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Production of Random Classes of Immunoglobulins in Brain Tissue during Persistent Viral Infection Paralleled by Secretion of Interleukin-6 (IL-6) but Not IL-4, IL-5, and Gamma Interferon

DEMETRIUS MOSKOPHIDIS,[1] KARL FREI,[2] JÜRGEN LÖHLER,[3] ADRIANO FONTANA,[2] AND ROLF M. ZINKERNAGEL[1]*

Departments of Pathology[1] and Internal Medicine,[2] Section of Clinical Immunology, University Hospital, 8091 Zürich, Switzerland, and the Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Universität Hamburg, 2000 Hamburg, Federal Republic of Germany[3]

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The activities of cytokines were determined in cerebrospinal fluid (CSF) and serum of mice persistently or intracerebrally acutely infected with lymphocytic choriomeningitis (LCM) virus (LCMV). In contrast to CBA/J (LCMV carrier) mice that responded with low levels of LCMV-specific antibody, high-responder NMRI (carrier) mice showed antibody production by B cells outside of lymphoid organs. The B cells that had infiltrated the brains of LCMV carrier mice exhibited no preferential immunoglobulin isotype or subtype virus-specific antibody production. Phenotypic analysis of the brain infiltrates in virus carrier mice revealed dominance of $CD4^+$ T cells in contrast to virtual absence of $CD4^+$ and dominance of $CD8^+$ in mice with acute LCM. In NMRI but not in CBA/J carrier mice, significant concentrations of interleukin-6 (IL-6) were detected in CSF and serum; IL-2, IL-4, IL-5, granulocyte-macrophage CSF (GM-CSF), and gamma interferon (IFN-γ) were not elevated. In contrast, during acute, lethal LCM, IL-6 and IFN-γ were found at high concentrations, and IL-4, IL-5, and GM-CSF were detectable in CSF and serum, but virus-specific antibody-producing cells were not (yet) detectable in the brain. Thus, distinct cytokine patterns are found in acute versus chronic LCMV infection of the brain: in LCM carrier mice, local random-class immunoglobulin production correlated with the absence of IL-2, IL-4, IL-5, and IFN-γ but active secretion of IL-6.

Some persistent viral infections induce chronic antibody production and the development of chronic immune complex disease accompanied by characteristic infiltrates of T cells and B cells in nonlymphoid organs and tissues such as the brain (1, 25). Fetal or neonatal persistent infection of the mouse with lymphocytic choriomeningitis (LCM) virus (LCMV) results in LCMV-specific immunologic tolerance of the T-cell compartment (16, 20). However, because of so-called split tolerance between T (areactive) and B (reactive) cells, antibodies against the virus and immune complex disease are often produced in aging LCMV carrier mice (2, 21). This antibody production is accompanied by the formation of extensive diffuse or more follicular infiltrates containing many specific antibody-producing plasma cells (AFC) in various organs such as the brain and kidney and fewer such cells in the liver (18). In the present study, we have characterized the antibody response in the brain of LCMV carrier mice and have evaluated which cytokines may regulate this ectopic antibody production in the central nervous system. In recent in vitro studies (reviewed in references 13, 19, and 22), interleukin-4 (IL-4) has been found to induce secretion of immunoglobulin G1 (IgG1) and IgE, IL-5 enhances the secretion of IgA, and gamma interferon (IFN-γ) augments the production of IgG2a and inhibits the IgG1-inducing effects of IL-4. IL-6 augments the antibody response by exerting its effects on all isotypes of IgM, IgG, and IgA. Our results reveal no Ig class or subclass preference and show production of IL-6 but not of IL-4, IL-5, and IFN-γ.

* Corresponding author.

MATERIALS AND METHODS

Mice. CBA/J, DBA/1, C57BL/10SnJ, C57BR/cdJ, B10.BR/SgSnJ, and AKR/J mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) or HARLAN-OLAC, Bicester, United Kingdom. NMRI mice were maintained locally.

Virus. The WE strain of LCMV (23) used was plaque purified three times. It was propagated and titrated as PFU in L929 cell monolayer cultures (15).

Carrier mice. Neonatal LCMV carrier mice were produced by intraperitoneal injection of 10^6 PFU of WE strain into mice less than 24 h old.

Enumeration of cells forming anti-LCMV antibodies. The procedure for enumeration of cells forming antibodies against LCMV in the spleens or in the plasma cell-rich infiltrates of kidneys and brains of LCMV carrier mice has been described in detail previously (18). Briefly, dispersed and counted spleen cells or leukocytes isolated from kidneys or brains were serially diluted and seeded onto LCMV-coated plates. After incubation for 5 h at 37°C, the cells were removed by rinsing the wells with phosphate-buffered saline containing 0.5% Tween 20. Optimally diluted rabbit anti-mouse IgM, IgA, IgG, or IgG isotype (IgG1, IgG2a, IgG2b, or IgG3) antiserum was added, and the mixture was incubated for 2 h at 37°C. The wells were again rinsed, and an antibody-alkaline phosphatase conjugate was added. The plates were left at room temperature overnight, and after further washing, 1 ml of 5-bromo-4-chloro-indolylphosphate-containing agarose was added to each well.

Blue spots corresponding to AFC were counted after incubation for 1 h at 37°C.

Assays for determination of IL-2, IL-4 (B-cell stimulatory factor 1 [BSF-1]), IL-5, IL-6 (BSF-2), IFN-γ, and granulocyte-

macrophage CSF (GM-CSF). LCMV carrier, intracerebrally (i.c.) infected, or control uninfected mice were ether anesthetized and exsanguinated; the serum was obtained after centrifugation of blood in Microtainer serum separator tubes (Becton Dickinson & Co., Rutherford, N.Y.). Cerebrospinal fluid (CSF) was collected from mice as described elsewhere (17), but the mice were first perfused intracardially with a solution of 0.9% saline containing 25,000 U of heparin per liter. Samples containing erythrocytes were discarded. Samples from 5 to 10 animals were pooled, centrifuged, and used for determination of cytokine activities.

IL-2 was measured with the CTLL-2 line (11). Samples were serially diluted in 96-well microtiter F-plates (Falcon, Becton Dickinson, Oxnard, Calif.), and 5×10^3 CTLL-2 cells per well were added and cultured for 48 h. For the final 6 h of incubation, 1 μCi of [^{3}H]thymidine ([^{3}H]TdR) (5 Ci/mmol) was added, and incorporation of TdR was measured in a liquid scintillation β-counter. The detection limit was 0.1 U of murine recombinant IL-2 (rIL-2) per ml (Genzyme, Boston, Mass.).

IL-4 was measured with CT.4S cells (12) (kindly provided by W. E. Paul, National Institutes of Health, Bethesda, Md.). Adherent CT.4S cells were obtained by vigorous rinsing, washed twice, and placed in 96-well microtiter F-plates at 5×10^3 cells per well in a final volume of 0.1 ml. Cells were incubated at 37°C in a humidified 7% CO_2 atmosphere. Serial dilutions of CSF or murine rIL-4 (Genzyme) were added at the initiation of the culture, [^{3}H]TdR (1 μCi per well; 5 Ci/mmol) was added at 48 h, and cells were harvested 16 h later. The activity of IL-4 was calculated on the basis of cellular growth obtained with a standard preparation of rIL-4. The detection limit of rIL-4 was found to be 0.32 U/ml (~20 pg/ml). Levels of IL-4 in serum could not be determined because of unspecific toxicity on CT.4S cells.

IL-5 was determined on B13 cells (24) (kindly provided by R. Palacios, Basel, Switzerland). Serial dilutions of samples were incubated with B13 cells (10^4 per well) in 0.1 ml of IMDM medium (10) containing 5% fetal calf serum. After 56 h, the cells were pulsed with 1 μCi of [^{3}H]TdR per well for the final 16 h of culture. The sensitivity of the assay was 0.1 U/ml with a murine rIL-5 standard (Genzyme).

IL-6 was titrated on 7TD1 cells as described elsewhere (9). Serial dilutions of test samples were incubated for 96 h with 7TD1 cells (500 per well). For the final 16 h of the 4-day culture, the cells were pulsed with 1 μCi of [^{3}H]TdR. When human rIL-6 (Boehringer Mannheim, Rotkreuz, Switzerland) was used as standard, the detection limit of IL-6 was found to be 0.1 U/ml.

IL-3 and GM-CSF were determined by using GM-CSF- and IL-3-dependent FDC-P1 cells (6). Duplicate serial dilutions of samples were incubated with FDC-P1 cells (2.5×10^3 per well). After incubation for 3 days, FDC-P1 cultures were pulsed with 1 μCi of [^{3}H]TdR per well for 6 h, harvested onto filter paper strips, and counted. Activities were given in units per milliliter, with 1 U being defined as the concentration resulting in the half-maximal response.

The activities of the various cytokines were defined by additional experiments with neutralizing polyclonal antibodies against the respective cytokines (data not shown).

IFN-γ was measured with a commercially available enzyme-linked immunosorbent assay (Holland Biotechnology, Leiden, The Netherlands) that allows the detection of 5 U/ml of sample.

Phenotypic analysis by flow microfluorometry. For cytofluorometric analysis, single-cell suspensions from spleens, brain infiltrate cells, or CSF were initially stained with monoclonal antibodies. Cells were suspended in 100 μl of supernatant from hybridoma cell lines secreting monoclonal antibodies. The antibodies used were KT3 (rat anti-CD3-TCR$^+$ cells) (27), GK1.5 (rat anti-CD4-T helper cells) (7), 53-6.7 (rat anti-CD8-cytotoxic T cells) (14), and RA3-3A1 (rat anti-B220-B cells) (4). Control samples were suspended in 100 μl of medium. After a 30-min incubation at 4°C, cells were washed with phosphate-buffered saline (2% fetal calf serum, 0.1% sodium azide) and incubated with fluorescein-conjugated goat anti-rat Ig (Tago, Burlingame, Calif.). A total of 10,000 viable cells were analyzed on an EPICS profile analyzer. The percentage of positive cells in each subset was calculated directly from the gated histograms.

RESULTS

AFC in organs of LCMV carrier mice. When LCMV carriers of various mouse strains were assessed for their abilities to produce virus-specific antibodies, both high-responder strains (NMRI, DBA/1, C57BL/10SnJ, C57BR/cdJ, and B10BR/SgSnJ) and low-responder strains (CBA/J and AKR/J) were identified (18; Table 1; see also Table 3). Correspondingly, with increasing age of mice, anti-LCMV AFC accumulated in parenchymatous organs such as brain and kidney in high responders but not at all or only much later in low responders.

Distinct cytokine patterns in virus carriers and acutely infected mice. NMRI carrier mice were analyzed as representatives of high anti-LCMV responders, and CBA/J carriers were analyzed as low responders. We found no IL-2, IL-4, IL-5, or IFN-γ activity above the detection limits in CSF and serum of NMRI and CBA/J virus carrier mice or in uninfected control mice of various ages (Table 2). In contrast, IL-6 activity in NMRI but not in CBA/J virus carrier mice was greatly increased in CSF but only moderately increased in serum compared with levels in uninfected mice (Table 2). In parallel with patterns in carrier mice, the pattern of cytokine activity was also determined in NMRI and CBA/J mice acutely infected i.c. As reported previously with ICR+/+ mice (17), high levels of IL-6 and IFN-γ were found in CSF, with a maximum on day 5 or 6.5, respectively. In addition, IL-4, IL-5, and GM-CSF activities were detectable in CSF but were low in serum of both mouse strains investigated (Table 2). Thus, virus carrier mice and acutely infected mice exhibited distinct cytokine patterns.

Isotype profiles of virus-specific AFC in organs of carrier or acutely infected mice. To obtain additional evidence for a role for distinct cytokines, the isotype profiles of virus-specific AFC were determined in various organs of NMRI and CBA/J LCMV carrier mice or in acutely infected mice (Table 3). No distinct pattern of LCMV-specific IgM, IgA, or IgG isotypes was found in brain and kidney compared with spleen in carrier mice. In mice acutely infected i.c. or intravenously, the numbers of LCMV-specific AFC in the spleen were high, without any preference for Ig isotype; in the CSF of i.c. infected mice with acute LCM, B-cell activity was low or just detectable.

Phenotypic analysis of infiltrates in brain or CSF of mice persistently or acutely infected with LCMV. Since IgG-producing B cells require T-cell help, mononuclear cellular infiltrates of carrier mice and of acutely i.c. infected mice were analyzed with respect to T-cell subpopulations (Table 4). As reported by others (3, 8), mice suffering from acute LCM exhibited 20% CD8$^+$ but virtually no CD4$^+$ T cells (<3%). In contrast, analysis of NMRI carrier mice revealed about 50% CD4$^+$ T cells in brain infiltrates and only about

TABLE 1. Numbers of cells producing IgM or IgG anti-LCMV antibodies in organs of mice[a] persistently infected with LCMV

Mouse strain (*H-2* type)	Organ	No. of AFC[b]					
		IgM			IgG		
		2 mo	8 mo	12 mo	2 mo	8 mo	12 mo
DBA/1 (*H-2*^{q})	Brain	~17	<[c]	<	~34	170 ± 10	294 ± 26
	Kidney	~25	~10	~80	~155	104 ± 16	180 ± 60
	Spleen	~30	~20	~75	335 ± 285	175 ± 105	210 ± 90
C57BL/10SnJ (*H-2*^{b})	Brain	<	~21	~49	<	232 ± 152	544 ± 64
	Kidney	<	<	~46	<	250 ± 150	438 ± 99
	Spleen	153 ± 108	215 ± 25	91 ± 34	170 ± 95	410 ± 70	321 ± 69
C57BR/cdJ (*H-2*^{k})	Brain	34 ± 12	~35	~68	183 ± 119	295 ± 95	500 ± 34
	Kidney	~15	~10	102 ± 34	~267	145 ± 95	350 ± 50
	Spleen	521 ± 227	360 ± 40	64 ± 38	696 ± 312	960 ± 0	488 ± 38
B10BR/SgSnJ (*H-2*^{k})	Brain	~4	30 ± 10	<	~132	315 ± 65	300 ± 100
	Kidney	~7	~12	~68	~28	192 ± 133	204 ± 68
	Spleen	~95	40 ± 0	90 ± 50	174 ± 79	130 ± 10	270 ± 150
AKR/J (*H-2*^{k})	Brain	<	<	<	~4	<	<
	Kidney	<	~12	<	<	19 ± 7	~17
	Spleen	~37	30 ± 10	<	47 ± 37	55 ± 15	55 ± 35

[a] Neonatal carrier mice established by infecting them within 24 h after birth.
[b] Per 10^6 trypan blue-excluding leukocytes (mean ± standard error of the mean in three to five mice).
[c] Below the detection level of 1 AFC per 10^6 mononuclear cells.

10% $CD8^+$ cells. Comparable results were obtained with infiltrates of kidneys (data not shown). CBA/J carrier mice of all ages had too few lymphocytes in the brain to permit analysis by fluorescence-activated cell sorting. This absence of lymphocytes was confirmed by histological analysis.

DISCUSSION

The results presented here illustrate the following points. (i) LCMV carrier mice can be grouped into those with many accumulations of lymphoid cells and specific B cells in parenchymatous organs (kidney and brain) and with great numbers of specific AFC and those with little infiltration and few specific B cells. (ii) Distinct cytokine patterns are found in acute versus chronic LCMV infection of the brain. (iii) No distinct pattern of LCMV-specific Ig isotype or subtype production by B cells that had infiltrated the kidney and brain was found. (iv) Phenotypic analysis of the kidney (not shown) or brain infiltrates in virus carrier mice revealed, besides plasma cells, dominance of $CD4^+$ T cells in contrast to virtual absence of $CD4^+$ but dominance of $CD8^+$ in mice with acute LCM.

Analysis of the capacity of cytokines to regulate cellular activities in vivo has been difficult because the biological

TABLE 2. IL-6 in the CSF and serum of mice persistently infected with LCMV

Mouse strain[a]	Age (mo)	Activity (U/ml)										
		IL-2		IL-4 in CSF	IL-5		IL-6		IFN-γ		GM-CSF	
		CSF	Serum		CSF	Serum	CSF	Serum	CSF	Serum	CSF	Serum
NMRI												
Virus carrier	6–8	<5[b]	<2[c]	<8	<2	<0.2	10/30[d]	16 ± 9	<25	<5	<5	0.9
	12	<5	<2	<8	<2	<0.2	260/160	51 ± 44	<25	<5	<5	1.3
	18	<5	<2	ND[e]	<1	<0.2	136	40 ± 20	<25	<5	<5	ND
Uninfected	12	<5	<2	ND	<3	<0.2	<5	10 ± 4	<25	<5	<5	1.0
Acutely infected (i.c.)[f]												
Day 5.0		<5	<2	ND	ND	11.8 ± 2	14,080	220 ± 12	<50	22.4 ± 4	20	1.5
Day 6.5		ND	ND	19.5	30	3.2 ± 0	7,200	706 ± 17	1,357	13.5 ± 2	6.3	2.0
CBA/J												
Virus carrier	6	<5	<2	<8	ND	<0.2	<5/<5	7 ± 1	ND	<5	<5	0.6
	12–14	<5	<2	ND	<1	<0.2	<5	9 ± 3	<25	<5	<5	ND
Uninfected	6	ND	ND	ND	ND	ND	<8	10 ± 5	<25	<5	ND	ND
	12	<5	<2	ND	<2	<0.2	ND	ND	ND	ND	<5	0.8
Acutely infected (i.c.)[f]												
Day 5.0		<5	<2	ND	ND	5.6 ± 1	12,800	157 ± 12	<50	23.1 ± 2	20	<2
Day 6.5		ND	ND	17.5	23	1.5 ± 0	4,400	1,190 ± 10	598 ± 12	11.0 ± 2	9.4	0.8

[a] Neonatal carrier mice were established by infecting them within 24 h after birth.
[b] CSF from 5 to 10 animals was pooled.
[c] Activities of cytokines in sera (mean ± standard error of the mean for 5 to 10 animals).
[d] Results represent two independent experiments.
[e] ND, Not done.
[f] Normal animals were inoculated i.c. with 100 PFU of LCMV (strain WE).

TABLE 3. Isotype profiles of virus-specific AFC in organs of mice acutely or persistently infected with LCMV

Mouse strain[a]	n	Age (mo)	Organ	No. of AFC[b]						
				IgG	IgG1	IgG2a	IgG2b	IgG3	IgM	IgA
NMRI										
Virus carrier	4	6	Brain	900 ± 180	150 ± 44	338 ± 106	170 ± 39	144 ± 39	200 ± 34	144 ± 16
			Kidney	1,359 ± 72	231 ± 28	331 ± 54	238 ± 22	293 ± 22	322 ± 72	175 ± 18
			Spleen	1,775 ± 202	388 ± 76	631 ± 100	369 ± 70	250 ± 24	693 ± 120	350 ± 38
	3	12	Brain	1,745 ± 209	499 ± 42	390 ± 12	343 ± 78	334 ± 86	309 ± 109	279 ± 89
			Kidney	850 ± 25	200 ± 12	208 ± 8	175 ± 12	175 ± 12	150 ± 0	142 ± 7
			Spleen	1,284 ± 258	358 ± 71	491 ± 118	200 ± 50	200 ± 14	325 ± 38	175 ± 14
Uninfected			Spleen	<[c]	<	<	<	<	<	<
Acutely infected[d]										
i.c., day 6.5	6		CSF	40 ± 0[e]	<	~20	~10	<	<	<
			Spleen	5,300 ± 500	938 ± 188	1,188 ± 38	950 ± 50	1,112 ± 88	1,450 ± 150	300 ± 100
i.v., day 8	4		Spleen	3,250 ± 150	1,050 ± 150	1,925 ± 275	1,539 ± 312	1,490 ± 88	1,150 ± 250	375 ± 82
CBA/J										
Virus carrier	4	12	Spleen	68 ± 21	12 ± 3	22 ± 6	14 ± 4	10 ± 2	30 ± 8	20 ± 4
Uninfected	3		Spleen	<	<	<	<	<	<	<
Acutely infected[d]										
i.c., day 6.5	6		CSF	40 ± 0	<	<	<	<	<	<
			Spleen	6,450 ± 450	1,170 ± 19	1,720 ± 182	1,475 ± 144	1,644 ± 106	2,140 ± 312	100 ± 25
i.v., day 8	4		Spleen	4,025 ± 725	1,128 ± 154	1,412 ± 212	1,2872 ± 306	1,182 ± 270	1,125 ± 425	300 ± 100

[a] Neonatal carrier mice established by infecting them within 24 h after birth.
[b] Per 10^6 trypan blue-excluding leukocytes (mean ± standard error of the mean).
[c] Below the detection level of 1 AFC per 10^6 mononuclear cells.
[d] Animals inoculated with 100 PFU of LCMV (strain WE). i.v., intravenously.
[e] CSF values from pools of three mice.

effects of cytokines are usually pleiotropic even in vitro (19). In general, it is possible to divide the cytokines into three categories: (i) a factor mainly involved in the activation of resting B cells (BSF-1/IL-4), (ii) a factor for the growth and maturation of activated B cells (B-cell growth factor (IL-5), and (iii) a factor for terminal differentiation of B cells into antibody-secreting cells (BSF-2/IL-6). Other cytokines (IL-1, IL-2, IL-3, and IFN-γ) originally thought to influence cells other than B cells are now known to modulate B-cell function. Some of these cytokines are important in the regulation of Ig class switch (reviewed in references 19 and 22). The presence of both virus-specific AFC and IL-6 in the CSF of those virus carrier mice suffering from immune complex disease supports the notion that this cytokine is involved in the regulation of local antibody production. In contrast to high-responder mice, the low-responder strain showed minimal production of antiviral antibodies, and IL-6 was not detected in CSF.

IL-6 induces the final maturation of B cells into Ig-secreting cells and augments IgM, IgG, and IgA production without obvious selection (19); this is not compatible with IFN-γ, IL-4, or IL-5 being detectable. However, the phenotypes of mononuclear cells in brain infiltrates of carrier mice (i.e., about 50% $CD4^+$) versus those of acutely infected mice (with less than 3% of $CD4^+$ T cells) contrasts with the absence of detectable IL-2, IL-4, IL-5, and IFN-γ in the CSF of carrier mice, since T helper cells are thought to produce these cytokines. This may be explained by postulating that

TABLE 4. Phenotypic analysis of cells from brain infiltrates and spleens of LCMV carrier or acutely infected mice[a]

Mouse strain	Source	% of positive cells				No. of MNC[b]
		T cells (CD3)	CD4	CD8	B cells (B220)	
NMRI						
Virus carrier (12 mo old)	Brain	59.8 ± 3.6	47.1 ± 0.2	9.8 ± 1.0	12.1 ± 1.8	(4.0 ± 0.7) × 10^5
	Spleen	31.7 ± 4.4	22.9 ± 2.9	9.6 ± 0.9	17.9 ± 1.0	(19.9 ± 2.2) × 10^7
Uninfected	Spleen	23.6 ± 0.9	16.8 ± 0.2	8.9 ± 1.2	32.5 ± 3.6	(20.2 ± 2.1) × 10^7
Infected i.c., day 6.5	CSF	21.5	2.8	20.2	5.6	(25.8 ± 2.3) × 10^6
	Spleen	22.3 ± 2.4	12.2 ± 0.6	14.9 ± 0.3	28.5 ± 1.7	(16.9 ± 4.8) × 10^7
CBA/J						
Virus carrier (12 mo old)	Spleen	24.0 ± 0.9	15.4 ± 1.2	9.6 ± 0.6	25.2 ± 3.9	(10.6 ± 1.4) × 10^7
Uninfected	Spleen	32.5 ± 2.3	18.1 ± 1.2	13.2 ± 2.0	21.8 ± 2.3	(9.4 ± 1.4) × 10^7
	CSF	26.9	3.2	24.4	6.6	(15.6 ± 2.9) × 10^6
Infected i.c., day 6.5	Spleen	32.8 ± 0.7	16.6 ± 1.0	15.5 ± 1.0	35.2 ± 0.9	(11.9 ± 2.5) × 10^7

[a] For details, see Table 2 and Materials and Methods. Values for spleen and brain represent the mean ± standard error of the mean of three individual determinations from separate mice. CSF values are from pools of three mice. The mean number of mononuclear cells obtained from CSF of uninfected mice was $<5 \times 10^4$/ml.
[b] Per organ or per milliliter of CSF. MNC, Mononuclear cells.

(i) cytokines are produced only early after induction of $CD4^+$ but not after long-term expansion in the periphery caused by persistent antigen (in late phases of T- and B-cell cooperation, IL-6 apparently suffices to have all major Ig classes produced locally and probably also systemically), (ii) lack of anti-LCMV $CD8^+$ T cells in carrier mice may influence overall levels of cytokines indirectly, and (iii) produced cytokines, e.g., IL-2 or IFN-γ, may be bound locally by receptors and therefore may not be measurable in CSF.

Preliminary histological evaluations of brain sections of LCMV carrier mice (17a) disclosed inflammatory mononuclear infiltrates in the subarachnoid spaces of strain NMRI but not strain CBA/J mice. These infiltrates were preferentially localized in the subarachnoid cisterns and perivascular Virchow-Robin spaces. Immunocytochemical analysis revealed that the infiltrates predominantly contained plasma and preplasma cells, $CD4^+$ cells, and some macrophages and $CD8^+$ T cells. While $CD4^+$ T cells were concentrated in the infiltrates, some $CD8^+$ T cells were found scattered throughout the brain parenchyma proper. Furthermore, many of the mononuclear cells in the infiltrates expressed transferrin receptor and IL-2 receptor, indicating their functionally activated state. The apparent close association of $CD4^+$ cells with plasma cell accumulations together with the expression of transferrin and IL-2 receptors in the infiltrates, as disclosed by immunocytochemistry, suggest a possible specific role for $CD4^+$ T cells.

Acute LCM is characterized by (i) massive infiltration of lymphocytes and monocytes into the meninges and choroid plexus, (ii) induction of the disease by $CD8^+$ T cells, and (iii) the presence of IL-6 and IFN-γ in CSF and serum (8, 9). Production of IL-6 was found in T-cell-dependent and apparently independent pathways involving meningeal macrophages, microglial cells, and astrocytes (10). The virtual absence of virus-specific AFC in the CNS 6 to 7 days after i.c. infection argues against an early and direct involvement of these cytokines in B-cell immune response in acute infection and is compatible with evidence implicating cytotoxic T cells in the pathogenesis of acute disease (3, 8). Unfortunately, because LCM is fatal by 6 to 8 days after i.c. infection, local virus-specific AFC cannot be analyzed at later times.

It is noteworthy that our results on virus-specific antibodies contrast somewhat with previously published results (5, 26), in which a preference for IgG2a or IgG1 in the sera of acutely infected mice or in C3H/HeJ LCMV carrier mice was documented. Experimental differences (determination of AFC versus serum antibody titers [5, 26]), mouse strain differences, and variations in the half-lives of Ig subclasses of anti-LCMV antibodies in the circulation and those forming immune complexes in carrier mice may explain this discrepancy.

In conclusion, the experiments presented here document the unique possibilities the central nervous system and CSF offer for analyzing immunologic mechanisms close to the site of action in persistent or acute virus infections. For the evaluation of cytokines, the low enzymatic activity in CSF is obviously a great advantage, particularly since the half-lives of cytokines in serum seem to be rather short. The evidence in LCMV carrier mice shows not only specific local antibody production against the etiologic agent, but also a correlation between local antibody production, the random classes of antibodies, and IL-6 activity levels in the CSF.

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