PHENOTYPIC ANALYSIS OF THE
INFLAMMATORY EXUDATE IN
MURINE LYMPHOCYTIC CHORIOMENINGITIS

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Lymphocytic choriomeningitis virus (LCMV)[1] characteristically causes a fatal neurological disease 6–7 d after intracerebral inoculation into adult mice (1). The onset of symptoms is associated with massive exudation of inflammatory cells into the cerebrospinal fluid (CSF), concurrent with a total breakdown of the barrier to protein transudation between the blood and the central nervous system (CNS). However, mice that are defective in the T cell compartment as a consequence of immaturity, absence of thymic function (nu/nu), treatment with antilymphocyte serum, or prior adult thymectomy, lethal irradiation and reconstitution with bone marrow, develop neither symptoms nor inflammation (1). In addition, adoptive transfer experiments using LCMV-infected, cyclophosphamide (Cy)-suppressed recipients (2) indicate that the induction of both meningitis and clinical impairment depends on the involvement of Thy-1$^+$, Ig$^-$, Lyt-2$^+$, donor LCMV–immune T cells that share class I MHC glycoproteins with the brain endothelium of the immunosuppressed recipients (1–6). Furthermore, direct inoculation of class I MHC-compatible, LCMV-specific, cloned CTL into the CNS of lethally irradiated, virus-infected mice is sufficient to induce fatal LCM (7).

The LCM model thus offers two major advantages for the study of T cell recruitment and localization in a virus disease. The first is that there are relatively few cells in normal mouse CSF (<10 per microliter), and samples free of other tissue elements can readily be obtained from the cisterna magna (1). The second is that cellular extravasation is a function of the cell-mediated immune response and does not reflect direct virus-induced damage (1, 5), as occurs, for instance, after infection with neurotropic influenza virus or vaccinia virus (8–10). The system has been used previously for quantitative studies of the inflammatory process, and to show that there is potent CTL and NK activity in the CSF of LCMV-infected mice (11, 12).

In the present experiments we have used both direct inoculation of LCMV

TABLE 1

Genotypes of Cell Surface Molecules for Mouse Strains Used in this Study

Mouse strain	Thy-1	Pgp-1	H-2
C57BL/6J (B6)	2	2	b
C57BL/6.Ka.Thy-1.1	1	2	b
BALB/c.H-2^b	2	1	b
(CBA/H × BALB/c)F$_1$	2	2*	kxd

* Note that CBA/H mice are Pgp-1.2, while CBA/J mice are Pgp-1.1 (13).

into immunologically competent adult mice, and adoptive transfer into virus-infected, immunosuppressed or unsuppressed recipients to characterize the inflammatory exudate by flow microfluorometry (FMF). This has allowed a unique analysis of: (a) the distribution of T cell subsets in a site of pathology, (b) the phenotypic characteristics of these T cells, and (c) the origin of specific and nonspecific participants in this inflammatory process.

Materials and Methods

Mice. The mice used in these experiments were all bred at the John Curtin School of Medical Research. The genotypes of the cell surface markers relevant to the present study are summarized in Table I.

Virus and Mouse Models. The neurotropic Armstrong (Arm) E350 strain of LCMV was grown in suckling mouse brain and the viscerotropic WE3 strain in BHK-21 cells (14). Virus stocks were diluted in cold gelatine saline and titrated by intracerebral inoculation into adult, outbred WEHI mice. Immune spleen cells for adoptive transfer experiments were obtained from mice that had been injected intravenously with 1,000 LD$_{50}$ of WE3 LCMV 8 d previously, the time of peak CTL effector function. Mice were injected intracerebrally with 1,000 LD$_{50}$ of Arm LCMV, and then (a) sampled 6 d later; (b) given 200 mg/kg of Cy (Endoxan Asta; Asta Werke AG, Bliefeld, Federal Republic of Germany) 4 d later followed by intravenous inoculation of 10^7 immune spleen cells on the next day. Samples of CSF were taken after a further 96 h; and (c) given 2.0 × 10^7 immune spleen cells 2 d after injection with virus, and then sampled at 48 or 72 h.

Both cell transfer protocols (b and c) have a mandatory requirement for Lyt-2$^+$, class I MHC-restricted, virus-immune T cells (4, 5; Doherty, P. C., J. E. Allan, and Rh. Ceredig, manuscript in preparation). Mice would normally die from LCM on day 7 (model a), at 5 or 6 d after cell transfer (model b) or at 4 d after cell transfer (model c).

CSF and Blood Collection. CSF was obtained from the cisterna magna of mice that had been anesthetized with 2,2,2-tribromoethanol and was exsanguinated (10). Peripheral blood lymphocytes were collected by cardiac puncture of mice under ether anesthesia. A final concentration of 0.38% sodium citrate was used. Lymphocytes were collected by centrifugation over Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden).

Culture Media and In Vitro Growth of CSF Cells. The medium used for the growth of CSF cells was DME, supplemented as described (15), containing 10% heat-inactivated FCS (Flow Laboratories, Sydney, Australia); this is referred to as mixed lymphocyte culture (MLC) medium. The CSF cells were washed and resuspended at 2–5 × 10^5/ml in MLC medium (in 24-well Linbro cluster plates, No. 76-063-05; Flow Laboratories). Then we added 1 ng PMA (Sigma Chemical Co., St. Louis, MO), per milliliter of medium, 300 ng ionomycin (Calbiochem-Behring Corp., La Jolla, CA) per milliliter, and 1:10 final concentration (80 U/ml) of IL-2-containing supernatant of PMA-induced EL-4 thymoma cells. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO$_2$ in air.

Limiting Dilution Analysis. The frequency of CTL precursors (CTLp) in CSF was determined using a protocol modified from that described previously (16). The stimulator

cells were thioglycollate-induced, anti-Thy-1.2 and complement-treated peritoneal macrophages that had been infected with WE3 LCMV, dispensed at 10^5 cells per well in 96-well Linbro plates, incubated for 48 h, and then UV-irradiated for 10 min (7,200 J/m^2) using a 30 W germicidal lamp (Philips Electronic Instruments, Inc., Mahwah, NJ). They were then cultured with graded numbers of CSF cells (16 replicates per dilution) in 80 μl of medium, to which 20 μl of IL-2-containing supernatant was added on day 0 and day 6. The contents of each well were split equally on day 8 or day 9, and incubated for 5–6 h with 5 × 10^3 LCMV-infected or uninfected ^{51}Cr-labeled target cells. Cultures were considered to be positive if the level of specific ^{51}Cr release was greater by 3 SD than that of the mean ^{51}Cr release for stimulators alone, or, if this was <5%, the latter was taken as the cut-off point.

Phenotypic Analysis by Flow Microfluorometry. Indirect immunofluorescence was used throughout, and all incubations were done at 4°C. Cells, either isolated freshly from CSF or cultured for 4–5 d in vitro, were stained as previously described (17). Thus 1–5 × 10^5 cells, depending on cell numbers obtained, were resuspended in 100 μl of supernatant from hybridoma cell lines secreting mAbs. The mAbs used were AT83, anti-Thy-1.2 (kindly provided by Dr. F. Fitch, University of Chicago, Chicago, IL); HO-22,1, anti-Thy-1.1 (18); 53.6.7, anti-Lyt-2 (19); GK-1.5, anti-L3T4 (20); PC61, anti-IL-2-R (21), IM7, anti-Pgp-1; RAM, anti-Pgp-1.1 and C71, anti-Pgp-1.2 (13). Control samples were resuspended in 100 μl MLC medium. After a 20-min incubation at 4°C, cells were spun through FCS and resuspended in 100 μl MLC medium containing 1:20 final dilution of FITC-labeled sheep anti-rat Ig second-step reagent (Silenus Laboratories, Melbourne, Australia). After a further 20-min incubation and washing through FCS, cells were finally resuspended in 0.3–0.5 ml PBS containing 0.3% paraformaldehyde and were analyzed by FMF.

FMF analysis was done using a FACS IV (Becton Dickinson & Co., Mountain View, CA) linked to a B-D Consort 40 computer. For each sample, 1–2 × 10^4 gated events were collected and stored as list mode files using the ACQ4 data acquisition program. For correlative analysis of list mode data, the DISP4 program was used for histogram generation, the DISP2D for contour plots, and the HIDDEN program for three-dimensional data display. Cells were analyzed using 600 mW of 488 nm laser light for detection of FITC signals. In all instances, the percent stained cells was determined after subtraction of signals from an equal number of control cells stained with the FITC-labeled second-step reagent alone.

Results

The Disease after Direct Inoculation of Virus. CSF was obtained from LCMV-infected B6 or (CBA/H × BALB/c)F$_1$ mice 6 d after intracerebral injection of 1,000 LD$_{50}$ of neurotropic Arm LCMV. The viability of such cells as shown by exclusion of trypan blue dye was >95% and the mean number of CSF cells obtained was 1.5 × 10^5 viable nucleated cells per mouse. The pooled CSF cells were stained by indirect immunofluorescence with mAbs to Thy-1.2, Lyt-2, L3T4, Pgp-1 and IL-2-R. Control cells, namely thymocytes for Thy-1, Lyt-2, and L3T4, or FDC-P1 cells (provided by Dr. A. Hapel, The John Curtin School of Medical Research) for Pgp-1 and IL-2-R, were included in each experiment. In addition, all populations were stained with the FITC-labeled sheep anti–rat reagent only.

The results of a typical experiment are given in Fig. 1, the reagent controls being shown in the upper panels and CSF cells from B6 mice in the lower panels. The dotted line to the left of each panel shows the profile of cells stained with the second-step reagent alone. Whereas thymocytes were 98% Thy-1$^+$, 86% Lyt-2$^+$, and 93% L3T4$^+$, CSF cells were only 36% Thy-1$^+$, 25% Lyt-2$^+$, and 30% L3T4$^+$. In contrast to staining for Thy-1 and Lyt-2, the CSF cells stained weakly
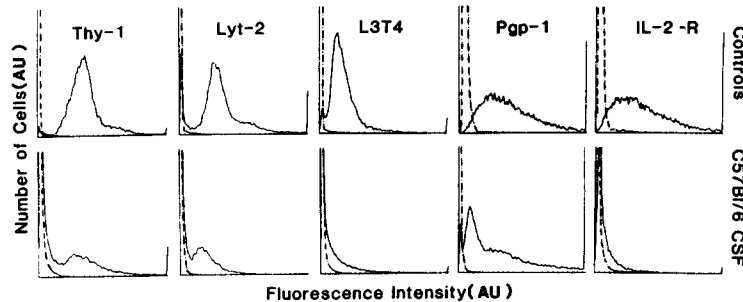
FIGURE 1.   FMF analysis of freshly isolated CSF exudate cells from C57BL/6 mice inoculated intracerebrally with LCMV 6 d previously. Each panel represents a fluorescence histogram of samples stained with mAbs to Thy-1, Lyt-2, L3T4, Pgp-1, or IL-2-R or with FITC-labeled anti-rat antibody alone (*dotted lines*). Controls (*top* panels) are adult C57BL/6 thymocytes (Thy-1, Lyt-2, L3T4) or FDC-P1 cells (Pgp-1, IL-2-R). (*Bottom* panels) The profiles of CSF cells at the same fluorescence gains as the controls.

TABLE II

Cell Surface Phenotype of Freshly Isolated CSF Cells from Mice Injected Intracerebrally with LCMV 6 d Previously

Exp.	Mouse strain	Percent positive				
		Thy-1 (AT83)*	Lyt-2 (53.6.7)	L3T4 (GK1.5)	Pgp-1 (IM7)	IL-2-R (PC61)
1	(CBA × BALB/c)F$_1$	20	11	14	92	ND
2	C57BL/6	36	25	30	34, 57‡	10
3	(CBA × BALB/c)F$_1$	42	37	31	37, 62‡	10

* mAbs are shown in parentheses.
‡ First value, weakly; second value, brightly stained cells. See text and Fig. 1 for details.

for L3T4 and the profile was not bimodal, suggesting that L3T4 was not being expressed on T cells (see below). Furthermore, the percent Thy-1$^+$ population was less than the sum of Lyt-2$^+$ and L3T4$^+$ cells, whereas, when normal lymph node T cells were stained, the percent Thy-1$^+$ cells is equivalent to this value (data not shown). The staining profile with Pgp-1 was triphasic, with a negative peak comprising 6.6% of cells, and two populations of positive cells. Thus, 37% showed relatively weak staining, whereas 57% were bright and had a wide range in intensity of staining. IL-2-R expression by freshly isolated CSF cells was low, with only 10% of cells staining above the level for the second-step reagent alone. Results similar to those shown in Fig. 1 were obtained on three successive occasions with CSF populations from C57BL/6 or (CBA × BALB/c)F$_1$ mice (Table II). In each case the profiles suggested that the majority of Thy-1$^+$ cells were Lyt-2$^+$; L3T4 expression on these freshly isolated CSF cells was consistently weak.

The frequency of LCMV-immune CTLp was determined by limiting-dilution analysis of CSF cells from these acutely infected mice. The results for three separate experiments are given in Table III. The frequency of CTLp generating effectors for LCMV-infected targets ranged from 1:2,000 to 1:3,500, being three to five times higher than that for uninfected target cells. The LCMV-specific component was surprisingly low, both in view of the dominance of Lyt-

TABLE III

Frequency of LCMV-immune CTL Precursors in CSF

Mouse strain	Target cell	Frequency of effectors for H-2-compatible targets	
		LCMV-infected	Uninfected
(CBA × B6)F$_1$	MC57G	1/3,021	1/16,466
CBA/H	L929	1/1,981	1/6,431
B6	MC57G	1/3,538	1/9,312

The CSF samples were taken from mice that had been injected intracerebrally 6 d previously with 1,000 LD$_{50}$ of Arm LCMV. The cultures were prepared as described in Materials and Methods, and incubated for 8–9 d at 37°C before adding 5 × 10^3 target cells per well. The results are for three separate experiments and linear plots (r = 0.9, 0.89, and 0.83 on infected targets) were obtained in each case.

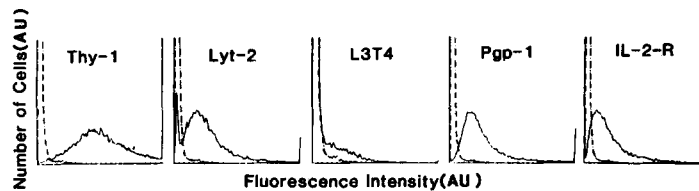

Fluorescence Intensity(AU)

FIGURE 2. FMF analysis of CSF populations equivalent to those analyzed in Fig. 1 that were cultured in IL-2 for 5 d after stimulation with PMA and ionomycin. Each panel shows samples stained with the FITC-labeled anti-rat antibody only (*dotted lines*) and staining with the mAb indicated in conjunction with the second-step reagent (*solid lines*).

2$^+$ class I MHC–restricted T cells in the immunobiology of this disease (3–6, 11) and previous estimates of virus-immune CTLp frequency (1:80 to 1:320) in mice injected intracerebrally with the more lytic vaccinia virus (22). The high frequency of cells lytic for uninfected targets is not surprising in view of the potent NK activity found in CSF of mice developing LCM following intracerebral inoculation of virus (12).

The next step was to grow the CSF T cells in vitro, so that the pattern of T cell subset distribution could be analyzed more clearly. CSF cells were thus washed in MLC medium and cultured at 3–5 × 10^5/ml medium containing 1 ng/ml PMA, 300 ng/ml ionomycin, and 80 U/ml IL-2. These culture conditions are optimal for the growth of both Lyt-2$^+$,L3T4$^-$ and Lyt-2$^-$,L3T4$^+$ T lymphocytes (23). Microscopic examination of the cultures at 24 h of incubation showed that ~50% of CSF cells were dead, as determined by the inability to exclude trypan blue dye. Subsequently, the cultures proliferated with a doubling time of ~15 h. Phenotypically, 5 d after the initiation of culture, the cells were 95% Thy-1$^+$, 82% Lyt-2$^+$, 9% L3T4, 91% Pgp-1$^+$, and 83% IL-2-R$^+$ (Fig. 2). Unlike the freshly isolated CSF cells, the percent Thy-1$^+$ population now approximated the sum of the percent Lyt-2$^+$ and L3T4$^+$ cells. These cultured cells also expressed Pgp-1 and IL-2-R, with the staining intensity of the latter somewhat lower than that of the former. Results similar to those shown in Fig. 2 were found in three separate experiments (Table IV). The T cell component of the inflammatory exudate is thus dominated by Lyt-2$^+$ lymphocytes, even though the frequency of LCMV-specific CTLp in freshly isolated CSF is low (Table III).

TABLE IV

Phenotypic Analysis of CSF Cells 5 d after
PMA + Ionomycin Stimulation

Exp.	Mouse strain	Percent positive		
		Thy-1 (AT83)*	Lyt-2 (53.6.7)	L3T4 (GK1.5)
1	(CBA × BALB/c)F₁	95	82	9
2	C57BL/6	97	85	7
3	C57BL/6	95	74	8

Cells from mice that had been injected intracerebrally with LCMV 6 d
previously were maintained in 80 U/ml IL-2 as described in Materials
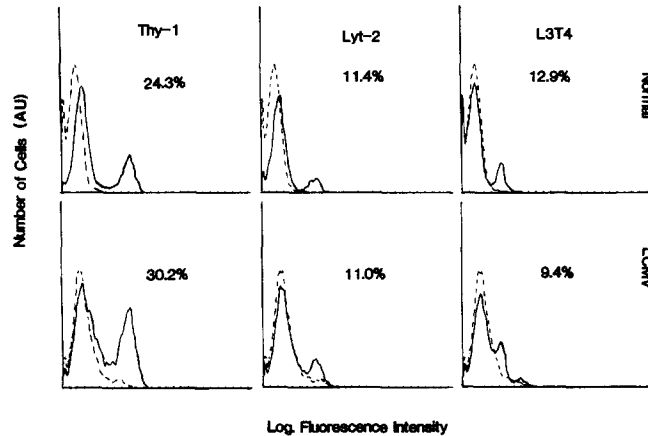and Methods.
* mAbs are shown in parentheses.

FIGURE 3.   FMF analysis of peripheral blood lymphocytes from LCMV-infected mice. Lym-
phocytes obtained after separation of blood over Ficoll-Paque were stained using biotinylated
mAbs and avidin-FITC conjugate. Each panel shows fluorescence histograms, on a logarithmic
scale, of cells labeled with the avidin-FITC conjugate alone (*dotted line*) or the indicated mAbs
(*solid lines*). Cells from normal (*top* panels) or LCMV-infected mice (*bottom* panels) were stained
and analyzed in the same experiment.

This dominance of the Lyt-2⁺ T cell subset does not reflect the situation in
blood. The FMF profile of circulating lymphocytes was assessed at 5 d after
intracerebral inoculation of LCMV. At this stage, the relative numbers of Lyt-
2⁺ and L3T4⁺ lymphocytes in blood were generally comparable for control and
LCMV-infected mice (Fig. 3). There thus seems to be selective recruitment, or
retention, of Lyt-2⁺ T cells in the CSF of adult mice developing LCM after direct
inoculation of virus (Figs. 1 and 2; Tables II and IV), even though many of these
T cells may not be virus specific (Table III).

LCM Resulting from T Cell Transfer into Cy-suppressed Mice.   Adoptive transfer
of LCMV-immune T cells into Cy-suppressed, virus-infected recipients was used
to assess the origins of the various cell populations in CSF. In general,
C57BL/6.Ka (Thy-1.1, Pgp-1.2) LCMV-immune spleen cells were injected into
BALB/c.H-2ᵇ (Thy-1.2, Pgp-1.1) recipients (Table V, Exps. 2 and 3). The CSF
cells were typed, using allele-specific mAbs to Thy-1 and to Pgp-1, at 4 d after

TABLE V

Phenotypic Analysis of CSF in the Cy-suppressed Transfer Model

| Exp.	Donor	Recipient	Percent positive					
			Thy-1.1 (HO-22.1)*	Pgp-1.2 (C71)	Thy-1.2 (AT83)	Pgp-1.1 (RAM)	Lyt-2 (53.6.7)	L3T4 (GK-1.5)
1	B/6	BALB/c.H-2ᵇ	ND	43	57‡	36	51	11
2	B/6.Ka.Thy-1.1	BALB/c.H-2ᵇ	44	66	0	32	ND	ND
3	B/6.Ka.Thy-1.1	BALB/c.H-2ᵇ	51	58	2.3	41	51	25

* mAbs are shown in parentheses.
‡ In this experiment, both donor and host were Thy-1.2.

cell transfer. The characteristics of this inflammatory exudate are summarized in Fig. 4. The data are represented as contour plot of fluorescence intensity (ordinate) versus forward light scatter (abscissa) and contour lines are drawn around areas of equal cell numbers. The majority of the T cells were of the donor Thy-1.1 phenotype, being ~30 times greater in number than those expressing the host Thy-1.2 marker (Fig. 4; Table V, Exps. 2 and 3). Furthermore, the concordance of the percent of cells staining for the donor Thy-1 and Pgp-1 alleles, taken together with the number of CSF cells that are Lyt-2⁺, indicates that all the donor T cells are probably Lyt-2⁺,Pgp-1⁺ (Fig. 4, Table V, Exps. 1 and 3), though the intensity of staining for Pgp-1 is approximately fourfold less than that found for the larger monocytes/macrophages (Fig. 4). Even so, the possibility that Pgp-1 is an activation marker for T cells was first raised by these experiments (Figs. 2 and 3), and has since been confirmed using a number of other protocols (24; Lynch, F., and Rh. Ceredig, manuscript in preparation).

The L3T4 marker was not present on the smaller cells that stained for the donor Thy-1.1 and Pgp-1.2 determinants (Fig. 4). However, L3T4 was expressed weakly on larger cells, which are presumably monocytes/macrophages and are also positive for the host Pgp-1.1 antigen (Fig. 3; Table V, Exps. 2 and 3). There is some precedent for this, as L3T4 has been shown to be expressed and synthesized by macrophages in both rat and man (25, 26).

Consequences of T Cell Transfer into Unsuppressed Mice. The almost total absence of Thy-1⁺ cells of host origin from the CSF of the Cy-suppressed recipients (Fig. 3, Table V) was surprising, especially in view of the low frequency of LCMV-specific CTLp in the inflammatory exudate of untreated mice inoculated with LCMV (Table III). Perhaps there is a greater proportion of LCMV-specific T cells in the Cy-suppressed mice, or the number of host T cells available for recruitment is greatly diminished as a consequence of the drug treatment. The FMF analysis was thus repeated using recipients that had not received Cy. They were injected intracerebrally with virus 48 h before the transfer of immune spleen cells, and CSF samples were taken 72 h later. Under these conditions the number of CSF cells is ~10 times greater than that in the mice that were not given immune spleen cells, augmentation being totally dependent on the participation of Lyt-2⁺ class I MHC–restricted virus-immune effectors (Doherty, P. C., J. E. Allan, and Rh. Ceredig, submitted for publication).

The CSF of BALB/c.H-2ᵇ (Thy-1.2) recipients was analyzed at 72 h after transfer of B6.Ka Thy-1.1 immune spleen cells. In this case, only 17% of small
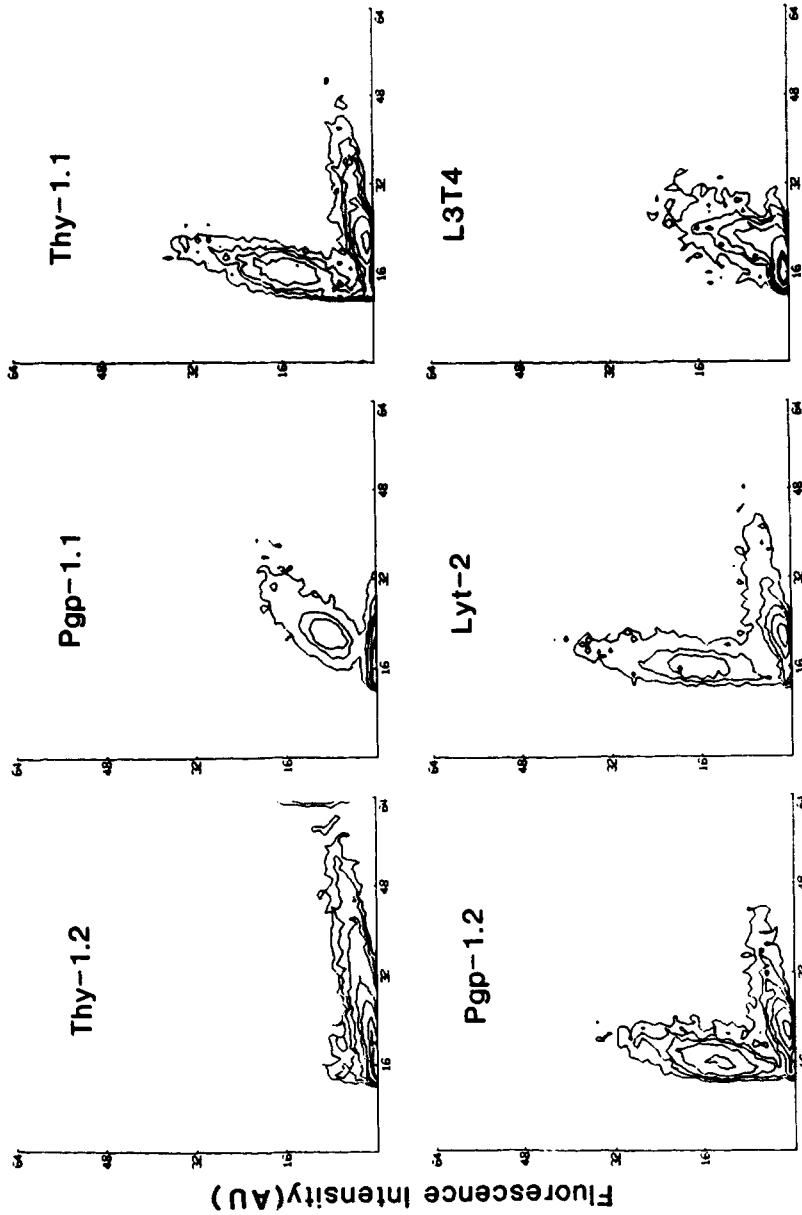
FIGURE 4. Fluorescence and forward light scatter correlative analysis of freshly isolated CSF from Cy-suppressed, virus-infected BALB/c.H-2^b (Thy 1.2$^+$, Pgp 1.1$^+$) mice that were injected intravenously 4 d previously with LCMV-immune B6 Ka.Thy1.1 (Thy 1.1$^+$, Pgp 1.2$^+$)-immune spleen cells. Each panel shows contour plots using the DISP2D program of fluorescence intensity (ordinate) and FLS (abscissa) of 20,000 cells stained with the mAbs indicated. Contour lines linking areas of equal cell numbers are shown for each reagent; typically lines were drawn at 10, 20, 30, 50, 100, 200, and 300 cells.
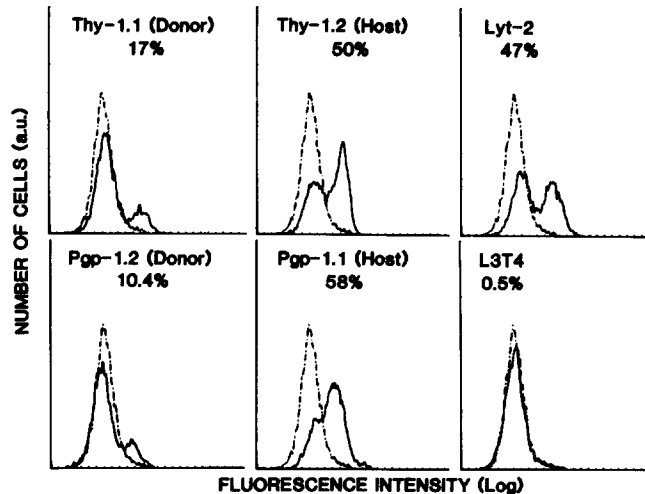
FIGURE 5. FMF analysis of CSF exudate from nonsuppressed, virus-infected BALB/c-H-2^b (Thy-1.2, Pgp-1.1) mice injected intravenously 3 d previously with LCMV-immune B6 Ka.Thy-1.1 (Thy-1.1, Pgp-1.2)-immune spleen cells. List mode data were analyzed using the DISP2D program and the fluorescence histogram of only small cells (gated on low FLS and 90° scatter) is shown in this figure. Each panel shows the fluorescence histograms on a logarithmic scale of cells stained with the FITC-labeled second-step reagent (*dotted lines*) or the indicated mAbs.

cells in the inflammatory exudate were of donor (Thy-1.1) origin, while 50% of CSF lymphocytes were of host Thy-1.2 phenotype (Fig. 5). FMF analysis also showed that most lymphocytes were Lyt-2$^+$ (47%), with only 0.5% expressing L3T4. As found with Thy-1, staining with anti-Pgp-1 mAbs showed that most (58%) lymphocytes were of host (Pgp-1.1) origin with only 10.4% donor (Pgp-1.2) cells. The reversal of the situation found previously for Cy-suppressed recipients (Fig. 3, Table V) was also found 48 h after transfer. At this time, FMF analysis of small cells gave 1% Thy-1.1$^+$, 0.5% Pgp-1.2$^+$ (both donor phenotype), 13.5% Thy-1.2$^+$, 13.3% Pgp-1.1$^+$ (host phenotype), 13.3% Lyt-2$^+$ and 0% L3T4$^+$ cells in the CSF exudate. Again, the T cell component of the inflammatory exudate is almost totally composed of Lyt-2$^+$ lymphocytes (Fig. 5). The dominance of donor T cells in the Cy-suppression model may thus reflect the immunosuppressive effects of the drug treatment (Fig. 4, Table V).

Discussion

The picture that emerges of cellular invasion in LCM is that the severe inflammatory process in CSF is triggered by relatively few LCMV-specific CTL, with the majority of T cells and monocytes/macrophages being recruited secondarily as a consequence of the initial MHC-restricted interaction between immune T lymphocytes and radiation-resistant APCs in the blood-brain barrier (3–6). A frequency of 1:3,000 LCMV-specific CTLp in an inflammatory exudate that consists of 1.5 × 10^5 cells would mean that only 45 CTLp are present in the cisterna magna. Of course, such an estimate is subject to two obvious constraints. The first is that many of the CTL present in CSF (11, 12) may not be capable of further proliferation under the culture conditions used. The second is that the T cells responsible for induction of the disease process may not be free in the CSF but intimately associated with virus-infected endothelium (5, 6) or other tissue elements. It should be noted, however, that LCM is a choriomeningitis, and there is little evidence of encephalitis in this acute disease process.

These findings are in accord with the results of Baenziger et al. (7), who demonstrated quite clearly that the direct inoculation of relatively few cloned,

virus-specific CTL into LCMV-infected brain can produce fatal LCM in lethally irradiated recipients. The inflammatory changes in mice with symptoms as a consequence of such manipulations were minimal (7). It thus seems reasonable to assume that the great majority of the extravasated cells in LCM are not required for the induction of neurological disease. Other, earlier experiments did not show any need for cortisone-sensitive cells (27) and treatment with, for instance, inhibitors of potential oxidative pathology mechanisms mediated by macrophages failed to modify the progress of LCM (28). The physiologic mechanisms underlying the development of symptoms in this T cell–mediated disease process are still not understood.

Perhaps the most intriguing finding from this analysis is that the T lymphocyte component of the inflammatory process is dominated almost entirely by the Lyt-2^+ T cell subset. This would not be surprising if the majority of the T cells were LCMV-specific, since the disease is initiated by Lyt-2^+ class I MHC–restricted effectors (3–6), but it is obvious that most of the T lymphocytes present are passively recruited bystanders. Circulating L3T4$^+$ T cells should be able to enter the CNS since there are resident class II MHC–positive macrophages located in the choroid plexus and meninges of normal mice (29) and, during LCM, the inflammatory exudate contains many monocytes and macrophages expressing class II MHC glycoproteins (30). Both populations could potentially target L3T4$^+$ cells (31) into the site of pathology, and induce further lymphocyte proliferation within the CNS.

One possible explanation for the observations is that the L3T4$^+$ cells become infected with LCMV and are eliminated by the Lyt-2^+ effectors in the site of pathology. However, from the limited analysis made to date, this does not seem to occur in blood or lymphoid tissue. An alternative explanation is that the CSF contains brain cell–derived factors that cause selective retention, or recruitment, of Lyt-2^+ T cells. Yet another possibility is that there is a lymphokine that selectively recruits and/or retains Lyt-2^+ cells. Immunohistochemical analysis of the distribution of T cell subsets in the meninges and choroid plexus would help to examine these points. However there would appear to be no absolute barrier to the invasion of L3T4$^+$ lymphocytes into the CNS; L3T4$^+$ T cells have been found in the cisterna magna of mice injected intracerebrally with vaccinia virus, and in association with the rejection of tumor cells growing in CSF (10, 32). Also, L3T4$^+$ T cells dominate the inflammatory process in the autoimmune disease, experimental allergic encephalomyelitis (34).

The present experiments point to the limitations of approaches that depend solely on the determination of T cell subsets. The fact that T lymphocytes of a particular subtype dominate an inflammatory process does not allow any conclusions concerning their functional importance since many can be passively recruited bystanders. Why this nonspecific component consists largely of Lyt-2^+ cells in LCM has yet to be elucidated.

Summary

The massive inflammation of the cerebrospinal fluid (CSF) which occurs in adult mice injected with lymphocytic choriomeningitis virus (LCMV) has been analyzed by flow microfluorometry (FMF). The great majority of the T cells

detected by direct examination of freshly obtained CSF were found to be Lyt-2^+, with an almost total absence of L3T4$^+$ lymphocytes. The Lyt-2/L3T4 ratio of lymphocytes in blood was within normal limits. Predominance of the Lyt-2$^+$ subset was confirmed by culturing the CSF cells after mitogenic stimulation. In addition, the T lymphocytes in CSF of cyclophosphamide-suppressed, virus-infected recipients that had been injected 4 d previously with LCMV-immune spleen cells were almost entirely donor Lyt-2$^+$ cells, while the nonlymphoid elements were exclusively of host origin. However this pattern of donor and host T cell distribution was reversed when the LCMV-infected recipients were not immunosuppressed.

The frequency of LCMV-specific CTL precursors in CSF taken immediately before the development of symptoms was as low as 1:3,000 cells. Thus most of the T lymphocytes extravasating into the CSF of mice with LCM are passive participants recruited as a consequence of the function of relatively few LCMV-specific effector T cells. The dominance of the Lyt-2$^+$ T cell subset in the CSF of mice with LCM is intriguing.

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