Virchows Arch (1997) 431:459–467

ORIGINAL ARTICLE

Nirit Assaf · Tsury Hasson · Hagit Hoch-Marchaim
Jacob Pe'er · Hadassah Gnessin
Martina Deckert-Schlüter · Otmar D.Wiestler
Jacob Hochman

An experimental model for infiltration of malignant lymphoma to the eye and brain

Received: 10 February 1997 / Accepted: 7 August 1997

Abstract Currently there is no adequate experimental model available whereby the lethal infiltration of malignant lymphoma to the eye and CNS can be studied. Variant S49 mouse lymphoma cells that exhibit cell-cell adhesion properties (named Rev-2-T-6) were inoculated intraperitoneally into Balb/C mice at the ages of 6–60 days postnatal. Mice inoculated between days 6–11 postnatal developed signs of eye and CNS involvement with an apparent peak (58% of mice) at day 7. None of the mice inoculated beyond day 11 exhibited such signs. Histological analysis of these sites revealed tumorous infiltrates into a variety of structures in the orbit, intraocular tissues, along the optic nerve and in the brain. Additional analysis of the histopathological data, based on the structures demonstrating the highest frequency of lymphoma infiltration, suggests preferred routes of lymphoma entry to the brain and eye. Thus, entry to the brain can occur mainly through the choroid plexus and cranial nerves or cranial nerve ganglia. Entry to the eye may occur from the brain (along the optic nerve), and through hematogenous infiltration of orbital structures. No data were found that would support retrograde infiltration of the lymphoma from the eye to the brain. These findings present an experimental model for addressing the molecular mechanisms that govern homing of malignant lymphoma to the eye and brain, as well as the development of experimental therapeutic modalities for malignant lymphoma in these organs.

N. Assaf · T. Hasson[1] · H. Hoch-Marchaim · J. Hochman (✉)
Department of Cell and Animal Biology,
Institute of Life Sciences, The Hebrew University of Jerusalem,
Jerusalem 91904, Israel
Tel.: (972)-2-6585113, Fax: (972)-2-5617918

J. Pe'er · H. Gnessin
Department of Ophthalmology, Hadassah University Hospital,
Jerusalem, Israel

M. Deckert-Schlüter · O.D. Wiestler
Institute of Neuropathology,
University of Bonn Medical Center, Bonn, Germany

[1] Deceased

Key words Metastasis · CNS lymphoma · Ocular lymphoma · S49

Introduction

Ocular lymphoma is a lethal disease [15, 39, 40, 52] caused mainly by two clinically distinct forms of non-Hodgkin's lymphoma: (1) non-Hodgkin's lymphoma of the central nervous system (NHL-CNS) and (2) systemic lymphoma metastatic to the eye. Hodgkin's lymphoma very rarely causes ocular and orbital disease [27, 42, 44]. The NHL-CNS form arises within the brain, spinal cord, leptomeninges or the eye, but then may spread throughout the CNS [3, 7, 43], with rare systemic spread outside the CNS [16, 38]. In contrast, systemic non-Hodgkin's lymphoma almost always arises outside of the CNS. The disease is aggressive, and most patients die within 1–5 years of diagnosis [35]. Although previously uncommon, ocular lymphoma is recognized with increasing frequency. This results from the increasing incidence of primary CNS lymphoma (PCNSL) in immunocompetent as well as in immunocompromised populations i.e., AIDS, renal transplant recipients, Wiskott-Aldrich syndrome and ataxia-telangiectasia [9, 17, 24, 31, 36]. Between 2% and 6% of AIDS patients are diagnosed with PCNSL; about 11% are diagnosed as such at autopsy [31]. About 20% of patients with PCNSL have ocular involvement, but 60%–80% of patients who initially present with ocular lymphoma develop subsequently clinically evident disease of the brain parenchyma or subarachnoid space [12, 40]. In a recent study, 24 patients with ocular lymphoma were reviewed; 23 had associated PCNSL [40]. Most cases of NHL-CNS that have been reported so far in AIDS patients are B cell lymphomas although, recently, there is an increasing number of T cell lymphomas of the CNS [14, 37]. The mechanisms of homing of lymphoma cells to the CNS and eye are not yet understood.

To our knowledge, there is currently no adequate experimental model available (of either T or B cell origin) that could mimic the infiltration of the eye and brain by

malignant lymphoma in humans. Such a model might be used to address the molecular basis of differential infiltration to these organs, as well as to investigate new therapeutic options for this malignancy. A model that partially relates to these questions was developed by White et al. [50, 51]. It is based on the direct heterotransplantation of cells from childhood leukemias and lymphomas to the anterior chamber of the eye in immunocompromised nude mice.

Here we report that following intraperitoneal inoculation of variant S49 mouse lymphoma cells into newborn mice, specific homing of these cells to the eye and brain takes place. This constitutes a novel experimental model for infiltration of lymphoma to the eye and CNS.

Materials and methods

S49 lymphoma, a malignant mouse T-cell lymphoma of Balb/c origin, has been adapted to growth in cell culture [22]. We have previously used these cells to select for highly tumorigenic suspension-borne variants named T-25 [18, 19]. T-25 cells developed progressive tumors (defined as a high-grade malignant lymphoma, small non-cleaved cell type, with a starry-sky appearance) with a median survival of about 14 days, following intraperitoneal (IP) inoculation of 10^7 cells into syngeneic mice. From T-25 cells we selected spontaneously developing substrate-adhesive variants, named T-25-Adh, which revealed impaired tumorigenicity (no tumors with up to 10^8 cells per mouse) and enhanced immunogenicity (protection against a challenge with tumorigenic T-25 cells) in immunocompetent mice [18, 19]. These cells were subsequently used to study different parameters of growth regulation and immunogenicity [6, 20, 21, 32]. T-25-Adh cells (that were selected for resistance to 0.5 mM ouabain as a marker) were subjected to UV irradiation and subsequent in vitro selection for suspension growing cells. This was followed by in vivo selection (six consecutive passages in Balb/c mice) for tumorigenic (non-cloned) revertants. These revertant cells (named Rev-2-T-6) were frozen in liquid nitrogen for subsequent use. They give rise to progressive, solid abdominal tumors following intraperitoneal inoculation into mature Balb/c mice. Rev-2-T-6 cells grow in suspension as cell aggregates. These cells were used in the present study.

Rev-2-T-6 cells were maintained in Dulbecco's modified Eagle's medium (Beth-Haemek, Israel) supplemented with 10% horse serum, 50 U/ml penicillin and 50 μg/ml streptomycin.

Rev-2-T-6 cells (3×10^6) were inoculated intraperitoneally into young (6–15 days postnatal), syngeneic Balb/c mice. Mice were checked daily for palpable abdominal tumors as well as for signs of eye and CNS involvement. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. Eyes and brains of sacrificed mice were fixed in buffered formalin, processed routinely and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin and subjected to histological analysis.

Cells were washed twice with phosphate-buffered saline (PBS). Samples (10^6 cells) were incubated for 30 min. at 4°C in Hank's balanced salt solution (HBSS) containing 3% horse serum and 0.1% sodium azide. The cells were then incubated with the appropriate antibody (see below) in HBSS for 45 min at 4°C. The following monoclonal antibodies against mouse cell adhesion molecules (CAMs) were used: purified rat anti-CD2, -CD18, -CD44 (Pgp-1), -CD49d (VLA-4a), -CD49e (VLA-5a), -ICAM-2, -LECAM-1(L-selectin), (Pharmingen); purified rat anti-ICAM-1(CD54) (Serotec); purified rat anti-NCAM (Immunotech); R-PE conjugated rat anti-CD4 (L3T4); FITC-conjugated rat anti-CD8a (Ly-2) (Pharmingen); rat anti-LFA-1 (supernatant of hybridoma cells, ATCC FD441.8).

Unless labeled first antibody was used, cells were washed with 5 ml HBSS and incubated with fluorescein isothiocyanate (FITC)-F(ab')2 fragment of rabbit anti-rat IgG (H+L) (Zymed) for an additional 45 min. After two washes with HBSS, the cells were fixed in 3.7% paraformaldehyde. The labeled cells were analyzed using a FACStar plus cell sorter (Becton Dickinson).

Results

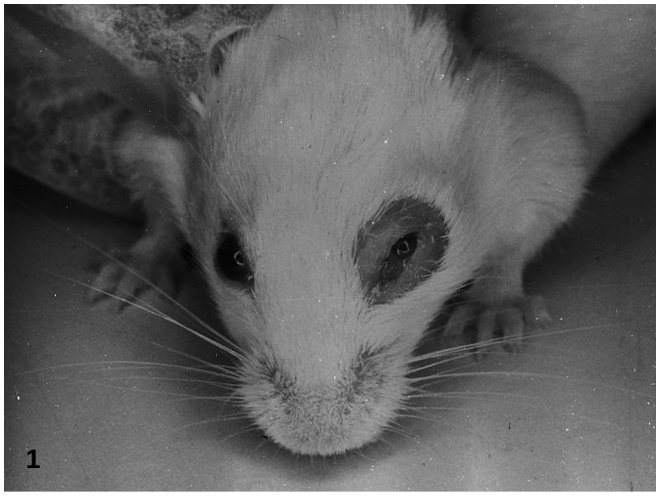
Rev-2-T-6 cells were derived from substrate-adherent, non-tumorigenic (immunogenic) variants of the S49 mouse lymphoma (see Materials and methods). These cells demonstrate cell-cell adhesion, growing in suspension culture as cell aggregates.

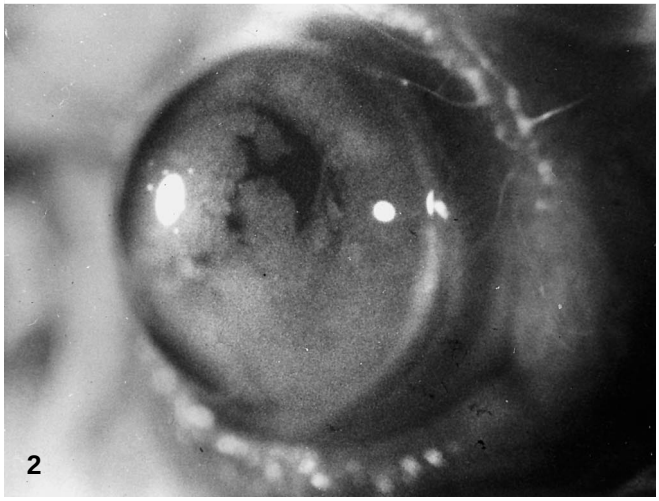
Upon intraperitoneal inoculation of Rev-2-T-6 cells into mature (2 months and older) syngeneic Balb/c mice, progressive, solid abdominal tumors develop. These are defined histologically as high-grade malignant lymphoma, small, non-cleaved cell type, with starry-sky appearance. Median survival of mature mice inoculated IP with 3×10^6–2×10^7 cells varies between 55–70 days post inoculation. Some inoculated mice can reach a weight of 45 g (due to tumor load), compared to 21 g for normal Balb/c controls.

As described above, IP inoculation of Rev-2-T-6 cells into mature Balb/c mice results in progressive, solid abdominal tumors. However, upon inoculation of these cells into newborn mice (age 6–11 days), up to 58% of the mice revealed also clinical signs of eye and CNS involvement. These signs may include: unilateral or bilateral involvement of the orbit and eyelids (Fig. 1); accumulation of lymphoma cells in the anterior chamber (AC) of the eye, thereby masking the posterior surface of the cornea (Fig. 2); retardation of animal growth (Fig. 3). The neurological signs manifested by affected mice included ataxia, spinning when held by the tail, and arched back. No signs of either eye or brain involvement appeared following intraperitoneal inoculation of parental, tumorigenic T-25 cells (see Materials and methods) into newborn mice. A summary of the frequency of clinical signs related to the age at inoculation of mice with Rev-2-T-6 cells is shown in Table 1. The eye signs, the neurological signs and the growth retardation all follow a similar pattern of decreasing involvement with increasing age at inoculation. The latest days of inoculation that still resulted in eye signs, neurological signs and growth retardation were 11, 9 and 9, respectively. None of these signs appear in mice inoculated later than day 11 postnatal (Table 1).

The onset of eye signs in mice inoculated on days 6, 7, 8, 9 and 11 with Rev-2-T-6 cells was in the range of 24–29, 17–56, 16–19, 20–40 and 24–30 days, respectively, with a median of 27, 23, 17, 25 and 28 days, respectively. The number of mice examined in each group was 5, 22, 4, 19 and 4, respectively.

Mice that demonstrated clinical signs (Table 1), and were not followed for survival (n = 22), were further submitted to histopathological analysis. This was carried out about 7–10 days following the onset of signs. Massive infiltration of the lymphoma into both orbit and eye, including the AC, uvea, vitreous body, retina and optic nerve, was revealed (Fig. 4). To identify the tissues that are most susceptible to infiltration by Rev-2-T-6 cells we analyzed 35 of the eyes (Table 2), and the 22 brains (see





below). Five eyes that were devoid of lymphoma infiltration and four eyes that were used to transfer lymphoma cells into culture (see below) were not analyzed. The structures with the highest frequency of involvement were the orbit (86%) and the optic nerve (86%). At the lower end of the scale, only one case of retina involvement was found. The cornea was devoid of any lymphoma cell infiltration. The data in Table 2 are consistent with the following routes of Rev-2-T-6 cell metastasis to the eye: (1) through the circulation (hematogenous); (2) along the optic nerve.

The significant involvement of the optic nerve (86%), and the presence of the neurological signs, although at low frequency (4 out of 119 mice inoculated at ages 6–11 days, see Table 1), prompted us to carry out a histopathological analysis of brains from the same mice. Figure 5 demonstrates infiltration of Rev-2-T-6 cells into brain tissue. It is noteworthy that in *all* cases where we found (histologically) infiltration of Rev-2-T-6 cells into the eye, these cells were also found in various structures of the brain.

When affected eye and brain tissues (4 and 1, respectively), were transferred into culture, masses of dividing cells dominated the population within a few days. These were characterized as Rev-2-T-6 cells according to their growth in suspension culture as aggregates and resistance to 0.5 mM ouabain (see Materials and methods).

Since mice inoculated with Rev-2-T-6 cells at the age of 13, 15 and 60 days did not demonstrate any symptoms related to eye and CNS involvement, or to growth retardation, it was of interest to find out whether these mice would still display infiltration to the eye and brain that could be detected histologically. To that effect, both brains and eyes of mice inoculated with Rev-2-T-6 cells at the ages of 13, 15 and 60 days (n = 5, 5 and 10, respectively) were subjected to histopathological analysis. Critically ill mice were killed when their weight, due to the abdominal tumor load, was 35–45 g. The above groups demonstrated infiltration to the brain in 1/5 (20%), 1/5 (20%) and 1/10 brains (10%), respectively, and infiltration to the eye in 2/10 (20%), 1/10 (10%) and 2/20 eyes (10%), respectively.

A more detailed histological analysis of the rostral as well as the caudal parts of the brain with regard to the frequency of structures infiltrated by the Rev-2-T-6 lymphoma was carried out (Table 3). The subarachnoid space was the site most often involved by the tumor (up to 95%). Choroid plexus (up to 64%), cranial nerves (up to 55%) and cranial nerve ganglia (55%) were also infiltrated. In all cases where lymphoma cells were detectable in the subarachnoid space, the choroid plexus and/or

Fig. 1 Unilateral involvement of the left orbit and eyelids following inoculation of Rev-2-T-6 cells into the peritoneum of newborn mice

Fig. 2 Photograph of anterior segment of Balb/c mouse eye showing heavy accumulation of lymphoma cells in the anterior chamber covering most of the posterior surface of the cornea

Fig. 3 *Left* Growth-inhibited mouse at age 38 days (inoculated at 7 days with Rev-2-T-6 cells), weight 9 g. *Right* Normal control mouse at age 38 days, weight 21 g

Table 1 Manifestation of clinical symptoms following intraperitoneal inoculation of Rev-2-T-6 cells into Balb/c mice from day 6 postnatal to 2 months of age. Mice were inoculated with 3×10^6 cells (days 6–15) and 1×10^7 cells (day 60)

Inoculation day	6	7	8	9	11	13	15	60
Eye symptoms [a]	6/11 55%	22/38 58%	4/13 31%	18/35 51%	4/22 18%	0/19 0%	0/11 0%	0/26 0%
Neurological symptoms [b]	1/11 9%	2/38 5%	0/13 0%	1/35 3%	0/22 0%	0/19 0%	0/11 0%	0/26 0%
Growth retardation [c]	5/11 45%	13/38 34%	4/13 31%	6/35 17%	0/22 0%	0/19 0%	0/11 0%	0/26 0%
Abdominal (palpable) tumors	4/11 36%	21/38 55%	6/13 46%	29/35 83%	16/22 72%	10/14 71%	9/11 82%	24/26 92%
Survival (days)[d]	21–56 (33) *n* = 9	16–80 (51) *n* = 30	15–65 (30) *n* = 12	14–90 (54) *n* = 25	64–70 (67) *n* = 3	72–90 (76) *n* = 6	60–65 (63) *n* = 4	50–67 (61) *n* = 14

[a] See Figs. 1, 2
[b] As described in the text
[c] Included in this category are mice that did not reach a weight above 14 g
[d] *Top* range of survival, *Middle* median survival, *bottom* no. of mice checked for survival

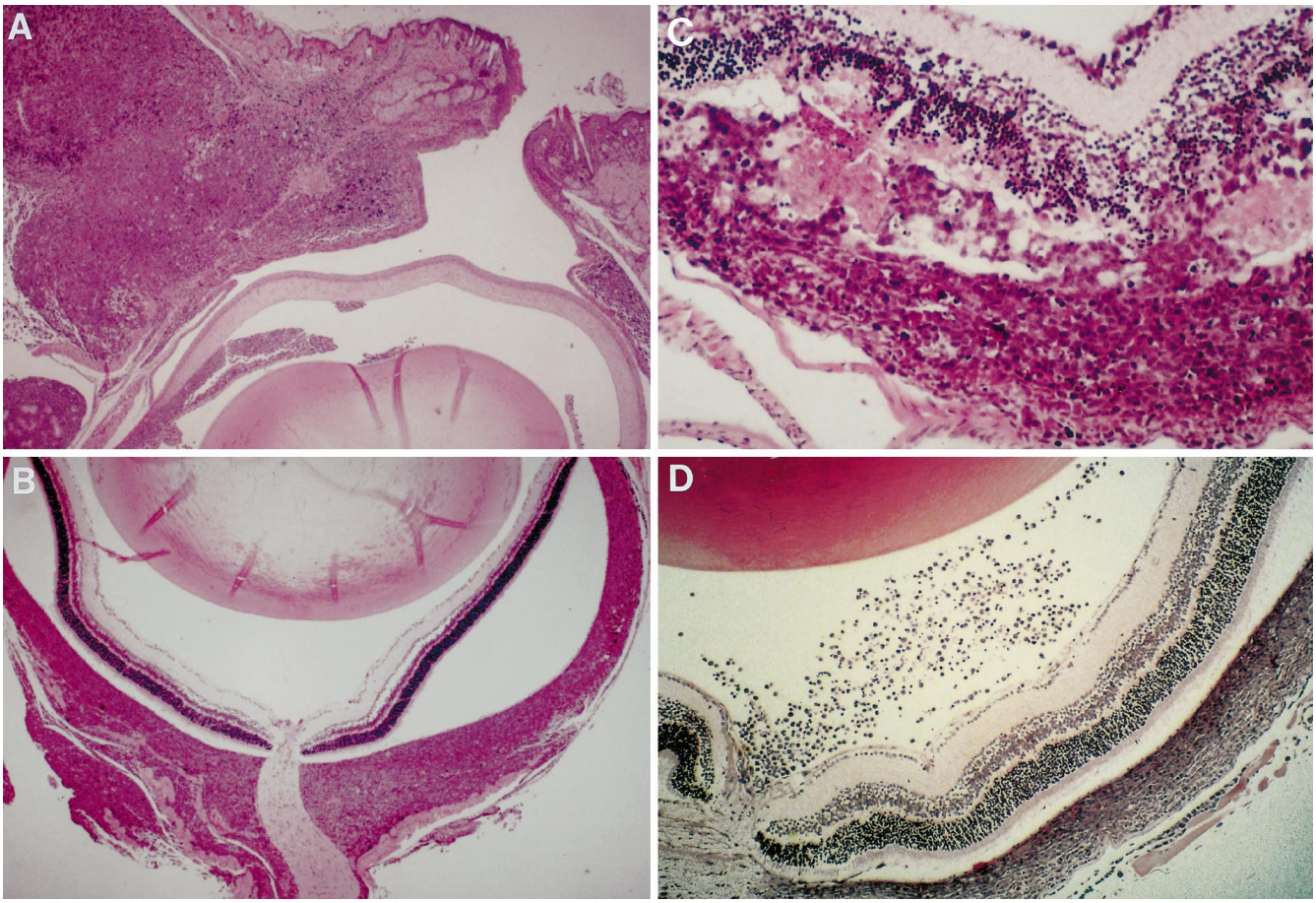


Fig. 4A–D Photomicrographs of ocular lymphoma following inoculation of Rev-2-T-6 cells into 7 days postnatal Balb/c mice (hematoxylin & eosin). **A** Histological section shows infiltration of lymphoma cells into the eyelid, conjunctiva and lacrimal gland as well as iris, ciliary body and anterior chamber (×36). **B** Histological section shows infiltration of lymphoma cells into the orbit (including extra-ocular muscles and lacrimal gland), as well as massive infiltration into the choroid and the meninges around the optic nerve (×36). **C** High-power photomicrograph shows lymphoma cells in the choroid, subretinal space and outer retina (×180). **D** Seeding of lymphoma cells into the vitreous body. Involvement of the choroid is evident as well (×90)

Table 2 Ocular structures infiltrated by Rev-2-T-6 cells following inoculation into young (6–11 days of age) Balb/c mice

Orbit 30/35 (86%)
General 28
Ocular muscles 28
Lacrimal glands 15
Optic nerve 30/35 (86%)
Meninges 30
Parenchyma 0
Bulbar Conjunctiva 20/35 (57%)
Eyelids 17/35 (49%)
Eyelids 16
Adjacent glands 1
Uvea 13/35 (37%)
Choroid- 13
Ciliary body 11
Iris 10
Intraocular (other than uvea) 10/35 (29%)
Anterior chamber 10
Posterior chamber 6
Sclera 11/35 (31%)
Vitreous 5/35 (14%)
Retina 1/35 (3%)
Cornea 0/35 (0%)

the cranial nerves and cranial nerve ganglia were also infiltrated. These findings demonstrate that the clinical signs of eye involvement are a convenient and reliable indicator of infiltration to both eye and brain by Rev-2-T-6 cells. Taken together with the high proportion (58%) of mice that develop signs of eye involvement, our findings offer an experimental model for the analysis of lymphoma metastasis to the eye and brain.

The brains, optic nerves and eyes of 21 mice that were previously found to demonstrate lymphoma cell infiltration to these sites (following the onset of clinical signs), were grouped according to different patterns of infiltration (Table 4). The prevalent pattern (no. 1) demonstrated bilateral involvement of the eyes and optic nerve. Patterns 2–7 show data of mice where one or more of these sites were not involved. This suggests some interesting points with regard to possible routes of infiltration to the eye and brain. Thus, from patterns 2–4 (6/21 mice, 29%), it may be concluded that one route is initiated in the brain and continues along the optic nerve and into the eye. From patterns 5–7 (4/21 mice, 19%), it may be concluded that the eye can also be infiltrated through hematogenous tumor spread, independent of the

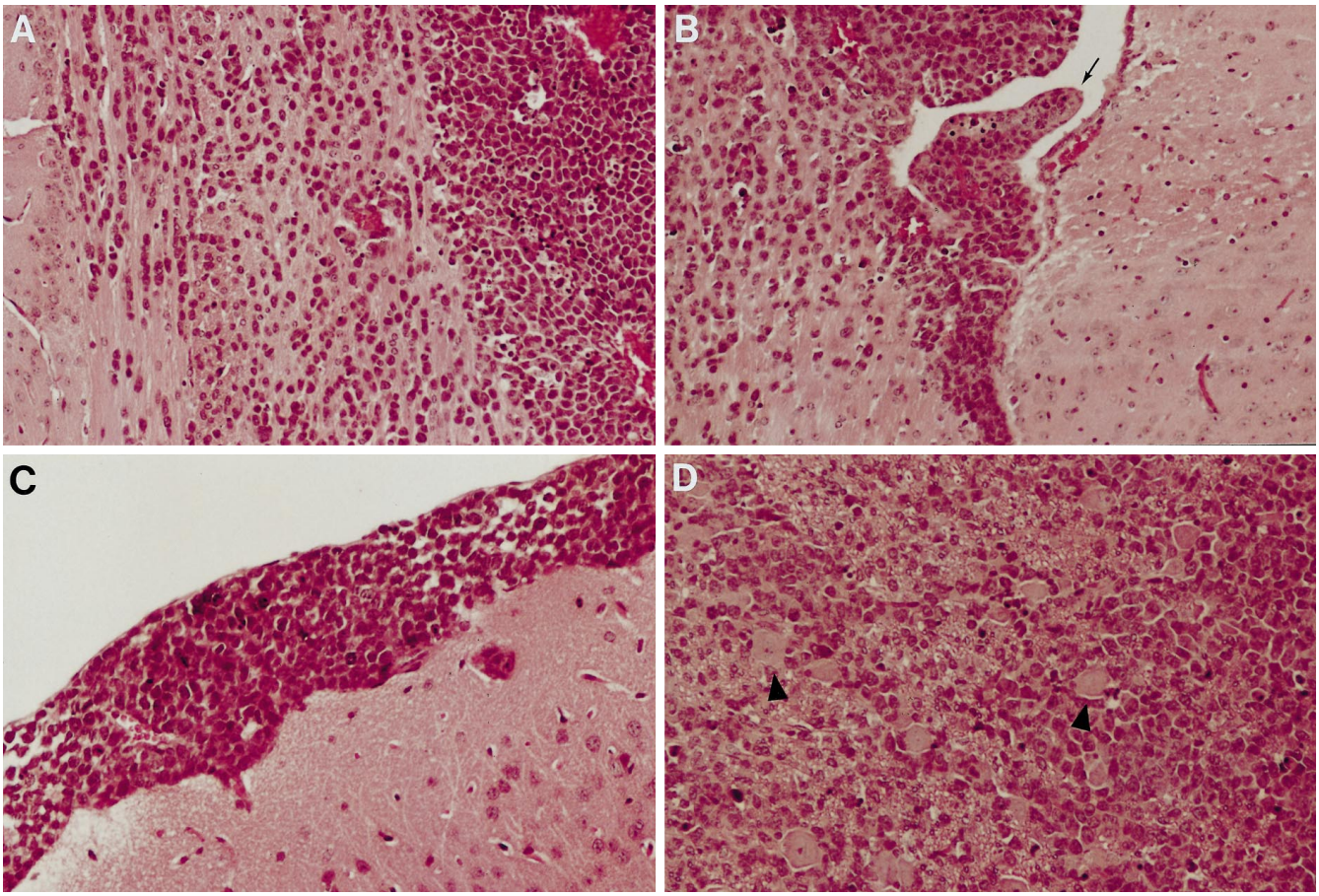


Fig. 5A–D Histopathological appearance of intracranial lymphoma infiltrates (H&E). **A** Tumor masses in the lateral ventricle (*right*) with diffuse infiltration of the adjacent periventricular brain and corpus callosum (×165). **B** Tumor infiltration of the choroid plexus (*arrow*). Note additional tumor manifestations in the ventricular lumen and the periventricular brain tissue (*left*) (×165). **C** Accumulation of tumor cells in the subarachnoid space without infiltration of the underlying cortex (×220). **D** Tumor manifestation in a cranial nerve ganglion. Local neurons are still detectable (*arrows*) (×220)

Table 3 Brain structures infiltrated by Rev-2-T-6 cells following inoculation into young (6–11 days postnatal) Balb/c mice

Regional lymph nodes were histopathologically analyzed in 6 animals, 2 of these have shown tumor involvement

Forebrain (n = 22)		Posterior fossa (*n* = 22)	
Subarachnoid space	(95%)	Subarachnoid space	(86%)
Choroid plexus	(59%)	Choroid plexus	(64%)
Cranial nerve ganglia	(54%)	Cranial nerves	(55%)
Corpus callosum	(45%)	Cranial nerve ganglia	(55%)
Perivascular system	(45%)	Ventricular system	(32%)
Ventricular system	(41%)	Cerebral cortex	(27%)
Periventricular infiltration	(41%)	Pons	(18%)
Cranial nerve involvement	(36%)	Cerebral white matter	(9%)
Myelin infiltration	(18%)	Skull bone	(5%)
Cortex	(18%)	Muscle	(0%)
Peripheral nerves	(14%)		
Basal ganglia	(9%)		

Table 4 Patterns of infiltration of Rev-2-T-6 cells into the eye, optic nerve and brain. Each pattern represents the findings in one mouse (2 eyes, 2 branches of the optic nerve and the brain)

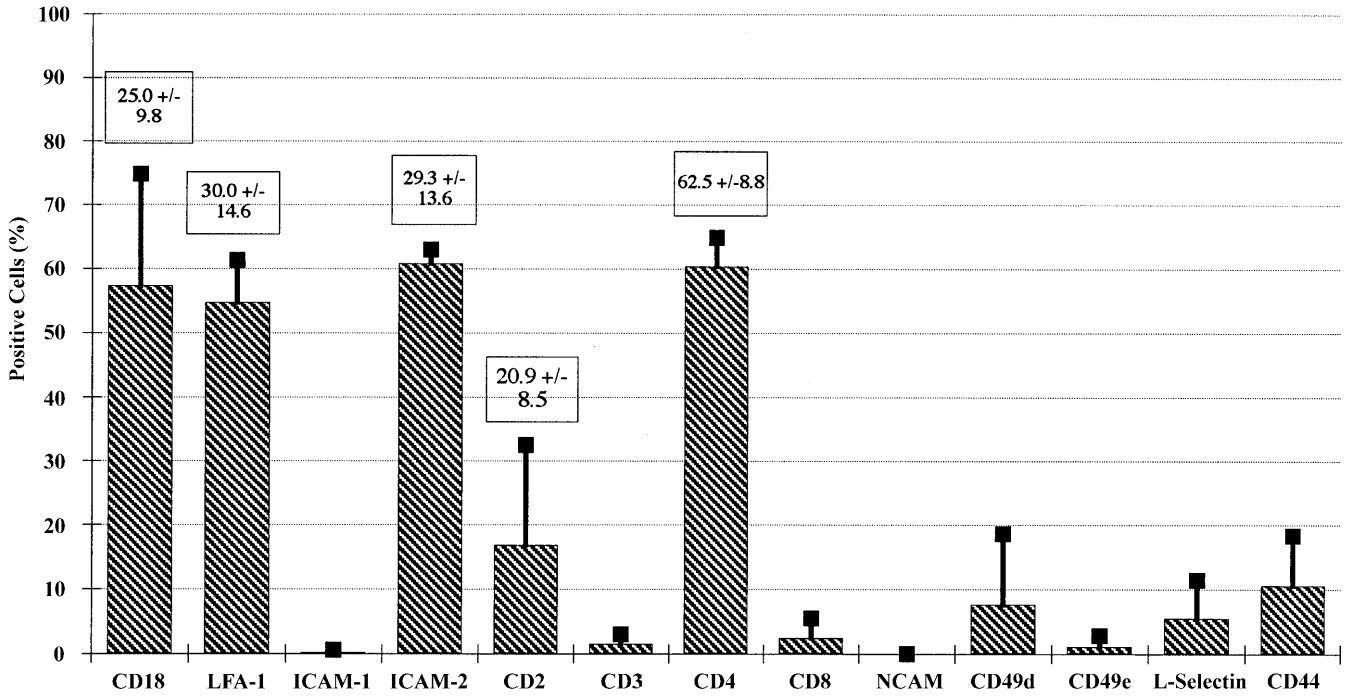
Pattern no.	Eye*	Optic nerve	Brain	No.of mice
1	+	+	+	11/21 (52%)
	+	+		
2	–	+	+	1/21 (5%)
	–	–		
3	+	+	+	3/21 (14%)
	–	–		
4	+	+	+	2/21 (10%)
	–	+		
5	+	–	+	1/21 (5%)
	+	–		
6	+	–	+	2/21 (10%)
	–	–		
7	+	–	+	1/21 (5%)
	+	+		

* Eye includes the orbit and intraocular tissues

optic nerve route. Indeed, this notion is strengthened by the finding that the four eyes of patterns 5–7 (that show no optic nerve involvement), demonstrate lymphoma infiltration into the orbit, but not to intraocular tissues.

Cell adhesion molecules (CAMs) are known to participate in the infiltration of lymphoid cells to the eye and CNS during inflammatory reactions [10, 51]. It was therefore of interest to characterize Rev-2-T-6 cells with regards to the expression of representatives of the different classes of CAMs. To that effect, a flow cytometry analysis was carried out with the following findings (Fig. 6): CD18, LFA-1, ICAM-2, and CD4 were expressed on the surface of about 60% of Rev-2-T-6 cells. CD2 was expressed in about 20% of cells. The mean fluorescence intensity (as a measure of fluorescence intensity per cell) of the CAMs expressed at the higher percentage of cells is also shown in Fig. 6. Other CAMs were either not detected (ICAM-1,

Fig. 6 Analysis of cell adhesion molecules (CAMs) on Rev-2-T-6 cells using flow cytometry. Histograms represent the percentage of cells that express the various CAMs. *Numbers in frames* mean fluorescence (representing fluorescence intensity per cell)



NCAM and CD49e) or were expressed in a low percentage of cells (CD3, CD8, CD44, CD49d and L-selectin).

Discussion

In the present study, we have developed a mouse model to explore the biology and therapy of lymphoma metastasis to the eye (including the orbit) and CNS. The significance of this model lies in its potential use for addressing the following issues: (1) determine exact patterns of lymphoma spread through the use of radiolabeled or genetically tagged cells; (2) identify molecular mechanisms (and genes) involved in metastasis to these sites; (3) develop novel experimental therapeutic modalities; (4) study a variety of parameters (including kinetics of cytokine profile) for early diagnosis of lymphoma spread in the eye and CNS; (5) investigate immune responsiveness of the eye and brain towards malignant lymphoma; (6) deliver genes of interest into the eye and brain. To that effect, we have recently introduced the human multi-drug resistance (MDR) gene into eye and brain infiltrating cells. Following intraperitoneal inoculation into young mice, multi-drug resistant tumors developed in both eyes and brain (J. Hochman, unpublished). These tumors can be used as targets for reversal of multidrug resistance by putative reversing agents. The following features render the model convenient: (1) the use of immunocompetent Balb/c mice (and not nude or scid mice as is the case in other related models – see below); (2) the relatively high percentage of mice (up to 58%) with eye and brain manifestation; (3) the ease of handling (IP inoculation); (4) the intervals from the date of inoculation (day 7) to the onset of clinical signs (about 3 weeks) and from the onset of signs until death (about 4 weeks) which allow one to investigate different approaches of intervention during the homing process to the eye and brain and during the spread of the lymphoma throughout these organs.

To our knowledge, no comparable model is currently available to study infiltration of lymphoma to the eye and brain. Other experimental models that have been introduced involve the brain or spinal cord but not the eye. Thus, Benke et al. [5] examined the in vivo behavior of adhesive ESb-MP tumor variants (derived from the ESb murine lymphoma). In the late stages of tumor growth, following subcutaneous or intravenous inoculation, 38% of ESb-MP tumor-bearing animals developed metastases around and within the spinal cord, causing a syndrome of hind-leg paralysis. However, no metastases were found in the brain despite detailed histological analysis. More recently, additional models of interest (albeit in immune-deficient mice) have been reported. In one study [13], retro-orbital inoculation of human B cell tumor (Daudi) cells into scid mice was carried out. Despite this route of inoculation, no infiltration into the eye was evident. Among the sites involved, cells metastasized to the cranio-ventral cerebral meninges and extended along the ventral and caudal aspects of the cerebrum and into the lateral ventricles. It is noteworthy that intraperitoneal inoculation of Daudi cells resulted in abdominal tumors but no involvement of the brain. In another experimental model [4], human T-cell lymphoblastic lymphoma cells were inoculated intravenously into beige-nude-xid (BNX) mice. Among the sites involved, there was invasion of the CNS (leptomeningeal and perivascular) but not the eye. The model of White et al. [51], which includes the direct inoculation of lymphoma cells into the AC of the eye of nude mice, cannot be used to study the routes of infiltration to the eye as well as the molecular mechanism(s) used by the infiltrating cells.

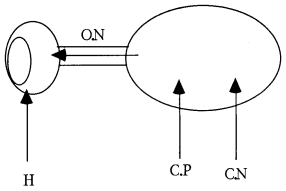


Fig. 7 A schematic model for infiltration of Rev-2-T-6 lymphoma cells to the brain and eye. *Arrows* depict the proposed major routes of infiltration (*C.N.* cranial nerves and cranial nerve ganglia, *C.P.* choroid plexus, *O.N.* optic nerve, *H.* hematogenous)

As major site of CNS infiltration, the subarachnoid space was involved in 95% of cases, followed by the choroid plexus (64%) and cranial nerves and ganglia (55%). These findings are consistent with two major routes of infiltration to the subarachnoid space: (1) through the choroid plexus (CP); (2) through the cranial nerves and cranial nerve ganglia (CN).

In the case of the eye, various possibilities for entry of the lymphoma cells exist: (1) through the brain and optic nerve; (2) through the carotid and ophthalmic arteries, directly to the orbit; (3) retrograde flow through anastomoses between the valveless facial and orbital veins [25].

With regard to our findings of orbit involvement in young mice, it is noteworthy that the orbit is one of the characteristic sites involved in young children with Burkitt's lymphoma [2, 44].

Both optic nerve and orbit were affected histologically in the majority (86%) of eyes. This observation is consistent with two independent (major) routes of infiltration to the eye: (1) from the brain along the optic nerve; (2) through the circulation (hematogenous infiltration; see above). These conclusions are supported by the different patterns of infiltration demonstrated in Table 4. Thus, pattern no. 2 (brain and one branch of optic nerve infiltrated without eye involvement), pattern no. 3 (brain, one branch of optic nerve and corresponding eye infiltrated) and pattern no. 4 (brain, two branches of optic nerve and one eye infiltrated), support the optic nerve (ON) pathway to the eye. Patterns 5–7, in which one or both eyes are infiltrated, one or both branches of the optic nerve are devoid of lymphoma cells and the brain is involved in all cases, support an independent, hematogenous (H) infiltration of the eye. It is noteworthy that during this study, we have *not* encountered patterns of infiltration to the eye with the following profiles: (1) eye pos-

itive, optic nerve and brain negative; (2) eye and optic nerve positive, brain negative. Such patterns could support a pathway of retrograde infiltration from the eye to the brain along the optic nerve. While such a route cannot be entirely excluded, its likelihood is low. The schematic model in Fig. 7 summarizes our current thinking on the infiltration of Rev-2-T-6 cells to the eye and brain. Two major routes (CP and CN) lead to the brain, and two major routes (ON and H) lead to the eye. Further analysis is needed to address this issue.

In mature immunocompetent mice, the normal recruitment of lymphocytes to the brain is an antigen-independent mechanism depending on the state of activation of lymphocytes [48]. Entry of immune cells into the brain is mediated by CAMs expressed by cerebral endothelial cells [30]. The basal level of lymphocyte binding to cerebral endothelial cells is much lower than binding to endothelia of other organs, since in the normal brain the expression of CAMs is downregulated. However, under inflammatory conditions, cytokines lead to an activation of T lymphocytes as well as an upregulation of CAMs on cerebral, endothelial cells, thus enabling hematogenous cells to enter the brain [23, 26, 33]. Anti-LFA-1 and anti CD18 were able to block the binding of mitogen-activated lymphocytes to brain endothelium in vitro. The time at which lymphocyte adhesiveness to the endothelium was greatest corresponded with the time at which highest levels of LFA-1 and VLA-4 expression were observed [34].

Monoclonal antibodies against LFA-1 and ICAM-1 inhibited experimental uveitis, suggesting a role for these molecules in mediating leukocyte recruitment to the eye [46, 49]. Since these CAMs participate in infiltration of lymphocytes to the eye and CNS, it is tempting to speculate that LFA-1 and ICAM-1 may also play a role in the metastasis of Rev-2-T-6 cells to the eye and brain. Furthermore, in view of our findings that the choroid plexus can serve as an important route of metastasis to the brain, it is noteworthy that NMRI mice constitutively express both ICAM-1 and VCAM-1 on the epithelium of the choroid plexus and ependymal cells of the ventricular wall [8].

As stated in the Introduction, the majority of CNS lymphomas are of the B cell type, while Rev-2-T-6 cells were derived from a mouse T cell lymphoma. Since LFA-1, ICAM-2 and CD2 are also expressed on some B cell lymphomas [28, 29, 41], one can not rule out the possibility that a mechanism (common to both T and B cell lymphomas) involving these CAMs (in particular LFA-1) may participate in metastasis to the CNS. Further experimentation will be needed to address this issue.

In the present model, metastasis to the brain and eye took place in mice inoculated no later than day 11 postnatal. This may suggest that a developmentally regulated process in the brain and eye, during a critical period between postnatal days 6 and 11, favors the initial infiltration into these sites. One possibility is selective leakage (towards Rev-2-T-6 cells) of the blood-brain barrier (BBB) at this period that coincides with the development of various parameters of a mature BBB [11, 47]. Another developmentally regulated process that might be relevant is the maturation of the vascular network in the eye, which takes place mostly during the first 2 weeks of postnatal development [1, 45]. Once infiltration has taken place, the lymphoma cells multiply and, depending on the initial dose that has crossed the barrier, will spread at a slower or faster pace in the affected brain and eye.

At present, we do not know what causes the growth arrest phenomenon in mice inoculated with Rev-2-T-6 cells at the ages 6–9 days. Since it correlates with the decrease, in time, of the eye and neurological signs (Table 1), one is tempted to speculate that the metastasis of Rev-2-T-6 cells to the eye and brain may involve the infiltration (and inhibition) of specific structures, along the hypothalamic-pituitary-skeletal axis, that are involved in the regulation of growth hormone secretion and action.

In conclusion, Rev-2-T-6 cells constitute a novel experimental model for metastasis of malignant lymphoma to the eye and brain. A better understanding of the molecular mechanisms that regulate the process of metastasis to the eye and brain, and of the complex interactions between the metastatic cells and host factors at these sites, will provide a biological foundation for the design of more effective therapy, as well as new options to deal with lymphoma metastasis to these organs.

Acknowledgements This article is dedicated to the memory of Tsury Hasson, a friend and a student. Supported in part by the Israel Cancer Association and by the Israel Science Foundation (J.H.).

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