with CNS lymphomas (ten biopsies, two autopsies) were stored at − 70° C. All tumors came from immunocompetent patients except for tumor number twelve which was obtained at autopsy from a patient with AIDS. Six micron sections from these tumors were placed on poly-L-lysine coated glass slides and fixed in acetone.

Other human tissue

Lymph nodes from two patients with non-malignant disease and non-tumor brain tissue contiguous to a primary brain tumor was similarly frozen and sectioned.

Mouse CNS lymphoma

Six- to eight-week-old male or female SCID/SCID CB17 mice (McLaughlin Labs, Great Falls, MT) were anesthetized with a ketamine-xylazine mixture. Five million cells of EBV (B95-8 strain) immortalized human B cells were injected into the right frontal lobe of four animals through a small burr hole using a No. 30 gauge needle. The animals were maintained in a sterile environment and sacrificed by decapitation at the earliest sign of disease. The brains were bisected, placed in OCT (Miles, Elkhart, IN) and frozen immediately at − 70° C. Four non-injected SCID mice were sacrificed and their brains stored at − 70° C.

Antibodies

Polyclonal rabbit anti-human IgG, IgM, Kappa and lambda light chains were purchased from Coulter Immunology (Hialeah, FL). Mouse monoclonal antibodies to LFA-1α (CD11A), LFA-1β (CD18), and ICAM-1 (CD54) were purchased from AMAC (Westbrooke, ME). Rat monoclonal antibody to murine ICAM-1 [31] was a gift from F. Takei (University of British Colombia). HECA 452 and MECA 79 were a gift from Dr. EC Butcher (Stanford, CA).

Staining

A three step avidin-biotin peroxidase staining was used (Vector Labs, Burlingame, CA). A sheep anti-rabbit antibody was used (Vector Labs) for the rabbit polyclonal antibodies. For the mouse monoclonals a sheep anti-mouse bridging antibody was

Table 1. The frequency of positive staining of tissue specimens from human and mouse CNS lymphomas, normal human and mouse brains by antibodies to adhesion molecules

	Human * CNS-L		Non-tumor human brain		LCL human/*SCID-M CNS-L		Non-tumor *SCID-M brain		Lymph node
	Tumor	bv	Brain	bv	Tumor	bv	Brain	bv	
IgG	3/12	0/12	0/2	0/2	4/4	0	0/4	0/4	2/2
IgM	9/12	0/12	0/2	0/2	4/4	0	0/4	0/4	2/2
LFA-1α (CD11a)	11/12	0/12	0/2	0/2	4/4	0/4	0/4	0/4	2/2
LFA-1β (CD18)	9/12	0/12	0/2	0/2	4/4	0/4	0/4	0/4	0/2
ICAM-1 (CD54)	9/12	6/7	0/2	0/2	4/4	0/4	0/4	0/4	2/2
HECA 452	0/12	0/12	0/2	0/2	0/4	0/4	0/4	0/4	0/2
MECA 79	0/12	0/12	0/2	0/2	0/4	0/4	0/4	0/4	0/2
MELA					0/4	4/4	0/4	0/4	0/2

* CNS-L – Central nervous system lymphoma. * SCID-M – Mouse with severe combined immunodeficiency.

used (Vector Labs). A blocking step with normal mouse serum was used. For the rat monoclonals, a rabbit anti-rat IgG or IgM was used (Vector Labs).

The intensity of staining of these tumors was graded from 0–3 (zero indicates no staining above background, 3 intense staining).

Results

All of the CNS lymphomas were of large cell histology (International Classification) [32]. Nine only expressed surface IgM, three only expressed surface IgG. Seven tumors expressed Kappa light chain and five lambda light chain. LFA-1α was expressed by 11 of the 12 CNS lymphomas (Table 1). Intense staining was present in the AIDS CNS lymphoma (Fig. 1A), and another tumor expressed moderate staining (Table 2). LFA-1β was expressed by nine tumors (Table 1), the most intense being that of the AIDS-related CNS lymphoma. ICAM-1 was expressed in nine of the 12 tumors (Table 1). Definite tumor blood vessels were observed in seven tumors, in six of these the vascular endothelium stained with CD54 (Fig. 1B) and none with CD11A and CD18 (Table 1). HECA 452, MECA 79 did not stain any blood vessels.

Table 2. Intensity of staining of human and human/SCID mouse CNS lymphomas with antibodies and adhesion molecules

		0	+1	+2	+3
Human CNS Lymphomas (N = 12)	LFA-1α (CD11a)	1	9	1	1
	LFA-1β (CD18)	3	8	1	0
	ICAM-1 (CD54)	3	7	2	0
Human/SCID Mouse CNS Lymphomas (N = 4)	LFA-1α (CD11a)	0	0	2	2
	LFA-1β (CD18)	0	0	2	2
	ICAM-1 (CD54)	0	0	2	2
	MELA	0	0	0	4

None of the non-tumor human brain biopsies stained with CD11A, CD18, CD54, HECA 452, or MECA 79. The normal lymph nodes showed staining of B cells with IgG, IgM as well as CD11A, CD18, CD54 and HECA 452, but not MECA 79 (Table 2).

Mouse brain staining: All four SCID/SCID CB17 mice developed symptoms between 11–14 days after intracerebral injection. In all four, highly infiltrating polyclonal brain tumors developed with significant subarachnoid disease. All of the tumors expressed IgG and IgM and stained with CD11A (Fig. 2A), CD18, and CD54 (Table 1). Normal brain tissue from SCID mice showed no staining with these antibodies (Table 1). Brain tumor blood vessels in SCID mouse brain did not show staining with CD54, but stained with rat anti-mouse ICAM-1 antibody (Table 1, Fig. 2B). Normal SCID mouse brain did not stain with rat anti-mouse ICAM-1 antibody. HECA 452 and MECA 79 showed no reaction with tumor bearing or normal mouse brains (N = 4).

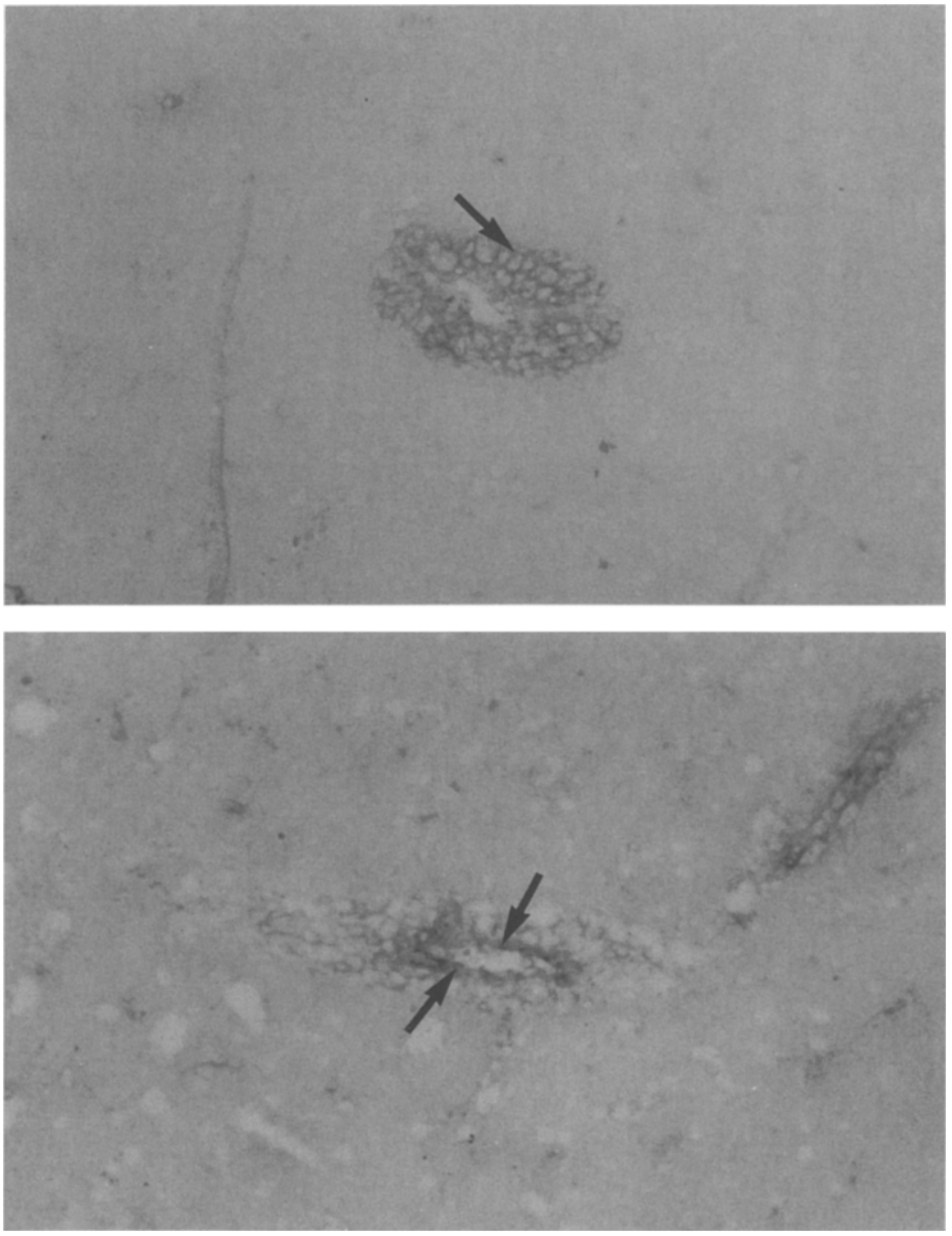

Fig. 1. A. Human CNS lymphoma tissue stained with CD11a. Arrow shows perivascular lymphoma cells with surface staining (400 × magnification). B. Arrows shows endothelium staining with CD54 antibody from the same tumor as in 1A (400 × magnification).

Discussion

We compared the reaction of a panel of antibodies to adhesion molecules (CD11a, CD18, CD54, HECA 452 and MECA 79) in 12 human CNS lymphomas to expression in non-tumor brain tissue from humans and mice and human tumor/SCID mouse chimeras. All human tumors expressed monoclonal surface IgM or IgG. The human tumor/SCID mouse chimeras were plyclonal for IgM or IgG. Eleven of the twelve human lymphomas reacted with CD11a; eight reacted with CD18. Six of the blood vessels seen in seven of the tumor specimens reacted with CD54. CD11a or CD18 and CD54

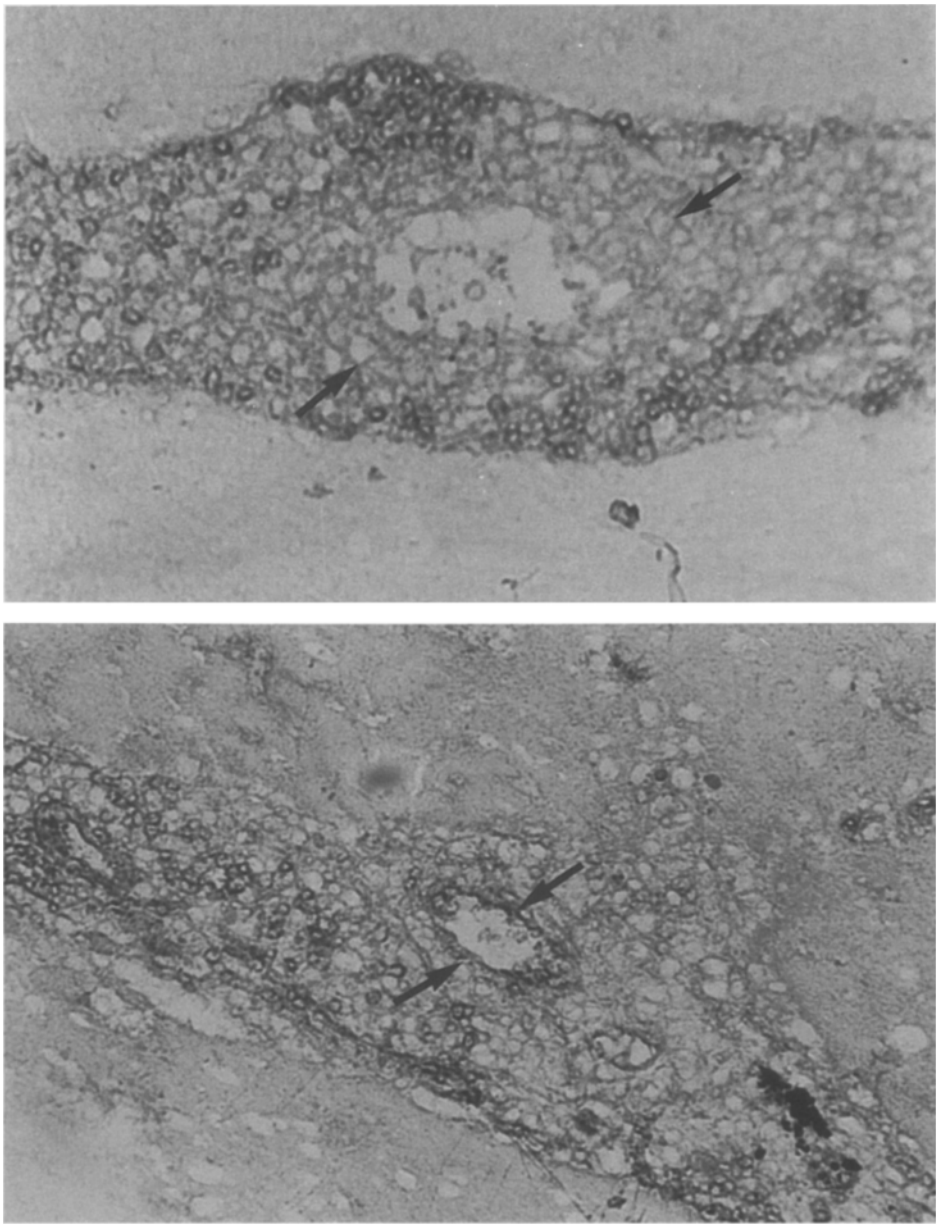

Fig. 2. A. Human LCL/SCID CB17 mouse CNS lymphoma stained with CD11a. Arrow shows tumor cells with surface staining (400 × magnification). B. Arrow shows endothelial staining from the same tumor stained with rat anti-mouse ICAM-1 (MALA-1) (400 × magnification).

showed no reaction with the two non-tumor human brains (Table 1). This is in keeping with other published studies [20, 21].

HECA 451 and MECA 79 did not react with the 12 human CNS lymphomas, human lymphoblastoid/SCID mouse tumors or non-tumor human or mouse brains (Table 1). This suggests that these addressin molecules play no role in human or human/mouse CNS lymphomas.

CD11a, CD18 and CD54 staining intensity was low (Table 2). Variable periods of time (20 to 60 minutes) elapsed between the time the tissue was obtained and snap frozen at $-70°$C. Delay in

freezing may have resulted in downregulation or lysis of some of these molecules.

Human lymphoblastoid cell-lines (LCLs) immortalized by EBV are known to express high levels of LFA-1α and LFA-1β and ICAM-1 [28–30]. The invasive brain tumors produced after injection of these cells in SCID mouse brain produce extensive lymphoproliferations [30]. These human LCLs/SCID mouse chimeras express human LFA-1α and LFA-1β and mouse ICAM-1 at levels higher than those in human tumor tissue (Fig. 2A). We offer two possible explanations for this difference. EBV-infected LCLs produce higher levels of LFA-1α, LFA-1β and ICAM-1 than human CNS lymphomas because they were grown in culture a phenomenon observed with Burkitt lymphoma [28–30, 33]. An alternative explanation is that the mouse brains were quick frozen at $-70°$ C within seconds of decapitation and hence less likely to have denatured adhesion molecules.

The AIDS CNS lymphoma exhibited the most intense staining with CD11a (Fig. 1A), CD18 and CD54. This high level of CD11a, CD18 and CD54 expression may be related to EBV infection of tumor cells which we have demonstrated by *in situ* hybridization [30, 34, manuscript in preparation]. Infection of tumor cells by Human Immunodeficiency virus (HIV) is another explanation. Infection of lymphocytes by HIV upregulates LFA-1, ICAM-1 expression [35]. CNS lymphomas from immunosuppressed patients exhibit EBV hybridization patterns that more closely resembles LCLs than Burkitt's lymphoma [36, 37]. The EBV hybridization pattern of our AIDS-related case of CNS-NHL was similar to that previously noted in AIDS associated CNS-NHL [34, 35]. This tumor might be an integrin expressing LCL rather than a true lymphoma. The monoclonality of this tumor by virtue of the expression of monoclonal immunoglobulin is not inconsistent with this view. LCLs grown in long term culture do express monoclonal immunoglobulin [38]. Establishing cell-lines from AIDS CNS-NHLs will help clarify this question.

Lymphoma invasion of cultured hepatocytes is dependent on LFA-1/ICAM-1 interaction and can be blocked by the antibody CD54 [22]. Our data suggest a role for LFA-1/ICAM-1 in the interaction between lymphoma and cerebral blood vessels [20, 21]. Increased expression of ICAM-1 in cerebral blood vessels was observed in gliomas and could be due to non-specific upregulation of factors released by tumors [40]. Further work is necessary to clarify the importance of this interaction by blocking it with CD54 antibody [20–22].

Immunosuppressed patients, deficient in T cells, develop lymphoproliferative conditions caused by immortalization of B cells by EBV [30, 39]. These lymphoproliferative states give highly infiltrative tumors [30, 39]. EBV transformed B cells express LFA-1α and β, can attach to cerebral blood vessels expressing ICAM-1 and colonize the CNS [20, 21]. Once in the brain, downregulation of LFA-1/ICAM could help protect these tumors from immune surveillance. Within the brain, the continuous division of LCLs may lead to lymphoma [38]. Understanding the mechanism of localization of CNS lymphomas to brains of immunocompromised subjects may lead to newer approaches to treating these tumors.

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