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Serological Evidence for Antigenic Variation in Brains of Mice Infected Persistently with the Virus of Lymphocytic Choriomeningitis

By

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With 3 figures and 5 tables

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In the course of the years the infection of mice with the virus of LCM* (13) has become a favorite model for the study of phenomena of great theoretical and practical importance like persistent viral infection, viral interference, immunological tolerance, immunopathology of virus diseases, and genetic control of immune reactions.

Life-long persistence of LCM virus in congenitally infected mice (14), combined with suppressed antiviral reactivity of the host, was attributed to immunological tolerance (2). This concept was accepted by many investigators including myself. More than a decade later this theory was disputed since deposition of pathogenic virus-antibody complexes was demonstrated in the kidneys of supposedly tolerant mice (8). At present, the opinions of different teams of researchers in this field are divided. Whereas some authors, especially VOLKERT and his colleagues (19, 4) hold on to the tolerance concept with convincing arguments, others, notably OLDSTONE and his coworkers (ref. in 7), contest its validity.

Own observations made during the last few years suggest that antigenic variation may influence antibody production in carrier mice tolerant towards the virus itself. Respective experiments will be described and commented on in the present paper.

Direct CF tests were used as the method of choice. They proved sufficiently sensitive to detect antigenic alterations in congenital carriers of LCMV.

* The following abbreviations will be used: LCMV = lymphocytic choriomeningitis virus; CF = complement fixation; AG = antigen(s); C = guinea pig complement; GPHIS = guinea pig hyperimmune serum; MHIS = murine hyperimmune serum; SD_{50} = calculated serum dilution allowing 50 % C fixation; AD_{50} = calculated Ag dilution giving 50 % C fixation; ac = anticomplementary.

Material and Methods

Mice: As in previous work (17) outbred NMRI mice and inbred CBA/J animals were used. Normal mice of these breeds failed to show any signs of disease or organ pathology. The feed consisted of commercial pellets (complete diet) and water was given in drinking bottles.

Virus strains: The wild mouse strain "W" and the cerebral passage strain "WCC" were employed (17). Both strains differ slightly in their organotropism in congenital carriers (cf. table 5), "WCC" multiplying to higher titer in the brain. It is not known whether this is an original property of this virus or one acquired by cerebral passage.

Persistently infected mouse colonies: Stocks of NMRI and CBA/J mice persistently infected with strain "W" were established in 1972. In both cases females regularly transmitted the virus to their embryos, presumably by transovarial infection (15), but their breeding efficiency decreased by more than 50 % in the course of 7 years. "W"-infected embryos, baby mice, and adults for the tests described below originated from these two colonies.

A breeding stock of NMRI mice carrying "WCC" virus, also started in 1972, gradually died out 6 years later, although its breeding capacity was only slightly inferior to that of the "W"-infected NMRI mice at the beginning (17). A new colony was therefore initiated from which the "WCC" carriers mentioned below descended (generations F_2 to F_5).

Sera used in CF tests: (a) GPHIS diluted 1 : 80, in this dilution reacts specifically with LCM viral antigen. The donors of this serum were infected and hyperimmunized with guinea pig organ virus (18). — (b) a pool of sufficiently potent individual sera from congenitally infected adult NMRI mice, in tables and text referred to as "carrier serum". These sera had shown relatively strong reactions with brain extracts from adult congenital carriers and negative ones with normal brain extract in screening tests. The donors were 67—185 days old and mainly carriers of "WCC". The rareness of such sera was a delaying factor in the present work. Carrier serum was used in dilutions of 1 : 3 or 1 : 4 as indicated in tables and figures. — (c) a serum pool from NMRI baby mice infected neonatally (ip) with "WCC" and bled 4 weeks later when most animals still showed retarded growth. This serum undoubtedly contained maternal antibody rather than antibody produced by the animals themselves. This can be concluded from its mode of reaction with embryo brain Ag (see tables 2 and 3) and from the fact that litter mates of the weanlings not killed at the age of 4 weeks lost most or even all of their serum antibodies within 3 months. The dilutions employed in CF tests were 1 : 3 and 1 : 4. — (d) MHIS obtained from NMRI females infected sc and, after a resting period of 1 month, hyperimmunized 5 times ip with 10 % carrier brain extract at 3—4 day intervals. The animals were bled 10 days after the last inoculation. — (e) a serum pool from contact-infected mother mice (NMRI) nursing litters infected neonatally with strains "W" or "WCC" and bled 4—6 weeks after inoculation of their baby mice. — (f) a pool of MHIS consisting of equal parts of serum d and 2 other MHIS obtained from NMRI females hyperimmunized in the same way as the donors of serum d with 10 % spleen and kidney extracts, respectively. Serum f was used in CF box tests recorded in table 5.

Preparation of organ extracts used as antigens: The procedure has been described elsewhere (17).

CF tests: The direct method (17) was employed and cold fixation for 18 hs at + 2—3 °C was practised throughout. In the tests shown in tables 1—4 and figures 1—3 the C dose was 1.5 units since brain and spleen extracts were either not or only slightly ac. In the box tests listed in table 5, in which various tissue Ag were tested, the C dose was raised to 2 units. These tests and those recorded in fig. 3 were carried out as described in (18).

The usual controls were included in every test (17, 18). In the calculation of the SD_{50}'s and AD_{50}'s the readings for the serum controls (buffered saline instead of Ag) and the Ag controls (saline instead of serum) were subtracted from those for the tubes with Ag plus serum. All sera used in these tests were not ac.

Since the above-mentioned serum b from congenital carriers reacted poorly with some brain extracts, especially those from small embryos, the AD_{50}'s of such Ag could not be calculated. The following procedure was therefore adopted: the Ag was tested in 3 tubes (undiluted, 1 : 2 and 1 : 4), keeping serum and C constant. In 3 comparable Ag controls serum was replaced by buffered saline. After the lysis period (30 min at 37 °C) the percentage of unlysed sheep erythrocytes remaining in every tube was estimated visually, judging both with cells in suspension and after sedimentation. By subtraction of the arithmetical mean

for the 3 Ag controls (nearly always zero) from that for the tubes with Ag plus serum the mean percentage of unlysed cells (briefly called "index" in the text) was obtained.

It should be noted that AD_{50} titers recorded in tables 2—4 and fig. 1 and 2 concern 20 % brain extracts, whereas those in tables 1 and 5 and fig. 3 were calculated for tissue, the reason being that in the latter tests 20 %, 10 % or 5 % extracts were made depending on the quantity of tissue available.

Results

Decrease of viral Ag in embryo brains from pregnant congenital carriers with increasing embryo age

In the course of this work many titrations of embryo brain extracts originating from congenitally infected pregnant females against GPHIS 1 : 80 were carried out. They could not all be made at the same time and with the same reagents, but we tried to keep experimental conditions as constant as possible, especially by using the same serum throughout and paying special attention to the C dose. The serum, deep-frozen in small quantities, was thawed and freshly diluted before every test.

When brain extracts of different mothers were titrated simultaneously, older embryos (judging from their mean weight) with some exceptions carried more viral Ag in their brains than younger ones.

A rare coincidence deserves to be mentioned here: Each of 3 NMRI litter sisters mated with a genetically unrelated male (all carriers of strain "W") was found at autopsy to carry one healthy embryo. Fortunately, these embryos differed in weight (219, 725, and 1,770 mgms., respectively). Their brains therefore were good material for a titration of viral Ag against GPHIS 1 : 80 under strictly comparable conditions. The AD_{50}'s of brain tissue in the order just mentioned were 1 : 52, 1 : 29 and 1 : 15.

Table 1

Decrease of viral antigen in embryo brains with increasing embryo weight

Pregnant mothers (congenital carriers)			Embryos		
Breed	Virus carried	No.	Mean embryo weight (mgms)	Mean AD_{50} of brain tissue	
				$-\log_{10}$	antilog
NMRI	W	6	78 - 197	1.654	1 : 45
		8	206 - 280	1.585	1 : 38
		7	311 - 510	1.473	1 : 30
		14	588 - 941	1.406	1 : 25
		14	1053 - 1777	1.305	1 : 20
	WCC	4	259 - 429	1.742	1 : 55
		4	660 - 865	1.660	1 : 46
		8	1006 - 1735	1.557	1 : 36
CBA / J	W	7	167 - 292	1.589	1 : 39
		9	307 - 529	1.409	1 : 26
		9	697 - 987	1.307	1 : 20
		8	1010 - 1260	1.351	1 : 22
		8	1266 - 1550	1.364	1 : 23

Table 1 records arithmetical means of all titration results, omitting those obtained with very small embryos whose brains were excised and weighed together with their capsules in order not to lose part or most of the extremely soft cerebral tissue. It can be seen in the table that in all 3 groups of pregnant congenital carriers a decrease of the embryo brain titer was inversely proportional to the weight of the embryos.

Comparison of the serological reactivity of maternal and embryonic brain antigens obtained from congenital carriers of strain "WCC"

Pregnant NMRI females furnishing these extracts were 51—121 days old at the time of autopsy. In this age span the viral Ag content of the brain is relatively low in congenitally infected mice (18).

Table 2

CF test with 20 % brain extracts from pregnant NMRI females (carriers of "WCC") and their embryos against GPHIS (1 : 80) and sera (1 : 3) from adult congenital carriers and weanlings infected neonatally with "WCC"

Pregnant mothers				Embryos				
No.	AD_{50} of brain extract with GPHIS	Reaction of brain extract with serum from		No.	Mean embryo weight (mgms.)	AD_{50} of brain extract with GPHIS	Reaction of brain extract with serum from	
		adult congenital carriers	weanlings infected neonatally				adult congenital carriers	weanlings infected neonatally
M 1	3.2 *	92 **	58 **	8	35 ×	13.7 *	0 **	75 **
M 2	1.5	53	33	9	46 ×	10.0	3	72
M 3	2.4	61	40	10	50 ×	12.0	1	70
M 4	2.2	12	6	12	77 ×	7.4	4	65
M 5	2.6	65	43	11	87 ×	10.6	0	74
M 6	2.0	60	24	10	90 ×	10.7	0	62
M 7	2.0	51	31	11	96 ×	6.4	0	38
M 8	2.5	46	29	9	259	11.2	5	65
M 9	1.4	10	3	12	317	10.4	5	74
M 10	1.5	22	13	12	429	6.3	13	46
M 11	1.5	17	9	9	789	5.9	61	51
M 12	1.2	18	7	7	1171	3.2	67	43
M 13	2.5	58	18	9	1222	5.5	52	47

* reciprocal of antilog of brain extract dilution; ** mean percentage of unlysed sheep erythrocytes in 3 tubes containing undiluted brain extract and dilutions 1 : 2 and 1 : 4, respectively; × brain capsule included in brain weight.

Design and results of the test are evident from table 2 which shows that embryonic brains invariably contained considerably more viral Ag than those of the mothers as indicated by the AD_{50}'s in presence of virus-specific GPHIS. With very young embryos from females 1—7 the difference might even have been greater had not the presumably inert brain capsule been included in the brain weight when the suspensions were made. Brains of small embryos again had a higher content of viral Ag than those from large ones.

The reactions of the two murine immune sera varied considerably. Carrier serum always had higher indices in presence of maternal brain Ag (mean

43 %) than serum from neonatally infected weanlings (mean 24 %). A similar result was obtained with brain extracts from relatively large embryos weighing 789—1,222 mgms. (means 60 vs. 47 %). In sharp contrast hereto, carrier serum reacted only very poorly with brain Ag from small embryos weighing 35—429 mgms. (3 % vs. 60 % for weanling serum) despite their relatively high virus content, which was confirmed by ic infectivity titration in mice.

Reactivity of the same sera as in Table 2 with brain extracts from carriers of strain "W"

An attempt was then made to determine whether results similar to those just described might not also be obtained with brain extracts from NMRI and CBA/J carriers of strain "W".

Since it would not have been feasible to record here all individual tests made with such antigens, arithmetical means of AD_{50}'s and indices from individual tests with embryo sets and older mice falling into certain weight or age groups (see table 3) were computed using the logarithmic method for the AD_{50}'s in presence of GPHIS. The number of individual tests per group varied between 3 and 9.

Table 3

CF test with brain extracts from embryos, baby mice, and adults carrying LCM strain "W" against sera (1 : 4) from adult congenital carriers and neonatally infected weanlings

Brain donors		AD_{50} with GPHIS 1 : 80	Reaction of brain extract with serum from	
Mouse strain	Developmental stage		adult congenital carriers	weanlings infected neonatally
NMRI	Embryos weighing 78 - 115 mgms.	5.8*	6**	43**
	139 - 170 "	5.1	11	41
	412 - 870 "	4.5	33	49
	900 - 1557 "	4.3	27	44
	1633 - 1777 "	3.5	32	42
	Suckling mice 1 day old	4.8	43	44
	2 - 3 days old	3.4	44	43
	8 "	2.3	40	30
	11 "	3.1	29	22
	14 "	4.0	48	41
	Young mice 28 days old	2.8	55	43
	33 "	2.7	46	43
	Adult mice 64 "	2.5	50	39
	135 - 142 days old	2.7	48	39
	148 - 160 "	3.8	59	45
CBA/J	Embryos weighing 29 - 57 mgms.x	6.2	7	24
	167 - 307 "	5.6	8	28
	417 - 529 "	5.0	9	37
	686 - 800 "	4.9	38	48
	928 - 1050 "	5.0	34	49
	1190 - 1550 "	4.2	26	37
	Suckling mice 1 day old	2.9	30	22
	14 days old	3.7	53	45
	Adult mice 57 - 62 days old	1.2	32	19
	76 - 86 "	1.5	34	18
	199 - 201 "	4.1	54	50

Asterisks: see Table 2.

As can be seen in table 3, the results were similar in principle to those listed in table 2. Again, carrier serum reacted less with embryonic brain Ag than weanling serum, whereas this trend was reversed with brain extracts harvested postnatally. Here too, mean indices calculated for carrier serum and Ag from embryos of different weight were inversely proportional to the AD_{50}'s obtained with GPHIS. There was no basic difference between NMRI and CBA/J mice in this respect.

Contrary to its behavior in presence of embryonic brain Ag from NMRI females, weanling serum reacted slightly less with cerebral Ag from small CBA/J embryos weighing 29—307 mgms. and rich in viral Ag than with Ag from heavier embryos with a mean weight of 686—1,050 mgms. and a lower AD_{50}.

Mode of reaction of GPHIS and murine carrier serum with brain extracts from congenitally infected NMRI baby mice

Previous titrations (18) indicated fluctuations of the cerebral Ag content in congenitally infected baby mice during the first few weeks of life. It was therefore decided to make a systematic study of the reactivity of brain extracts obtained from such animals in presence of GPHIS and of murine carrier serum.

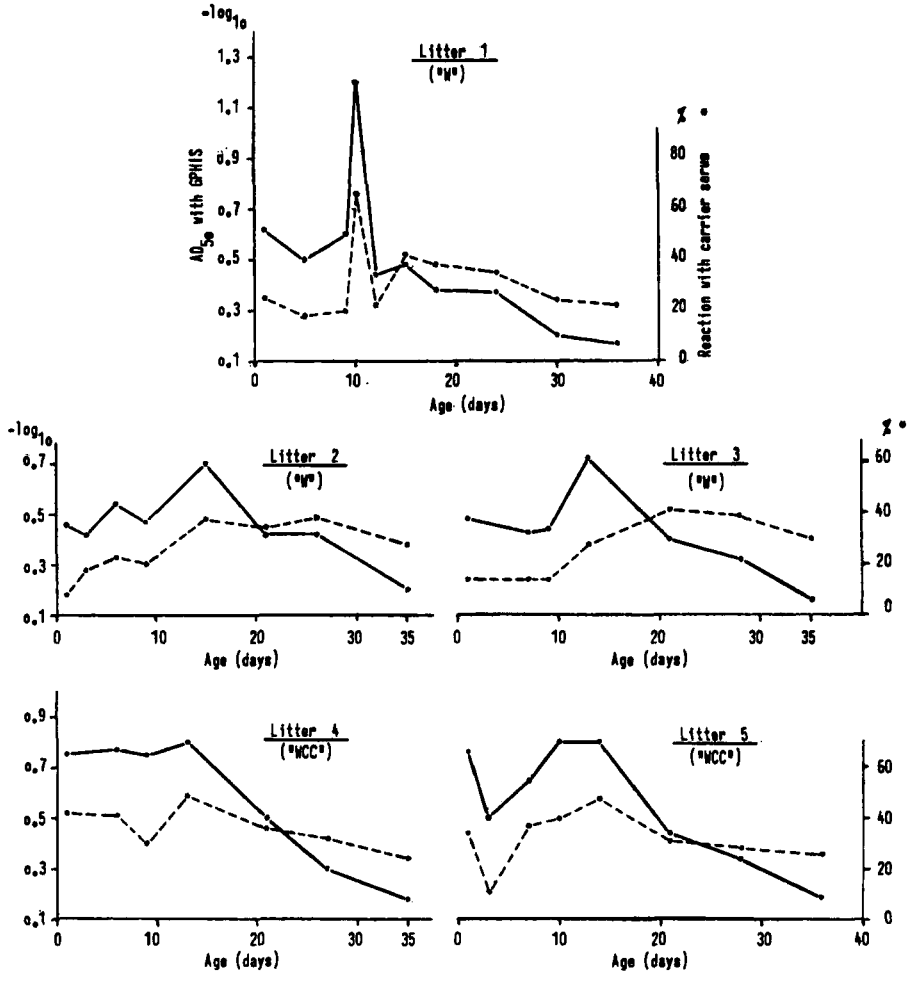
18 sizable litters were selected for this purpose, 10 carrying strain "W" and 8 strain "WCC". They were nursed by their mothers. Baby mice from each litter were chloroformed at intervals (see fig. 1), killing two at a time while the animals were still very small and one when they had grown larger. 20 % extracts of their brains were titrated individually against GPHIS 1 : 80 and carrier serum 1 : 4. The AD_{50}'s of the extracts in presence of each serum were determined and their indices with carrier serum calculated. In the 5 graphs in fig. 1 showing curves obtained from 3 "W"-infected and 2 "WCC"-infected litters the AD_{50}'s with GPHIS (standing for 20 % brain extracts) are graded on the left ordinate and the indices obtained with carrier serum on the right one.

A postnatal increase of viral Ag in the brain, often following an initial drop, was observed with all 18 litters. Up to the time when the viral Ag curve (unbroken line) obtained with GPHIS reached its highest peak or a plateau lasting for several days, a distinct, though rarely perfect parallelism between this curve and the one obtained with carrier serum (broken line) was noted in most cases. After culmination the solid line would descend more or less rapidly and then be crossed by the broken line, henceforth running above. These crossings occurred between days 15 and 23 with an arithmetical mean of 19 days in the case of "W"-infected litters, and between days 18 and 31 (mean 24 days) with curves obtained from "WCC"-carrying litters.

The very high peaks reached on day 10 by the curves from litter 1, confirmed in a repeat test, are exceptional. They are nevertheless shown here because a nearly perfect parallelism between the two curves exists in this case. Responsible for the high peak is a single baby mouse.

Comparison of the antigenic properties of brain extracts from infantile and adult congenital carriers

To obtain a more complete picture of the antigenic behavior of 20 % brain extracts from congenitally infected NMRI mice of different age, such extracts from 82 adult carriers, in addition to 143 extracts from infantile carrier mice, were titrated individually against GPHIS 1 : 80 and carrier serum 1 : 4.



* Mean percentage of unlysed sheep erythrocytes in 3 tubes containing undiluted brain extract and dilutions 1:2 and 1:4, respectively

Fig. 1: Reactivity of 20 % brain extracts from litter mates of 5 congenitally infected NMRI litters in CF test with GPHIS 1 : 80 (———) and serum 1 : 4 from adult congenital carriers (— — —)

The animals were persistently infected either with strain "W" or with "WCC". Individual brain titers were grouped according to the age of the tissue donors as indicated on the abscissa in fig. 2. Arithmetical means, computed from individual AD_{50}'s of brain extracts from mice falling into the various age spans, are recorded graphically in fig. 2, where the unbroken line connects the mean AD_{50}'s obtained with GPHIS and the broken line those with carrier serum. The picture is distorted because more space had to be allotted to Ag from infantile mice than to those from adults.

In fig. 2 it can be seen that the two curves run practically parallel from days 1 to 15, the mean AD_{50}'s with GPHIS lying far above those with carrier serum. The slopes of the curves differ drastically between 15 and 45 days, giving the impression that the two sera were not measuring exactly the same

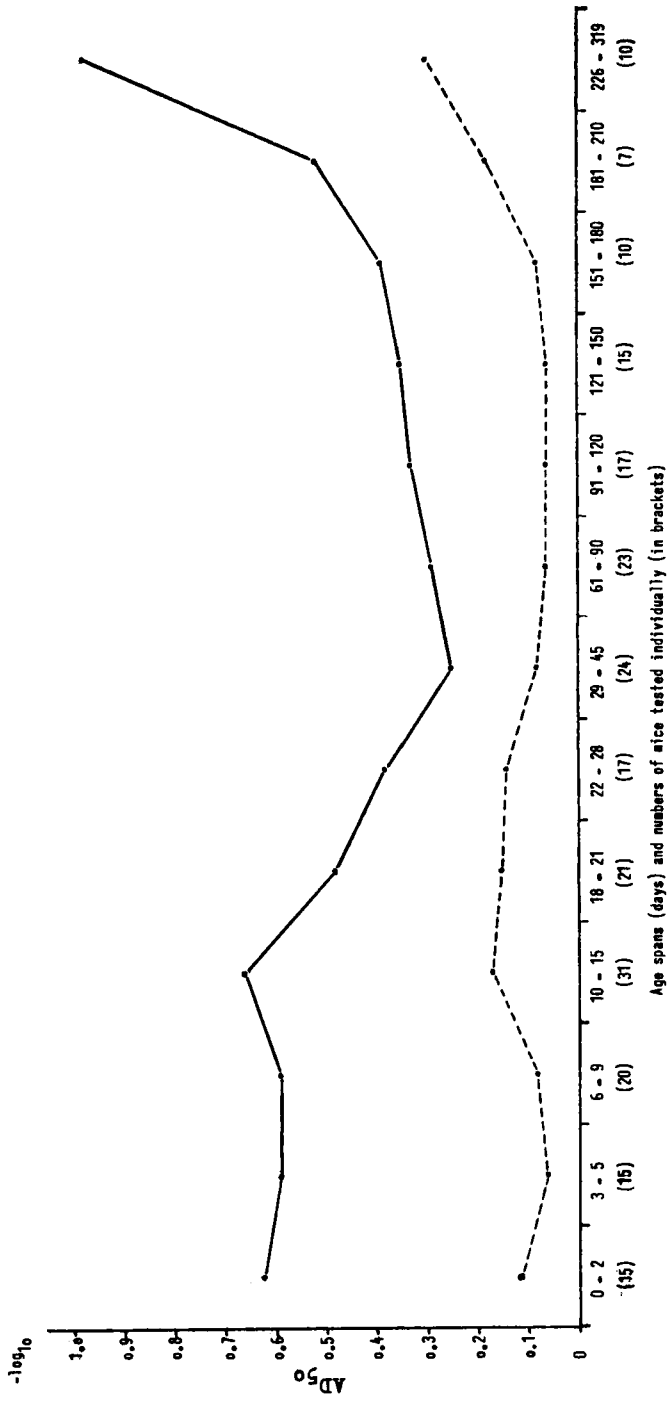


Fig. 2: Reactivity of brain antigens from congenitally infected mice of different age with GPHIS (———) and serum from adult congenital carriers (— — — —)

Ag. Between days 61 and 180 the broken line stays nearly level, while the GPHIS curve rises slowly. Thereafter, both curves climb more steeply, their slopes again differing greatly with Ag from the oldest mice tested.

CF box tests with murine immune sera and brain antigens from infected embryos, baby mice, and adult carriers

In previous box tests (18) MHIS reacted in unusually high dilutions with cerebral Ag from adult congenital carriers. Brain Ag from embryos and infantile mice has not previously been studied in this respect. Our collection of Ag from mice in different stages of development offered an opportunity to fill this gap.

Numerous box tests were carried out with such Ag using a method described previously (18). From the results of several tests with the same sera (a, d and e in Material and Methods) and various brain Ag falling into a given age group (see table 4) arithmetical means were calculated. In contrast to carrier serum, both murine sera did not differentiate between brain extracts from embryos of different weight in preceding box tests. From the results of these tests arithmetical means were computed covering the entire weight ranges of the embryos as indicated in column 2 (Nos. 1—3) of table 4.

Table 4

Reactivity of murine immune sera with brain extracts from infected embryos, baby mice and adult carriers in CF box tests

Antigens (20 % brain extracts)			Serum from adult NMRI females			
			hyperimmunized with carrier brain extracts		infected by contact, not boostered	
No.	Origin	AD_{50} with GPHIS	SD_{50}	AD_{50}	SD_{50}	AD_{50}
1	Embryos weighing 118-1770 mgms. from NMRI females carrying strain "W"	5.1 *	227 *	4.0 *	49 *	3.5 *
2	Embryos weighing 46-1222 mgms. from NMRI females carrying strain "WCC"	11.4	220	11.8	36	9.4
3	Embryos weighing 53-1550 mgms. from CBA/J females carrying strain "W"	4.6	264	4.0	51	3.4
4	NMRI baby mice, 1-7 days old, infected congenitally with strain "W"	5.1	282	3.4	52	3.2
5	dtto., 8-14 days old	6.5	436	5.0	69	3.4
6	Adult congenital carriers (NMRI) of strain "W", 2-5 months old	2.7	740	1.7	89	1.6
7	Adult congenital carriers (NMRI) of strain "WCC", 7-9 months old	10.0	1740	10.2	282	9.2
8	NMRI baby mice infected neonatally with "WCC", killed 4-10 days later	17.4	363	18.3	48	17.3
9	Adult NMRI mice infected neonatally with "WCC", 2 to 3 months old	3.7	753	3.4	144	3.0

* reciprocals of the calculated antilogs.

Table 4 shows that both murine sera reacted in higher dilutions with brain Ag from adult mice than with those from young animals including embryos. A comparison of the SD_{50}'s with the corresponding AD_{50}'s indicates that this result is not correlated with the quantity of viral Ag present in the

respective extracts. Both immune sera reacted alike in principle. They merely differed in potency. There was a gradual increase of the SD_{50} titers in parallel to the age of the Ag donors.

Comparison of the serological reactivity of MHIS with spleen and brain extracts from congenital carriers of different age

In previous work different curves were obtained from titrations of CF antigen in spleens (17) and brains (18) of congenital carriers of different age against GPHIS. It was therefore of interest to find out how MHIS (serum d in Material and Methods) would react in CF box tests with such Ag.

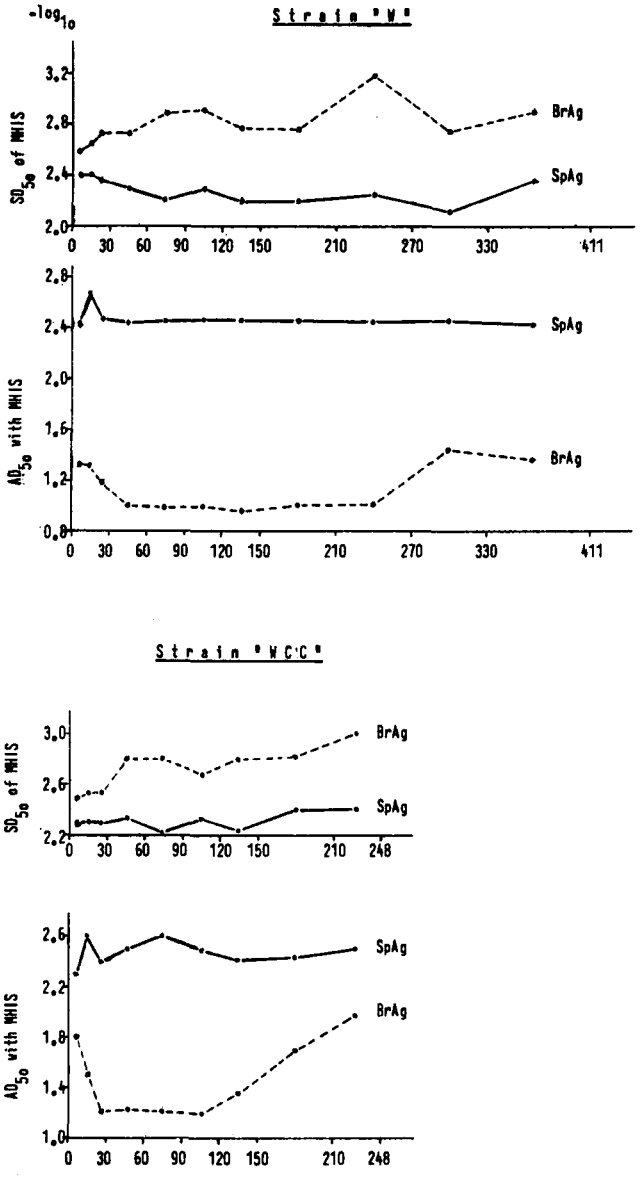


Fig. 3: CF box tests with murine hyperimmune serum vs. spleen and brain antigens from NMRI mice of different age infected congenitally with LCM strains "W" or "WCC"

Ag donors were NMRI mice carrying either strain "W" or strain "WCC". The results are presented graphically in fig. 3, dots in the curves indicating arithmetical means calculated from individual box tests with Ag from 6—9 animals per age group. Spleen and brain extracts were always titrated simultaneously. AD_{50}'s are calculated for tissue since spleen and brain extracts differed in concentration (10 and 20 %, respectively).

The SD_{50} curves in fig. 3 indicate that MHIS reacted differently with spleen and brain Ag from carriers of either virus strain. As expected in view of previous results (18), the SD_{50}'s were generally higher in presence of brain extracts than with spleen Ag in spite of the much greater Ag content of the spleens as shown by the AD_{50} curves whose shape resembles that obtained several years ago using GPHIS (18).

After a peak in suckling mice the AD_{50} of the spleen remained remarkably constant for many months in carriers of strain "W", while the curve concerning carriers of "WCC" shows some fluctuation. The AD_{50} curves obtained with brain extracts from both kinds of carriers are similar in principle. A high in baby mice is followed by an extended low lasting much longer in carriers of strain "W" than in those of "WCC". Thereafter, a rise of the Ag content is evident in both cases, reaching another peak at about 10 mos in carriers of strain "W".

In both "W"-infected and "WCC"-carrying animals the brain/spleen ratio (vertical distance between the two SD_{50} curves expressed in $\log_{10}$) was lowest (about 0.2) in suckling mice. It increased considerably with age, reaching a maximum of nearly 1.0 in 7—9 mos old animals carrying strain "W" and of 0.6 in 7—8 mos old carriers of "WCC". Older "WCC"-infected mice were not available at the time of this experiment. It is clear from the course of the SD_{50} curves that brain Ag are mainly responsible for this age-linked variation of the brain/spleen ratio.

Mode of reaction of MHIS with extracts of various organs or tissues from adult congenital carriers

The CF box tests described here are an extension of previous tests with MHIS and brain, spleen and kidney Ag from congenitally infected NMRI mice (18). In these experiments the SD_{50} of MHIS was always highest in presence of brain extracts in spite of their relatively low Ag content.

The assumption that this discrepancy might be due to an Ag-inactivating effect of enzyme(s) present in spleen and kidneys but missing or low in cerebral tissue (RNA'se?) was not substantiated by subsequent experiments in which normal spleen extracts were added to relatively potent brain Ag, varying their ratio, the time and temperature of incubation as well as pH. The basic mechanism therefore remains obscure.

In the box tests shown in table 5 serum f described in "Material and Methods" was employed. This serum was tested as outlined previously (18) against extracts made from organ pools from groups of 5 or 4 male or female carriers of "W" or "WCC" as indicated in the table. The concentration of the extracts varied between 20, 10 and 5 % depending on the quantity of tissue available. Consequently, the AD_{50} titers were calculated for tissue rather than extract. The tiny adrenals from each group of females (blank spaces) did not furnish enough Ag for a box test. Some of the females had borne healthy litters about 2 mos before they were taken into the experiment.

All extracts were also titrated against GPHIS 1 : 80, the AD_{50}'s being in fair agreement with those obtained with MHIS.

The AD_{50}'s listed in table 5 give information on the quantitative distribution of CF antigen in the bodies of congenital carriers. The Ag content varied greatly with different organs and tissues.

SD_{50}'s were frequently in disproportion to the corresponding AD_{50}'s. High SD_{50}'s in combination with low or very low AD_{50}'s were noted for brain extracts and those of skeletal muscle (biceps femoris). Less striking examples

Table 5

Reactivity of murine hyperimmune serum with organ extracts from congenital carriers in CF box tests

Tissue donors: / Organ	Carriers of strain "W"				Carriers of strain "WCC"			
	5 males, 212 days old		4 females, 152 days old		5 males, 191 days old		4 females, 146 days old	
	SD_{50}	AD_{50}	SD_{50}	AD_{50}	SD_{50}	AD_{50}	SD_{50}	AD_{50}
Spleen	123*	431*	204	412	115	316	191	372
Lymph nodes	141	219	200	431	166	257	257	355
Thymus	200	227	195	279	200	232	224	309
Brain	355	26	759	10	417	105	794	28
Spinal cord	457	9	191	7	224	40	676	9
Heart	257	50	240	26	170	54	257	51
Lung	257	120	288	119	275	214	275	257
Liver	123	209	219	117	166	126	209	219
Pancreas	178	148	?**		166	385	144	457
Adrenals	269	158			229	224		
Kidneys	110	145	229	136	162	115	191	272
Urinary vesicle	257	60	316	29	525	110	214	40
Biceps femoris	537	7	776	7	646	13	661	12
Brown fat	316	27	355	27	214	50	263	65
Small intestine	105	85	251	107	102	178	182	178
Large intestine	214	214	209	209	178	417	257	407
Testes	436	58			316	59		
Epididymis	229	112			209	417		
Prostate	275	110			195	93		
Ovaries			275	86			251	355
Uterus			200	112			191	355
Mammary gland (dry)			245	58			200	29

* reciprocals of antilogs of serum or Ag dilutions; AD_{50}'s concerning tissue, not organ extracts; ** extract hemolysing; Note: Extracts of salivary glands and seminal vesicles were strongly ac and the results therefore not conclusive.

are extracts of spinal cord, heart, urinary bladder, brown fat, testes, and mammary gland. On the other hand, relatively low SD_{50}'s combined with high AD_{50}'s were obtained with Ag extracted from spleen, lymph nodes, thymus, pancreas ("WCC"), large intestines ("WCC"), epididymis ("WCC"), ovaries ("WCC"), and uterus ("WCC"), the remaining Ag taking an intermediate position.

Extremes in this box test are: biceps femoris (mean SD_{50} 1 : 645; mean AD_{50} 1 : 7) and spleen (mean SD_{50} 1 : 157; mean AD_{50} 1 : 421).

Discussion

From the data presented above it can be concluded that in brains of growing embryos from congenital carriers a decrease of viral Ag, detectable with virus-specific GPHIS (table 1), is accompanied by a qualitative antigenic alteration demonstrable with serum from adult congenital carriers (tables 2 and 3). This change was not recognized by murine immune sera from adult NMRI mice infected either experimentally or by contact. Serum from neonatally infected weanlings, which obviously contained maternal antibodies, also failed to detect it in brains of NMRI embryos (tables 2 and 3).

Postnatal multiplication of LCMV in brains of congenitally infected sucklings is followed by a second antigenic variation presumably equal or similar in nature to the first one in embryos. It could be demonstrated with carrier serum in combination with GPHIS (fig. 1 and 2) but also with MHIS (fig. 3) and did not parallel the viral Ag content of the brain extracts. The curves concerning brain Ag in fig. 3 suggest that further variations may occur later in life.

MHIS failed to disclose clear-cut antigenic changes in spleen Ag obtained from the same animals which furnished the brain extracts (fig. 3).

The antigenic alteration occurring postnatally appears to cause an immune reaction of the host, both humoral and cellular. It will be shown in a following paper that specific CF antibodies appear at the age of about 6 weeks in sera of NMRI mice carrying "WCC" and somewhat later in carriers of strain "W". Indirect evidence for a cellular immune reaction was obtained from observations made in our persistently infected colonies of NMRI mice. In carriers 35—120 days old (mostly in the age span of 40—60 days) abrupt deaths were observed, predominantly among carriers of "WCC" (18). This trend continued in the new "WCC"-infected colony mentioned above in "Material and Methods". The animals would look perfectly healthy on the days before the attack. On 3 occasions such mice were seen dying in typical convulsions, which are generally attributed to a cellular immune reaction of the host. They were never observed in carriers older than 4 mos or in virus-carrying sucklings, which sometimes show less characteristic signs of disease (18).

It seems unlikely that antigenic variation in congenital carriers is confined to the brain. Extracts of other tissues, perhaps those with high SD_{50}/AD_{50} ratios resembling that of the brain (table 5) might furnish similar results in tests with carrier serum. Scarcity of the latter has prevented further experimentation.

The nature of the antigenic alteration in congenital carriers is as yet unknown. It must be closely connected with the virus itself but not involve the viral genome. This can be concluded from the remarkable serological stability of murine LCMV upon natural and experimental passage.

Since carrier sera often contain antibodies to host Ag, it was at first held possible that antigenic virus-host complexes might be responsible for antibody formation in congenital carriers. This hypothesis, however, was not substantiated by our own later observations.

Interference by a foreign virus, for instance, an unidentified leucosis agent presumably present in NMRI mice (17) is more difficult to rule out. Theoretically, it might play a role in the reaction of carrier serum with murine organ extracts containing both LCMV and the hypothetical tumor agent. However, there is circumstantial evidence against this possibility: (a) The curves in fig. 1 and 2 obtained with GPHIS and with carrier serum run nearly

parallel in certain age ranges, especially in young sucklings and again in mice older than 2 mos. (b) Interaction by a leucosis virus would be expected to be less effective in the brain than in the spleen. (c) Numerous other investigators engaged in LCM research have, to our knowledge, not reported disturbing effects by vertically transmitted latent leukemia viruses in spite of their presence in practically all strains of laboratory mice (10) and also in L cells of murine origin (1) with which some LCM experts have worked.

More recently the possibility has been considered that antibody production in carrier mice may be directed against defective interfering (DI) virus particles and their degradation products rather than against complete virus (S particles).

Multiplication of LCMV in primary cultures of murine lymph node cells (16) and in L cells (5) occurs in waves. DI particles, which are non-infectious but antigenic and inhibit the synthesis of complete virus (6, 22, 12) are now thought to be responsible for cyclical multiplication of LCMV (3, 12, 22). They have also been demonstrated in organs of neonatally infected carriers including the brain (11). The ratio between S and DI virus was found higher in old mice than in infantile animals.

Cyclical multiplication of LCMV in brains of congenitally infected mice starts in embryos and continues postnatally. This is indicated by the CF antigen titers in presence of GPHIS reported elsewhere (18) and those recorded in tables 1—3 and fig. 1 and 2 above, the amplitudes differing greatly in very young and older animals. Viral Ag titers are high in brains of small embryos with which carrier serum reacts very poorly. Slowly progressing formation of DI particles may lower the specific Ag titer and, perhaps, gradually reduce the immunosuppressive effect of S particles. At this early stage the immunologically immature animal is still unable to make antibodies, but a relatively weak immune response does take place some time (see above) after the first postnatal Ag peak in sucklings.

Objections to this concept may come from biochemists whose analyses showed no difference in polypeptide composition between DI and S virions (20). However, the density of DI particles was found slightly lower than that of S virus (21, 20), indicating an incomplete viral genome. It should be mentioned in this connection that in DI particles of closely related Pichinde virus the 22 S piece of the RNA is missing (9) and this or a similar loss may also be found in DI LCMV. Both kinds of particles were shown to be antigenic (21).

Summary

This paper deals with the controversial problem of immunological tolerance in carrier mice infected congenitally with LCM virus, in particular with the antigenic basis of the relatively weak humoral immune response, which induced some investigators to doubt the existence of tolerance in such animals.

The reactivity of serum from congenital carriers, various immune sera from non-tolerant mice, and virus-specific hyperimmune serum from guinea pigs was compared in quantitative CF tests, in which brain extracts from carriers of different age, including embryos, were used as antigens.

The results of these tests indicated repeated qualitative antigenic alterations, evidently correlated with cyclical multiplication of the virus in the brain. This process started in embryos and continued postnatally.

In contrast to the brain, there was little evidence of antigenic variation in the spleen. However, the result of an experiment with other organ extracts raised the suspicion that this phenomenon may not be confined to the brain.

The possibility is discussed that the humoral immune response in congenital carriers, tolerant towards complete infectious virus, might be directed against non-infectious defective interfering (DI) particles, now generally thought to be responsible for cyclical multiplication of LCM virus.

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Zusammenfassung

Serologische Hinweise auf Antigenvariationen im Gehirn von persistent mit dem Virus der lymphocytären Choriomeningitis infizierten Mäusen

Vorliegende Arbeit befaßt sich mit dem umstrittenen Problem der Immuntoleranz bei congenital mit dem LCM-Virus infizierten Mäusen, im besonderen mit der relativ schwachen humoralen Immunreaktion, die manche Forscher veranlaßt hat, die Existenz von Immuntoleranz bei solchen Tieren zu bezweifeln.

Die Reaktionsweise von Serum congenital infizierter Virusträger, verschiedener Immunsera von nicht toleranten Mäusen und virusspezifischem Hochimmunserum von Meerschweinchen wurde in quantitativen Komplementbindungstesten verglichen, in denen Gehirnextrakte von Virusträgern verschiedenen Alters, einschließlich Embryonen, als Antigene dienten.

Die Ergebnisse dieser Teste ließen auf wiederholte qualitative Antigenveränderungen schließen, die offenbar mit der zyklischen Virusvermehrung im Gehirn in Zusammenhang stehen. Der Prozeß begann im Embryo und setzte sich postnatal fort.

Im Gegensatz zum Gehirn war in der Milz nur wenig Antigenvariation zu erkennen. Jedoch erweckte das Resultat eines Versuchs mit anderen Organextrakten den Verdacht, daß dieses Phänomen nicht auf das Gehirn beschränkt sein dürfte.

Die Möglichkeit wird diskutiert, daß die humorale Immunreaktion bei congenital infizierten Virusträgern, die gegenüber dem intakten infektiösen Virus tolerant sind, gegen nicht infektiöse, defekte, interferierende Viruspartikel (DI particles) gerichtet sein könnte, die heute als verantwortlich für die zyklische Vermehrung des LCM-Virus angesehen werden.

Résumé

Indications sérologiques sur des variations d'antigène dans le cerveau de souris infectées de façon persistante avec le virus de la chorioméningite lymphocytaire

Le travail présent s'est occupé du problème controversé de l'immunotolérance chez des souris infectées congénitalement avec le virus LCM. On a considéré en particulier la réaction immunitaire humorale relativement faible qui a amené certains chercheurs à douter de l'existence d'une immunotolérance chez de tels animaux.

On a comparé dans des tests quantitatifs de réaction de fixation du complément les formes de réaction de sérums provenant de porteurs du virus infectés congénitalement, de différents immunsérums de souris non tolérants et de sérums spécifiques du virus provenant de cobayes fortement immunisés. Des extraits de cerveau de porteurs du virus d'âges différents, y compris des embryons, ont servi d'antigènes. Les résultats de ces tests ont permis de constater des modifications qualitatives répétées de l'antigène qui sont probablement en rapport avec la multiplication cyclique du virus dans le cerveau. Le processus débute chez l'embryon et se poursuit après la naissance. On a constaté peu de variation de l'antigène dans la rate par rapport au cerveau. Le résultat obtenu avec un essai utilisant d'autres extraits d'organes a permis de penser que ce phénomène n'était pas limité au cerveau.

On discute la possibilité que la réaction immunitaire chez des porteurs du virus infectés congénitalement et qui sont tolérante vis-à-vis du virus infectieux intact pourrait ne pas être dirigée contre une particule virale (DI particles) non infectieuse, défectueuse et interférente qui aujourd'hui passe pour être responsable de la multiplication cyclique du virus LCM.

Resumen

Evidencia serológica de variaciones antigénicas en cerebros de ratones infectados persistentemente con el virus de la coriomeningitis linfocitaria

El trabajo presente se refiere al problema discutido de la inmunotolerancia en los ratones infectados por vía congénita con el virus de la LCM, en especial a la inmunorreacción humoral relativamente débil, la cual indujo a ciertos científicos a poner en duda la existencia de la inmunotolerancia en semejantes animales.

Se comparó la reactividad del suero de los portadores infectados por vía congénita, de inmunosueros diversos de ratones no tolerantes y de sueros hiperinmunes de cobayas, específicos de virus, en pruebas cuantitativas fijadoras del complemento, en las que se usaron como antígeno extractos de cerebro de portadores de virus de diferentes edades, incluso embriones.

Los resultados de estas pruebas señalaron hacia alteraciones antigénicas cualitativas repetidas, las cuales se hallan relacionadas evidentemente con la multiplicación cíclica del virus en el cerebro. Este proceso se inició en los embriones, continuándose de manera postnatal.

En contraste con el cerebro, se apreció solo una evidencia pequeña de variación antigénica en el bazo. Sin embargo, el resultado de un ensayo con otros extractos de órganos despertó la sospecha de que este fenómeno no se debe limitar al cerebro nada más.

Se discute la posibilidad de que la respuesta inmunitaria humoral en los portadores de virus infectados por vía congénita, que son tolerantes frente al virus infeccioso intacto, podría estar dirigida contra las partículas defectuosas interferentes (DI) no infecciosas, las cuales se admiten hoy en día como responsables de la multiplicación cíclica del virus de la LCM.

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