ANTIVIRAL ANTIBODY–PRODUCING CELLS IN PARENCHYMATOUS ORGANS DURING PERSISTENT VIRUS INFECTION

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In a variety of experimental and natural illnesses of animals and man involving the central nervous system (CNS)[1] immunoglobulin (Ig) is produced intrathecally (1–4). Intracerebral Ig production has been seen in acute and subacute infectious diseases (5–9) but is more often observed when the courses are chronic. In multiple sclerosis, the infectious nature of which is debated but not established (10), antibodies with sundry specificities have been detected (11–13), while during illnesses with known etiologies antibodies against the causative agents are predominantly formed (14, 15). In certain slow virus diseases this phenomenon seems to be a regular feature, and in subacute sclerosing panencephalitis (SSPE) (16–18), progressive rubella panencephalitis (19, 20), and visna (21, 22), antibodies directed against measles, rubella, and visna viruses, respectively, have been shown to be produced intracranially.

For humoral immune responses, several cell types must cooperate; hence, antibody production in the CNS would require that these cells not only migrate there but also assemble locally. In cases of multiple sclerosis, the CNS has been reported to contain tissue resembling the Ig-secreting medullary regions of the lymph nodes (23), but whether cells of the immune system are similarly arranged in a chronic brain disease caused by a persistent virus infection seems to be unknown. Nor do we know how cells of the immune system find their way into the CNS, and no explanation can at present be given for the apparent longevity of certain B cell clones under these conditions (24, 25).

After connatal or neonatal infection of a mouse, the lymphocytic choriomeningitis virus (LCMV) persists lifelong in high concentrations in essentially all organs (26). Inability to eliminate the virus is assumed to result from LCMV-specific immunologic tolerance of the T cell compartment (27); antibodies against the virus may be produced and form complexes with virus that are thought to cause an immune complex disease (ICD) often seen in aging carrier mice (28). In the parenchymatous organs of these mice, including the CNS, there are

[1] *Abbreviations used in this paper:* AFC, antiviral antibody–forming cell; CNS, central nervous system; ICD, immune complex disease; IU, infectious unit; LCMV, lymphocytic choriomeningitis virus; PAP, peroxidase-antiperoxidase; SSPE, subacute sclerosing panencephalitis.

accumulations of mononuclear cells (29–31) that may be so extensive as to resemble lymphomas. We now report that these infiltrates contain numerous plasma cells but also lymphocytes and mononuclear phagocytes, and, by use of a recently developed procedure (32, 33), we have demonstrated in the same organs cells forming LCMV-specific Ig (antiviral antibody–forming cell; AFC). Accumulations of lymphoid tissue, and numbers of AFC in parenchymatous organs, as well as ICD were found to be quantitatively correlated among mouse strains. The LCMV carrier mouse should prove to be a useful model with which to study the ectopic production of antiviral antibodies during slow virus diseases with and without involvement of the CNS.

Materials and Methods

Mice.   CBA/J (CBA), C3H/HeJ (C3H), AKR/J, C57BR/cdJ, and B10.BR/SgSnJ (all H-2^k), C57BL/10SnJ (B10) (H-2^b), and DBA/1LacJ (H-2^q) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and SWR/Ola (SWR) and B10.G/Ola (B10.G) mice (both H-2^q) from OLAC 1976, Ltd. (Blackthorn, Bicester, United Kingdom). NMRI inbred mice, originally obtained from the Medical Research Council Laboratory Animals Centre, Carshalton (Surrey, United Kingdom) were bred in this institute by continual brother-sister mating; their haplotype has been determined (personal communication) by K. Fischer-Lindahl (Basel) as H-2^q. Gray house mice (haplotype unknown) came from our own colony originating from wild animals trapped in northern Germany.

Virus.   The WE strain LCMV (34) was used after it had been plaque purified three times; it was propagated and titrated in L cells and expressed as mouse infectious units (IU), IU being numerically identical with 50% mouse infectious dose (35).

Carrier Mice.   The neonatal carrier status was induced by inoculating intraperitoneally 10^7 IU of LCMV 24 h or less after birth (36). The carrier house mice came from a colony established 10 yr ago with organ homogenate from a persistently infected wild mouse.

Histologic and Immunohistologic Procedures.   Pieces of kidneys, livers, and brains were excised from the same organs that were subsequently processed for the enumeration of AFC. After fixation either in acid formaldehyde (37) or Bouin's solution, they were embedded in Paraplast, and sections were stained with hematoxylin-eosin, periodic acid–Schiff, or Giemsa stain. In a few instances brains were fixed by perfusion and embedded in glycol-methacrylate (Technovit; Kulzer, Friedrichsdorf, Federal Republic of Germany), and semithin sections were stained with toluidine blue.

For demonstrating Ig, the peroxidase-antiperoxidase (PAP) technique (38) was used. Sections were rehydrated and treated with 0.01% protease type VII (Sigma Chemical Co., Deisenhofen, Federal Republic of Germany) or 3 M urea (Merck, Darmstadt, Federal Republic of Germany) in 0.05 M Tris-buffered salt solution at pH 7.6. Overnight incubation with suitably diluted affinity-purified antibodies against heavy chains of either mouse IgG raised in rabbit (Jackson, Avondale, PA) or mouse IgM raised in goat (Pel-Freez, Rogers, AR) was followed by incubation with antibody directed against rabbit or goat Ig (Nordic, Tilburg, The Netherlands), respectively. The PAP complex (Dakopatts, Glostrup, Denmark) was then applied, and peroxidase was visualized by the method of Graham and Karnovsky (39) using 3,3′-diamino-benzidine-tetrahydrochloride (Fluka, Buchs, Switzerland) as cosubstrate.

Detection of Cells Forming Anti-LCMV Antibodies.   AFC were released enzymatically from parenchymatous organs and enumerated by use of a solid-phase immunoenzymatic technique. Trypsin (Difco Laboratories, Detroit, MI) was prepared according to Wallis and his colleagues (40) and diluted before use to 0.25% with Eagle's MEM (no serum). Kidneys, livers, and brains (and for control purposes spleens) from carrier mice were cut into small cubes and subsequently digested by 3 (livers, brains, and spleens) and 12 (kidneys) successive 15-min periods of gentle agitation with trypsin solution at 25°C. Released cells were concentrated by centrifugation and leukocytes were separated by Ficoll-Isopaque density centrifugation (41) and counted as living on the basis of trypan

blue exclusion. To search for AFC in the circulation, blood was collected by venipuncture and leukocytes were separated by use of Ficoll-Isopaque. Subsequently, AFC were enumerated as has been described for the spleen (33). Leukocytes were serially diluted and seeded onto the surfaces of virus-coated 2 × 2-cm wells of 25-square polystyrene dishes. After 5 h of incubation at 37°C, the cells were rinsed off with PBS containing Tween 20. Optimally diluted rabbit anti–mouse Ig (IgM + IgG + IgA) (Zymed Laboratories, San Francisco, CA), anti-IgM (Miles Scientific, München, Federal Republic of Germany), or anti-IgG (Zymed Laboratories) was added to the wells, which were incubated again for 2 h. They were rinsed and antibody–alkaline phosphatase conjugate (Tago, Burlingame, CA) was added. The plates were left at room temperature overnight and, after further rinsing, agarose containing the p-toluidine salt of 5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemical Co.) was pipetted onto the surfaces. After incubation for 1 h at 37°C, blue spots corresponding to AFC could be counted.

Detection of Cells Producing Antibodies of Undetermined Specificities. The assay was performed as described above for AFC, except that the antigen with which the wells were coated was goat anti–mouse H- and L-chain–specific Ig (IgG + IgM + IgA) (Zymed Laboratories) instead of purified LCMV.

Results

Histological and Immunohistochemical Observations in Carrier Mice. Aging mice of 7 of the 11 strains included in this study, namely NMRI, SWR, DBA/1LacJ, B10, C57BR/cdJ, B10.G, and B10.BR/SgSnJ developed a characteristic syndrome, which had previously been known as late-onset disease (29), and has more recently been identified as ICD (28). Because it has been extensively reviewed (27), no details will be presented here. Persistently infected C3H, CBA, AKR/J, and house mice remained outwardly healthy throughout the period of observation and histologically presented at most low-grade alterations.

In addition to the changes caused by deposition of antigen-antibody complexes, in carrier mice undergoing ICD essentially all organs contained mostly nodular infiltrates of mononuclear cells that varied considerably in size and structural organization. Here we confine ourselves to the organs chosen for the quantitative determination of cells producing antiviral antibodies. In the kidney the infiltrates were mostly localized in the perivascular space of the arcuate arteries and veins in the juxtamedullary zone (Fig. 1), from where they extended into the loose connective tissue of the pelvis. Areas with small lymphocytes containing darkly stained nuclei were separated from areas populated predominantly by larger cells with bright nuclei and varying degrees of cytoplasmic basophilia. Besides lymphoid cells, macrophages and histiocytes could be recognized, and mitotic figures were frequently seen. The peripheral zones were characterized by the accumulation of numerous plasma cells. In larger infiltrates, newly formed small blood vessels were seen passing through. In the liver the infiltrates' predominant localization was the perivascular space of the portal vein; in structure they were similar to the ones in the kidney but, as a rule, smaller in size.

In the CNS the infiltrates were predominantly localized in the leptomeninx, from where they extended with the Virchow-Robin spaces into the brain; occasionally they were found independent of vessels in the parenchyma itself. As in the other organs, they consisted of plasma cells, small and large lymphocytes, and mononuclear phagocytes. In shape, they varied considerably, probably as a consequence of the complex anatomical situation created by the deep, narrow, and ramified fissures separating the single parts of the brain and the subarachnoid
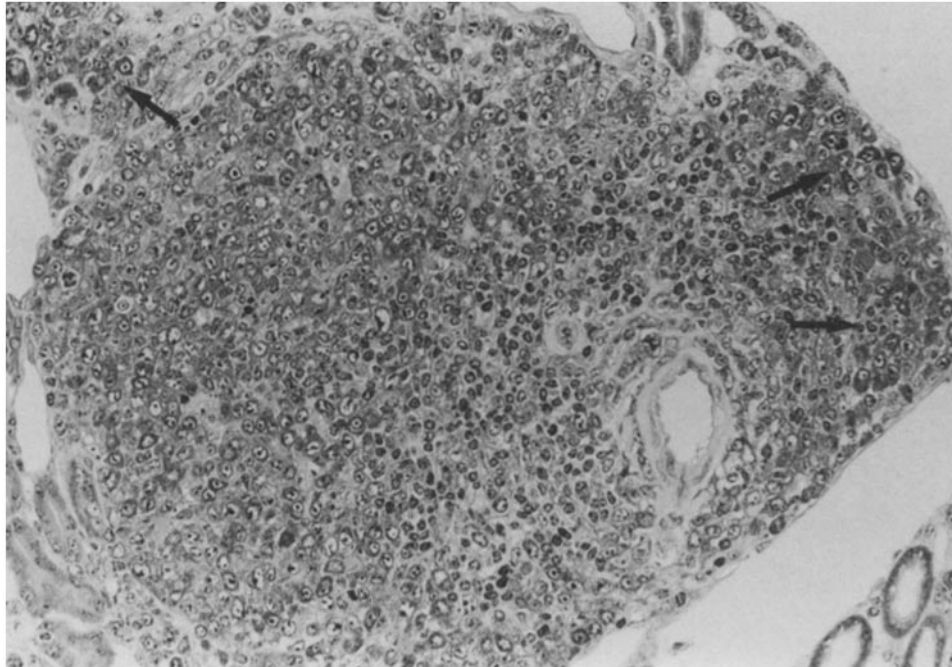
FIGURE 1. Kidney of a 12-mo-old LCMV NMRI carrier mouse with a large nodular infiltrate in the perivascular space of an arcuate artery. Around the blood vessel there are mainly small lymphocytes with dark nuclei, while elsewhere larger lymphocytes with bright nuclei of varying sizes and abundant basophilic cytoplasm predominate. The arrows point to accumulations of plasma cells in the periphery of the infiltrate. Giemsa stain. × 300.

space as it accompanies each vessel together with the leptomeninx into the depth of the tissue (Fig. 2a).

With increasing age of the carrier mice and parallel with the severity of the ICD, the lymphoid infiltrates expanded in both number and size. In mice not suffering from ICD, lymphoid infiltrates were never found.

Immunohistochemical staining for Ig revealed small numbers of plasmacytes secreting IgM (Fig. 3), although many cells in the inner parts of the infiltrates exhibited IgM surface staining. In contrast, IgG-secreting plasma cells were numerous, especially in the periphery (Figs. 2b and 4). Further findings with a large number of monoclonal antibodies revealed that all cell types believed to be needed for antibody production were present in these infiltrates (J. Löhler, D. Moskophidis, and F. Lehmann-Grube, manuscript in preparation).

Antibody-producing Cells in Organs of Carrier Mice. The demonstration of production of Ig in the focal accumulations of mononuclear cells in the organs of carrier mice led to an investigation of its specificity. Tissues were dispersed by digestion with trypsin, and the ability of separated leukocytes to form anti-LCMV antibodies was determined. Representative findings for five mouse strains are presented in Tables I and II. Relatively few leukocytes produced IgM class antibodies, although it is noteworthy that there were any. Many more leukocytes released from parenchymatous organs of carrier mice produced virus-specific IgG class antibodies, and there was a clear quantitative correlation with the
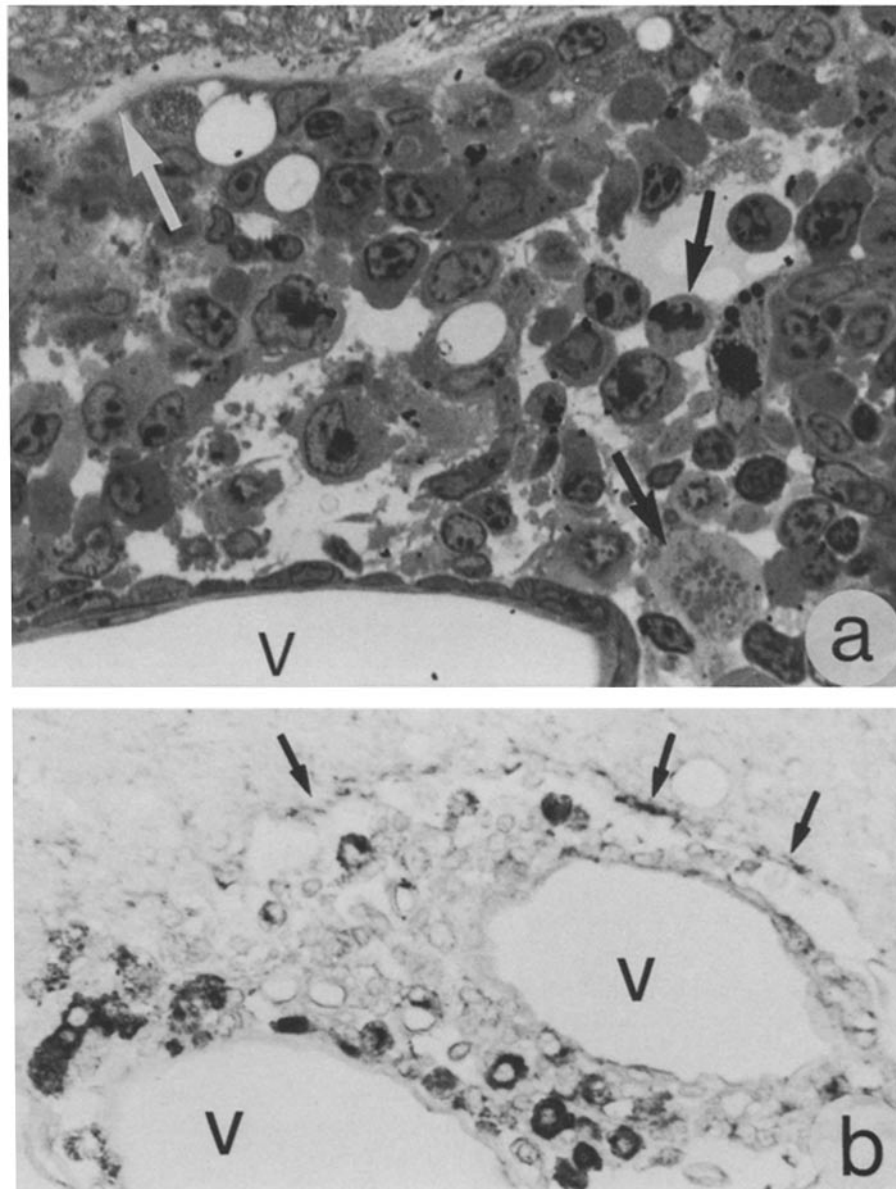
FIGURE 2. Brain in the region of the fissura hippocampi of a 14-mo-old LCMV NMRI carrier mouse. (a) The perivascular, subarachnoid space of a cerebral vein (V) is infiltrated by lymphocytes, plasma cells, immature preplasma cells, and macrophages. The black arrows point to mitoses and the white arrow marks the membrana limitans gliae superficialis of the brain tissue. Semithin section stained with toluidine blue. × 1,000. (b) IgG-producing plasma cells are localized predominantly in the periphery of the infiltrate around leptomeningeal vessels (V). IgG is also deposited in astrocytic foot processes of the membrana limitans gliae superficialis (arrows). PAP technique, no counterstaining of nuclei. × 500.
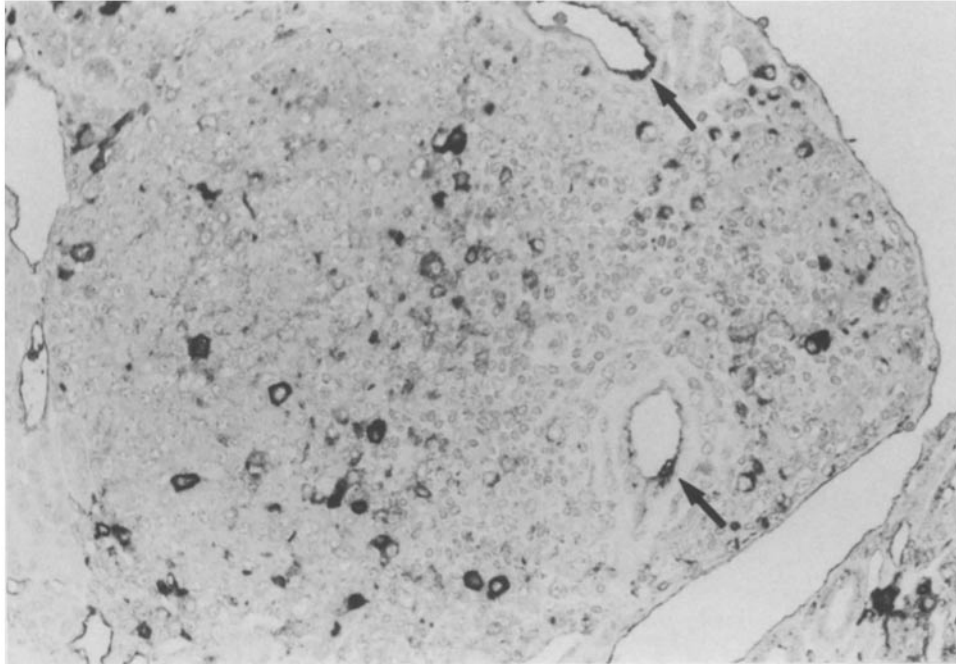
FIGURE 3. Kidney of a 12-mo-old LCMV carrier mouse stained for IgM. Sequential section of the infiltrate depicted in Figs. 1 and 4. Small numbers of IgM+ plasma cells are scattered throughout the infiltrate, but the area around the artery is conspicuously free. In the walls of blood vessels there is subendothelial deposition of IgM (arrows). PAP technique, slight counterstaining with hemalaun. × 300.

appearance of lymphoid infiltrates (which, in turn, was correlated with ICD), meaning that numbers of AFC were higher in NMRI, SWR, and B10.G mice than in C3H and CBA mice and increased with the animals' age. As for the organs, the figures vary greatly, with comparable numbers in spleen, kidney, and brain. The organs of connatal carrier house mice were essentially devoid of AFC (Table III). In all mice, the blood was free of cells producing antibodies against the virus.

The observations on further mouse strains thus investigated, together with the histologic findings, are summarized in Table IV. As a rule, carrier mice possessing the haplotype H-2^q had extensive cell infiltrates and high numbers of AFC in parenchymatous organs and developed severe ICD, while carrier mice of haplotype H-2^k had few and small infiltrates and low numbers of AFC, and exhibited minimal degrees of ICD. There are, however, noteworthy exceptions: C57BR/cdJ and B10.BR/SgSnJ mice, although of H-2^k haplotype, were high responders for all three criteria; apparently, the major histocompatibility gene complex is not alone responsible for antibody production in LCMV carrier mice.

It was, a priori, likely that not all plasma cells of the infiltrates would form antibodies against LCMV. Their proportion among the total of active elements (meaning cells producing antibodies of any specificity) was determined in persistently infected NMRI and B10.G mice (Table V). It was low in the spleen,
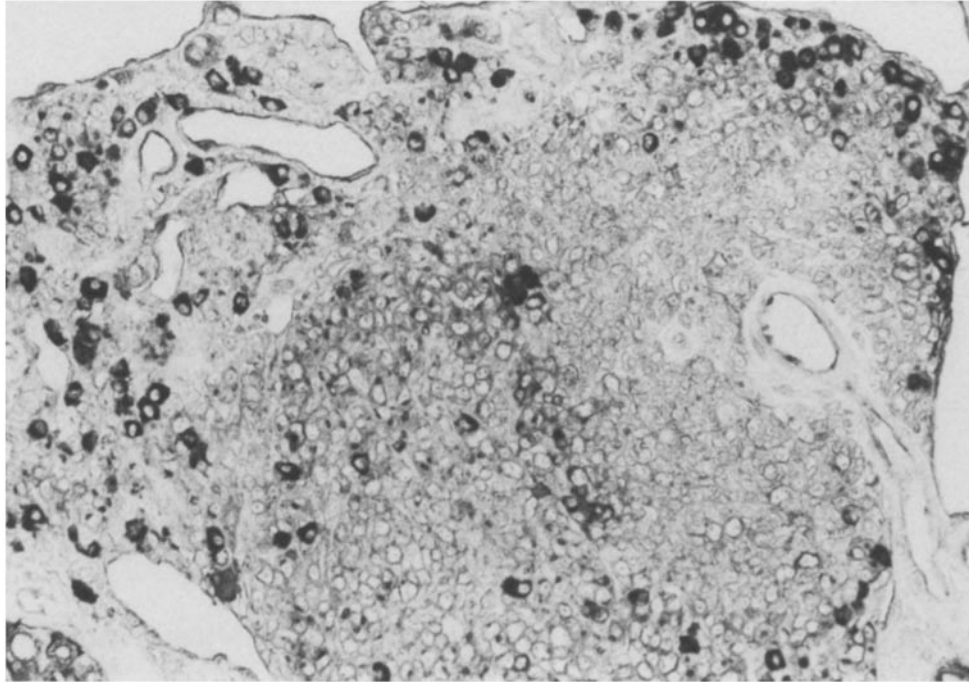
FIGURE 4. Kidney of a 12-mo-old LCMV carrier mouse stained for IgG. Sequential section of the infiltrate depicted in Figs. 1 and 3. Numerous mature IgG$^+$ plasma cells and immature preplasma cells with faint IgG-specific labeling of their cytoplasm are concentrated in the periphery and the inner parts of the infiltrate, respectively. As in the case of IgM$^+$ cells (Fig. 3), a zone around the artery is free of labeled cells. PAP technique, slight counterstaining with hemalaun. × 300.

relatively high in kidney and liver, and highest in the brain, where in individual mice, up to 90% of all Ig-forming cells formed antibodies directed against LCMV.

The data of Table V were obtained by use in the first overlay of an antiserum containing antibodies against mouse IgG, IgM, and IgA. In these experiments, cells producing LCMV-specific IgM and IgG antibodies were counted in parallel (not shown). The sums of their numbers were slightly lower than the numbers determined using the antiserum directed against three classes of mouse antibodies, indicating that few of the active cells produced IgA antibodies.

Discussion

It has long been known that, during an infectious disease or under experimental conditions, Ig may be formed outside the lymphatic tissue. In a thorough review published in 1968, Heremans (4) summarized the then-available evidence for antibody formation in a number of different tissues. Of the CNS he wrote "although proof is lacking, one may presume that the monoclonal immunoglobulins found in CSF from patients with infectious or parasitic diseases of the central nervous system represent intrathecally synthesized antibodies directed against the offending antigen." In the meantime, further details have become known, but direct proof for antibody production within the CNS is still lacking.

Table 1

Numbers of Cells Producing IgM Anti-LCMV Antibodies in Organs of Mice Persistently Infected with LCMV

Mouse strain	Organ	Age (wk)						
		7	19	26	31	36	42	58
NMRI	Brain	<*	~3	~28	~35	~52	~5	60 ± 45
	Kidney	<	~15	<	68 ± 26	~26	~45	262 ± 130
	Liver	5 ± 1‡	~5	~3	115 ± 54	55 ± 22	~45	~41
	Spleen	204 ± 132	~38	68 ± 23	285 ± 122	190 ± 48	280 ± 90	~160
	Blood	<	<	<	<	<	<	<
SWR	Brain	<	~2	<	~5	~5	<	ND
	Kidney	<	<	~5	<	<	~6	ND
	Liver	<	<	<	<	<	~6	ND
	Spleen	~34	70 ± <1	30 ± 6	~12	<	<	ND
	Blood	<	<	<	<	<	<	ND
B10.G	Brain	~20	~9	ND	17	ND	84 ± 16	ND
	Kidney	<	38 ± 12	ND	~67	ND	<	ND
	Liver	~30	20 ± 10	ND	41 ± 27	ND	180 ± 120	ND
	Spleen	215 ± 175	275 ± 85	ND	38 ± 14	ND	500 ± 320	ND
	Blood	<	<	ND	<	ND	<	ND
C3H	Brain	<	<	<	~10	~10	~5	ND
	Kidney	<	<	<	~15	<	<	ND
	Liver	<	<	<	<	~5	<	ND
	Spleen	<	33 ± 9	~3	175 ± 5	40 ± <1	180 ± 40	ND
	Blood	<	<	<	<	<	<	ND
CBA	Brain	<	<	<	<	~10	<	<
	Kidney	<	<	~10	<	<	~20	~10
	Liver	<	<	<	<	<	<	<
	Spleen	2 ± <1	<	<	24 ± 12	<	40 ± 10	<
	Blood	<	<	<	<	<	<	<

Neonatal carrier mice established by infection within 24 h after birth.

* Below detectability.

‡ Numbers of antibody-producing cells per 10^6 trypan blue–excluding leukocytes, means ±SE in three to six mice.

The question then is whether the findings presented here prove that antiviral antibodies are produced within the CNS (and within other parenchymatous organs) of LCMV carrier mice.

LCMV carrier mice are apparently devoid of LCMV-specific cytotoxic T lymphocytes, which seems to be the basis of their inability to eliminate the virus (27), but virus-specific antibodies are produced and form complexes with virus that are held responsible for a late-appearing ICD (28). Whereas the virus titers are comparable in the organs of carrier mice of different strains (42), circulating antibodies vary considerably and are correlated with circulating immune complexes (43); presumably, the quantity of the latter is a function of the quantity of the former. As it is likely that the amounts of circulating immune complexes determine the severity of the illness they cause, a correlation probably exists between circulating antibodies and ICD, an assumption borne out by findings in two mouse strains (44).

Organs of carriers of certain mouse strains often contain extensive cell infiltrates harboring many plasma cells, which have been regarded as an expression of pathologic cell-mediated immune reactions (31) or of some immunoproliferative disease process (30). Possibly these infiltrates are the basis for the sometimes-

TABLE II
Numbers of Cells Producing IgG Anti-LCMV Antibodies in Organs of Mice Persistently Infected with LCMV

Mouse strain	Organ	Age (wk)						
		7	19	26	31	36	42	58
NMRI	Brain	~9	273 ± 169	308 ± 119	564 ± 270	619 ± 109	646 ± 287	643 ± 479
	Kidney	<*	~193	~125	278 ± 93	~80	307 ± 116	1,070 ± 761
	Liver	8 ± <1‡	45 ± 9	~21	~235	99 ± 26	140 ± 49	90 ± 38
	Spleen	136 ± 108	148 ± 45	378 ± 83	655 ± 59	363 ± 104	560 ± 163	687 ± 348
	Blood	<	<	<	<	<	~4	<
SWR	Brain	55 ± 2	102 ± 86	60 ± 21	520 ± 259	555 ± 332	654 ± 210	ND
	Kidney	~60	264 ± 25	100 ± 54	~138	320 ± 110	323 ± 199	ND
	Liver	~10	103 ± 20	~20	~25	<	~143	ND
	Spleen	296 ± 80	284 ± <1	367 ± 29	~50	58 ± 17	167 ± 79	ND
	Blood	<	<	<	<	<	<	ND
B10.G	Brain	40 ± <1	819 ± 299	ND	608 ± 42	ND	1,353 ± 87	ND
	Kidney	~15	450 ± 250	ND	600 ± 1	ND	625 ± 125	ND
	Liver	40 ± 20	70 ± <1	ND	164 ± 38	ND	215 ± 65	ND
	Spleen	520 ± 180	510 ± 190	ND	438 ± 14	ND	655 ± 335	ND
	Blood	<	<	ND	<	ND	<	ND
C3H	Brain	<	<	<	<	55 ± 35	~10	ND
	Kidney	<	<	~10	35 ± 15	<	15 ± 5	ND
	Liver	<	4 ± 0.5	<	<	<	~5	ND
	Spleen	<	62 ± 6	30 ± 10	95 ± 25	25 ± 15	105 ± 15	ND
	Blood	<	<	<	<	<	<	ND
CBA	Brain	<	<	<	<	~10	<	<
	Kidney	<	<	<	~109	~5	20 ± <1	<
	Liver	<	<	<	61 ± 28	~40	10 ± <1	<
	Spleen	8 ± <1	<	<	37 ± 5	<	35 ± 25	<
	Blood	<	<	<	<	<	<	<

Neonatal carrier mice established by infection within 24 h after birth.
* Below detectability.
‡ Numbers of antibody-producing cells per 10^6 trypan blue–excluding leukocytes, means ±SE in three to six mice.

TABLE III
Numbers of Cells Producing IgM and IgG Anti-LCMV Antibodies in Organs of House Mice Persistently Infected with LCMV

Organ	Age of mice (wk)									
	IgM					IgG				
	8	26	44	64	77	8	26	44	64	77
Brain	<*	<	<	<	55 ± 40‡	<	<	~40	<	<
Kidney	<	<	<	<	~14	<	<	<	<	<
Liver	<	<	<	<	<	<	<	<	<	<
Spleen	<	<	~20	~22	<	<	<	<	<	<
Blood	<	<	<	<	<	<	<	<	<	<

Connatal carrier mice from a colony established 9 yr ago with organ homogenate from a persistently infected wild house mouse.
* Below detectability.
‡ Numbers of antibody-producing cells per 10^6 trypan blue–excluding leukocytes, means ±SE in two mice.

TABLE IV

Extent of Infiltrates, Quantity of Cells Producing Antibodies Against LCMV (AFC) and Degree of ICD in Parenchymatous Organs of LCMV Carrier Mice of Different Strains

Mouse strain	H-2	Infil-trates*	AFC[‡]	ICD[§]
CBA/J	k	+	+	+
C3H/HeJ	k	+	+	+
AKR/J	k	+	+	+
C57BR/cdJ	k	++	++	++
B10.BR/SgSnJ	k	++	++	++
NMRI	q	++	++	++
SWR/Ola	q	++	++	++
DBA/1LacJ	q	++	++	++
B10.G/Ola	q	++	++	++
C57BL/10SnJ	b	++	++	++
House mouse		−	(+)	−

* +, Small nodules in the interstice not causing displacement of parenchymatous tissue; ++, infiltrates that are so large as to displace parenchymatous tissue and to be visible (in stained sections) with the unaided eye.

‡ (+), No or very few AFC; +, low numbers of AFC with uncertain quantitative relationship with the animals' age; ++, AFC in essentially all organs, the numbers of which increase with the animals' age.

§ +, Distension of mesangial space, where lumps of homogeneous PAS-positive material accumulate, little loss of mesangial cellularity, and occasionally focal or segmental sclerosis of glomeruli; ++, fully developed severe lupus-like glomerulonephritis leading to complete hyalinization of glomeruli.

TABLE V

Ratios of Cells Producing Antibodies against LCMV to Numbers of Cells Producing Antibodies with Any Specificity in Organs of Mice Persistently Infected with LCMV

Mice	Organ	Antibody-producing cells*		
		LCMV-specific	Total	Ratio
NMRI (n = 3, 42 wk old)	Brain	603 ± 205[‡]	873 ± 166	0.69
	Kidney	459 ± 180	2,242 ± 630	0.21
	Liver	190 ± 80	820 ± 141	0.23
	Spleen	533 ± 40	14,617 ± 949	0.04
	Blood	<[§]	133 ± 17	—
B10.G (n = 2, 40 wk old)	Brain	1,467 ± 34	2,375 ± 175	0.62
	Kidney	700 ± 100	7,800 ± 1,900	0.09
	Liver	440 ± 180	1,600 ± 140	0.28
	Spleen	1,220 ± 600	37,500 ± 750	0.03
	Blood	<	135 ± 35	—

Neonatal carrier mice established by infection within 24 h after birth.
* IgM + IgG + IgA.
‡ Numbers per 10^6 trypan blue–excluding leukocytes, means ±SE.
§ Below detectability.

expressed view that carrier mice are prone to develop lymphomas (45, 46). We have analyzed the focal cell accumulations in the parenchymatous organs of LCMV carrier mice histologically and, by use of a collection of monoclonal antibodies, immunocytologically, and have come to the conclusion that they represent functionally active lymphatic tissue (manuscript in preparation). This finding, and the existence of large quantities of virus in the same organs suggested the possibility that the lymphoid infiltrates in the parenchymatous organs of LCMV carrier mice might participate in the humoral antiviral immune response. This possibility was explored by histological inspection and use of a procedure for localization and counting of cells producing antiviral antibodies. Our investigation shows that in persistent LCMV infection (a) a correlation exists in mice of different strains and different ages between the degree of ICD on one hand and numbers and extent of lymphoid cell infiltrates on the other, and that the magnitude of the latter (as well as the severity of the ICD) increases with the animals' age; (b) liver, kidney, and brain contain AFC that correspond in numbers with numbers and dimensions of the cell infiltrates; (c) of the cells that generate antibodies of any specificity, the proportion generating antibodies directed against LCMV is highest in the CNS; and (d) a minority of AFC produce IgM, which is in line with the previous finding that immune complexes in the kidneys of LCMV carrier mice may contain IgM (47).

To our knowledge, this is the first example of a persistent virus infection in which localization and counting of antiviral antibody–forming cells in parenchymatous tissue has become possible, and we will continue using this model to study antibody production in organs not belonging to the lymphatic system, especially the CNS, in virus persistence. At present many questions remain unanswered. How, for instance, do the cells forming the lymphoid aggregates enter the CNS? Why is the proportion of cells forming LCMV-specific antibodies higher in the CNS than in other organs? Why is the switch to IgG in an environment in which the immunogen has been present for long periods of time not complete? The virologic and immunologic knowledge about the LCMV is well advanced (27, 48, 49), and we expect that further work will soon give us answers. One conclusion can be drawn now; the continuous presence of a powerful immunogen seems to induce the lymphatic system to expand into organs where it normally does not belong. In the carrier mouse, the virus is essentially ubiquitous, which may explain why AFC are found in liver and kidney as well as in the brain. The same may turn out to be the case in illnesses in which the virus is distributed among various tissues, for instance visna (50), cytomegalovirus disease (51), and Aleutian mink disease (52), a speculation that should be amenable to testing by methods used here. These should also yield an answer to the question whether, in conditions with predominant or even exclusive localization of the virus in the brain, such as SSPE or progressive rubella panencephalitis, antibody production occurs in this organ; in both these diseases infiltrates were demonstrated in the CNS and assumed, but not proved, to contain cells contributing to the specific antibodies found in the cerebrospinal fluid (53, 54). It would also be of interest to determine the organs in which the large quantities of measles virus–specific Ig that are often present in SSPE (55) are produced.

As pointed out in the introduction, ectopic antibody production, especially in

the CNS, is not a domain of persistent virus infections but has also been found in other infectious diseases, for instance neurosyphilis (14, 15), tuberculous meningitis (7), during the acute phases of mumps (5, 6) and Japanese flavivirus encephalitis (9), during and after poliomyelitis (56), and after herpes simplex virus encephalitis (57). Parainfluenza and Sindbis virus–infected mice have been proposed to serve as animal models (58, 59). In all these instances, too, it would be desirable to obtain more direct evidence for intracranial antibody production than has been so far possible.

Summary

In mice persistently infected with lymphocytic choriomeningitis virus (LCMV), the parenchymatous organs contain infiltrates of mononuclear cells, the sizes and numbers of which vary between strains and become more numerous and extensive when the animals grow older. Histologically, these were found to possess a tissue-like structure, and by use of immunohistologic procedures they were shown to contain plasma cells secreting IgM and IgG. Cells of kidneys, livers, brains, and spleens of LCMV carrier mice were dispersed by digestion with trypsin, leukocytes were separated by density gradient centrifugation, and numbers of cells producing antibodies against LCMV were determined by use of a solid-phase immunoenzymatic technique. In all these organs, cells producing LCMV-specific IgM and IgG antibodies were demonstrated, the latter more numerous than the former. Their numbers correlated with numbers and extent of the lymphoid cell infiltrates. The blood of the same mice was essentially free of antiviral antibody–forming cell. The proportion of cells producing LCMV-specific antibodies to all cells producing Ig of any specificity varied between organs, being lowest in spleen, intermediate in liver and kidney, and highest in the brain, where in individual mice up to 90% of all active cells produced virus-specific antibodies. The LCMV carrier mouse should prove to be a useful animal model to investigate antibody production in parenchymatous organs during persistent virus infections.

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