Zbl. Vet. Med. B, 28, 133—145 (1981)

ISSN 0514—7166/InterCode: ZVRBA2

Institute of Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, University of Munich, W. Germany
Director: Prof. Dr. DDr. h. c. Anton Mayr

Factors Influencing Specific Antibody Formation in Mice Persistently Infected with LCM virus*

By

ERICH TRAUB

With 2 figures and 4 tables

(Received for publication November 14, 1980)

This paper supplements a preceding one (8) in which serological evidence for antigenic variation in congenital carriers of LCMV was reported. The opinion was expressed that antigenic alteration(s) *in vivo* may elicit the weak humoral immune response which led American investigators to doubt the existence of immunological tolerance in such animals (4).

In the present report certain other factors influencing antibody formation in carrier mice will be dealt with. It has been found that besides the long known immunosuppressive effect of infectious LCMV the genetic constitution of the host as well as the virus strain carried by the animals play a role. Moreover, the significance of non-specific (anti-host) antibodies reacting in direct CF tests with organ extracts from normal mice (6) will be discussed.

Direct CF tests were used for the demonstration of specific and non-specific Ab in carrier sera. The latter were also tested by indirect CF which detects specific Ab only.

Material and Methods

Mouse and virus strains: These have been specified previously (6, 7).

Sera: a) Sera from congenital carriers (Fig. 2), 1 month old or older, were collected and tested individually by direct and indirect CF. Since younger mice did not furnish enough serum for individual testing, serum pools were made from several or all litter mates in such cases. After exsanguination from the heart in deep ether anaesthesia, 20 % extracts were prepared of brains from individual animals or littermates, corresponding to the serum pools from sucklings. All of these extracts were shown to contain viral Ag by DCFT with GPHIS 1 : 80. The figures in brackets below the abscissa in Fig. 2 concern serum pools from litter mates in the age span of 7—30 days, and individual sera in the older age groups.

* Abbreviations used: LCMV = lymphocytic choriomeningitis virus; Ab = antibody(ies); Ag = antigen(s); C = guinea pig complement; CF = complement fixation; DCFT = direct CF test; ICFT = indirect CF test; ac = anticomplementary; GPHIS = guinea pig hyperimmune serum; GPNS = normal guinea pig serum.

b) Serum pools used in the DFT shown graphically in Fig. 1 originated from 2 batches of congenital carriers of strain "W" (NMRI and CBA/J) and 1 batch of normal NMRI mice. Each batch of 40 females, about 6 weeks old at the start of the experiment, was divided into 4 groups of 10 animals each. The mice of one group from each batch were not inoculated. Those of the other 3 groups were infected sc with 0.2 ml. 10 % homologous spleen, kidney or brain extract from adult congenital carriers of strain "W" as indicated in Fig. 1. All animals showed no definite signs of illness after this treatment. Starting one month after the first injection the mice were given 5 ip inoculations with 0.2 ml. 10 % spleen, kidney and brain extract, respectively, at 3—4 day intervals. These homologous organ extracts were freshly prepared every time from tissue taken from adult congenital carriers. All mice, including those of the untreated control groups, were exsanguinated 10 days after the last inoculation, pooling the blood groupwise.

All sera and pools were heated at 58 °C for 30 min prior to use.

Organ extracts: Extracts of spleens (10 %), kidneys (10 %) and brains (20 %) from adult congenital carriers (NMRI) made with isotonic salt solution (6) were used as Ag. Suitable specimens were selected from many deep-frozen organ extracts obtained from individual animals and previously titrated against GPHIS 1 : 80. Criteria for the choice were: about equal distribution of Ag from "W" and "WCC" carriers, relatively high Ag content, and missing or low ac effect, the latter being particularly disturbing with kidney extracts from carriers or normal adults. Satisfactory extracts were pooled and the pools titrated against GPHIS 1 : 80, checking ac activity in serial Ag dilutions without serum. Comparable Ag pools were prepared from the same organs of normal adult NMRI mice. All pools were deep-frozen at — 25 °C and cleared by centrifugation before use.

Optimal dilutions of the organ extracts serving as Ag in DCFT and ICFT were determined in preceding tests against known positive carrier serum and normal murine control serum, both originating from NMRI mice. Brain extracts were usually used undiluted due to their lower Ag content compared with spleen and kidney extracts.

Other materials employed in CF tests: Small test tubes (internal diameter 0.7 cm., height 7.5 cm.) served as containers in all tests. Rabbit hemolysin and preserved C were purchased from Behringwerke AG, Marburg/Lahn. Sheep blood (preserved with Alsever solution) was obtained from animals of the Institute. Diluents for the reagents and their dosage were mentioned elsewhere (6).

Direct CF tests: Because of the very low Ab content of many carrier sera it was not possible to use the serum dilution method and calculate SD_{50} titers. The C dilution method was therefore employed, limiting the number of C dilutions to 3 due to the small amounts of serum obtainable from individual mice.

Sera from individual carriers of different age including serum pools from sucklings (Fig. 2) were diluted 1 : 2 and tested simultaneously against undiluted 20 % carrier brain extract (3 tubes), undiluted 20 % normal brain extract (3 tubes) and buffered saline instead of antigen (3 tubes) representing the serum controls to check the ac effect of the serum. Ag controls were included in every test (3 tubes with carrier brain extract and 3 tubes with normal brain extract), in which serum was replaced by saline. In 3 more tubes (C controls) saline was substituted for serum and Ag. Into the 3 tubes of every set C dilutions were pipetted corresponding to 1.5, 1.75 and 2 units, respectively, as shown by a preceding C titration.

The mixtures were subjected to cold fixation for 18 hs at + 2 to + 3 °C before addition of sensitized sheep erythrocytes and subsequent incubation for 30 min at 37 °C in a waterbath. After the lysis period the percentage of unlysed cells in the 3 tubes of every set was estimated visually before and after sedimentation of the cells. The mean fixation index was determined, subtracting the readings for the serum and Ag controls from those for the tubes with serum plus Ag.

The same procedure was applied in the DCFT recorded in Fig. 1, except that 5 instead of 3 different doses of C (1, 1.25, 1.5, 1.75 and 2 units) were used since more serum was available.

Indirect CF tests: A technique described previously (1, 7) was modified in some respects. In the experiment shown in Fig. 2 carrier sera were tested against spleen Ag from congenital carriers.

Undiluted serum and optimal Ag dilution were pipetted in 0.025 ml. amounts into each of 6 tubes using graduated 0.1 ml. pipettes. The mixtures were incubated for 1 h in a

waterbath at 37 °C. Thereafter, tubes 1—5 received 0.05 ml. GPHIS 1 : 40 and tube 6 the same amount of GPNS 1 : 40. C dilutions were added in 0.05 ml. doses corresponding to 1.5 units in tubes 1 and 6, 1.75 units in tube 2, 2 units in tube 3, 2.25 units in tube 4 and 2.5 units in tube 5 as shown by a preceding C titration at 37 °C for 30 min. A color control containing carrier serum under test + Ag + buffered saline instead of guinea pig serum and C was included in every case to facilitate the reading of the results. For C fixation the mixtures were kept in a waterbath at 37 °C for 30 min and again so for lysis after addition of sensitized sheep cells (0.1 ml. per tube).

After the final incubation the percentage of unlysed erythrocytes present in every tube was estimated as described above and the arithmetical mean calculated for tubes 1—5. There was always complete hemolysis in tube 6 indicating that carrier sera did not fix C directly under the conditions of this test.

A positive murine control serum, 2 normal mouse sera and a series of tubes in which buffered saline was substituted for carrier serum were included in every test.

To obtain the "mean ICF index" of a carrier serum the mean percentage of unlysed cells in tubes 1—5 was expressed in per cent of the overall mean for the corresponding tubes containing the two normal sera and saline instead of murine serum, respectively.

Serum pools from carriers inoculated repeatedly with infectious tissue extracts (Table 1) were tested in the same way as just described against spleen, kidney and brain Ag. They also failed to fix C directly at 37° C, tube 6 showing complete hemolysis in every case.

This was different with the much more potent sera from mouse groups 9—11 in Table 1. When they were titrated against spleen, kidney and brain Ag, tubes containing serum dilutions 1 : 10 to 1 : 320 plus Ag were set up in duplicate, adding to one series GPHIS 1 : 40 and to the other GPNS 1 : 40. There was evidence of direct C fixation by murine immune sera in presence of all 3 Ag, most marked in tubes containing brain Ag. Complete lysis in the Ag controls ruled out ac effects of the Ag.

Before calculating the SD_{50} titer of a serum the estimated mean percentage of unlysed erythrocytes in the tubes with GPNS was subtracted from those in the tubes with GPHIS.

Absorption of carrier serum with normal mouse brain: Relatively potent carrier sera with strong non-specific ("anti-host") components were selected for this purpose. For every test a pool of 2 or 3 individual sera was made, which had not been ac and shown similar reactions in a preceding screening test.

Normal mouse brains were obtained from exsanguinated adult NMRI and CBA/J mice, respectively, and deep-frozen *in toto*. In some absorption tests such brains were used without chemical treatment. They were halved lengthwise. Each half was ground without sand and suspended in 0.9 ml. dilution 1 : 2 of a carrier serum under test. The suspension (roughly 20 %) was kept for 18 hs at + 2 to + 3 °C and then for 2 hs at 37 °C, shaking continuously. Thereafter it was cleared by centrifugation, the supernatant representing the absorbed serum.

In the majority of the absorption tests powdered normal mouse brain was employed, which had been extracted with acetone and ether according to the method used by CASALS and BROWN (3) for the preparation of arbovirus hemagglutinin. Ground brain tissue (without diluent) was extracted twice with acetone (c. p.), once with a mixture of equal parts of acetone and ether (DAB 7, Ph. Eur., Hoechst), and 3 times with ether alone. Traces of ether remaining in the final sediment after centrifugation were evaporated in a desiccator connected with a vacuum pump. The dry sediment was ground to a fine powder in a mortar and stored in quantities of 100 mg. in small centrifuge tubes in the deep-freeze. This amount approximated the dry weight of one half mouse brain.

For absorption 0.9 ml. carrier serum 1 : 2 were added to 100 mg. homologous brain powder. The mixture was vigorously shaken at room temperature for 15 min and incubated and centrifuged as just described for wet brain.

In the Ab titration (DCFT with cold fixation) unabsorbed and absorbed serum were tested undiluted and in two-fold serial dilutions against undiluted 20 % brain extracts from adult congenital carriers and normal adults, respectively. In a dilution series representing the serum controls brain extract was replaced by buffered saline. In the Ag controls saline was substituted for serum. CF indices were obtained as described above, subtracting the readings for the controls from those for the tubes with serum plus Ag. The percentage of eliminated Ab was calculated from the CF indices obtained with absorbed and non-absorbed serum.

Results

Antibody production by congenital carriers and normal mice inoculated repeatedly with organ extracts from adult congenital carriers

In an experiment, whose results are shown in Fig. 1 and in Table 1, the effect of booster inoculations in congenital carriers of strain "W" (NMRI and CBA/J) was investigated. For comparison, normal NMRI mice were

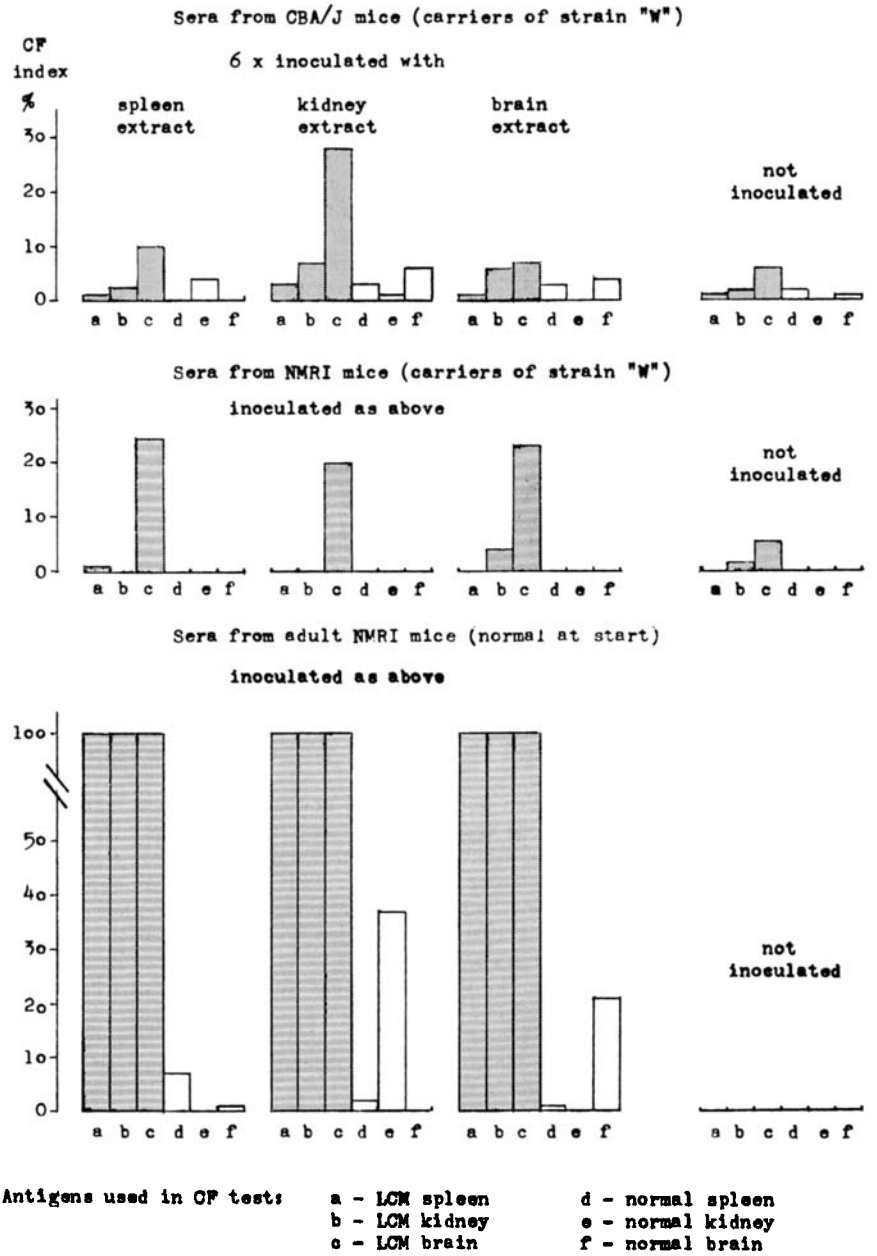

Fig. 1. Direct CF test with sera from congenital carriers and normal mice inoculated repeatedly with 10 % spleen, kidney or brain extracts from congenital carriers of strain "W"

treated in the same way as the carriers. Details have been described in "MATERIALS and METHODS". Serum pools from all mouse groups including inoculated animals were tested by DCF (Fig. 1) and by ICF (Table 1) using spleen, kidney and brain extracts as Ag.

It can be seen in Fig. 1 that sera of congenital carriers from our persistently infected CBA/J and NMRI stocks did not react strictly alike to the inoculations with tissue extracts. In CBA/J mice a booster effect above 10 % was recognizable only in animals treated with kidney extracts, whereas all 3 groups of inoculated NMRI carriers showed a slight increase of specific Ab. In every case the rise could only be detected with LCM brain Ag. Inoculated NMRI carriers failed to develop non-specific Ab, whereas a very slight boost of such Ab appears to have occured in inoculated CBA/J mice. A more distinct and mostly organ-specific increase of non-specific Ab took place in inoculated normal mice as shown by the respective columns in Fig. 1.

A marked immunosuppressive effect in NMRI carrier mice becomes evident when one compares the modest increase of specific Ab in their sera with the high Ab content in sera of treated normal NMRI animals, whose SD_{50} titers are listed in brackets in Table 1.

As Table 1 shows, virus-specific Ab could not be demonstrated by ICF in the sera from untreated and inoculated CBA/J carriers. This result is not in full agreement with that of the DCFT in Fig. 1. On the other hand, all sera from inoculated NMRI carriers did show a slight increase of ICF antibodies demonstrable with all 3 Ag used.

Table 1

Indirect CF test with sera from congenital carriers and normal mice inoculated repeatedly with 10 % spleen, kidney or brain extracts from congenital carriers of strain "W"

Serum pools (from 10 mice per group)				Indices and SD_{50}'s in ICFT (%)		
Group No.	Breed	Status at start	6 x inoculated with	spleen Ag	kidney Ag	brain Ag
1	CBA / J	congenital carriers	spleen extract	100	104	105
2	"	"	kidney extract	103	97	103
3	"	"	brain extract	102	98	104
4	"	"	not inoculated	102	98	101
5	NMRI	"	spleen extract	86	67	94
6	"	"	kidney extract	78	69	68
7	"	"	brain extract	80	73	85
8	"	"	not inoculated	94	89	100
9	"	normal	spleen extract	1 : 168 * (1 : 421) **	1 : 105 (1 : 368)	1 : 170 (1 : 1010)
10	"	"	kidney extract	1 : 180 * (1 : 337) **	1 : 160 (1 : 288)	1 : 95 (1 : 905)
11	"	"	brain extract	1 : 106 * (1 : 268) **	1 : 79 (1 : 227)	1 : 76 (1 : 705)
12	"	"	not inoculated	100	99	100
13 x	"	congenital carriers	not inoculated	1 : 5.1	1 : 3.8	1 : 2.5

* SD_{50} in serum titration (ICFT); ** SD_{50} in DCF box tests; x pool of relatively potent carrier sera.

The much greater Ab response in repeatedly injected normal mice compared with that in congenital carriers was confirmed by ICF as shown by the SD_{50} values listed in Table 1.

Tests for serum antibodies in congenital carriers of different age by direct and indirect CF

In this experiment numerous individual sera from NMRI mice carrying strain "W" or "WCC", and from CBA/J carriers of strain "W" were tested by direct CF against undiluted 20 % brain extracts from adult carriers and normal adults. Parallel indirect CF tests were carried out using carrier spleen extract diluted 1 : 5. From individual indices arithmetical means were calculated for mice of various age groups as indicated on the abscissae in Fig. 2.

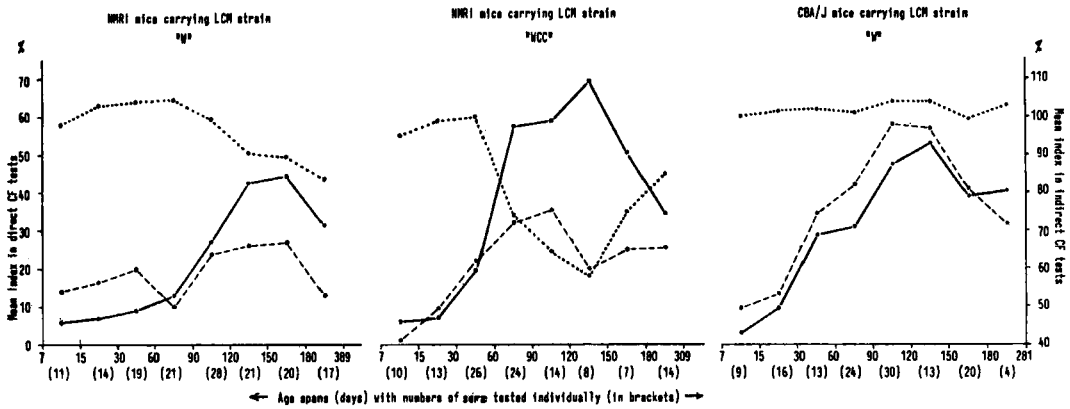

Fig. 2. Reactivity of sera from congenital carriers of different age in direct CF tests with LCM (——) and normal (- - -) brain extracts, and in indirect CF tests with LCM spleen antigen (. . .)

The curves in Fig. 2 show marked differences between sera from animals of the same breed (NMRI) carrying different strains of virus ("W" or "WCC"), and also between sera from mice of different breeds (NMRI and CBA/J) carrying the same virus ("W").

In the DCFT sera from young NMRI carriers of strain "W", 7—60 days old, reacted better with normal than with infectious brain extract. This trend was reversed at the age of 3 mos. At about the same time Ab demonstrable by ICF started to appear as shown by a slow drop of the dotted line in the respective graph. It should be recalled here that, in contrast to DCF, high ICF indices mean small amounts of Ab. The DCF curves relating to LCM and normal Ag reached a maximum between 150 and 180 days and then regressed. The vertical distance between these curves indicates the presence of specific Ab. It is noteworthy that Ab detectable by ICF was still on the increase at 389 days, when the amount of specific DCF antibody had dropped considerably.

Sera from carriers of "WCC" originated from animals of the new colony mentioned previously (8). Their reactivity differed in two main respects from that of the "W" sera. First, specific Ab appeared earlier and reached higher titers than in carriers of strain "W". Secondly, there was a distinct parallelism in the formation of DCF and ICF antibodies in this case. It started at the age of about 45 days, peaked between 120—150 days and then decreased at a similar rate.

The mode of reaction of sera from CBA/J carriers differed fundamentally from that of NMRI carrier sera. Above all, ICF antibodies were missing in CBA/J animals of any age. This confirms earlier observations made in this laboratory (1, 7). The presence of specific Ab demonstrable by DCF is at least very uncertain, since the curve for Ab reacting with normal brain Ag runs above the curve for specific Ag up to the age of about 6 mos. It is for two reasons doubtful whether the reversal at 180—281 days has any significance. First, there were only 4 such animals available. Secondly, any evidence for the presence of ICF antibodies in these sera was missing. The fact that formation of specific DCF and ICF antibodies began about simultaneously in NMRI carriers suggests that this would also have happened in CBA/J mice in the case of synthesis of specific Ab.

The broken lines in Fig. 2 indicate that production of non-specific Ab reacting with normal brain extract was more marked in CBA/J than in NMRI carriers.

CF antigen content of spleens and brains of adult NMRI mice infected neonatally either with strain "W" or with "WCC"

Searching for a possible explanation for the relatively poor Ab production by carriers of strain "W", CF antigen was titrated in the spleens and, for comparison, in the brains of adult NMRI mice which had been inoculated ip on the first day of life with 0.1 ml. 10 % brain extract from congenital carriers of strain "W" or "WCC". Available were 10 mice infected neonatally with strain "W" and 11 animals infected with "WCC". They were 67—71 days old at the time of sacrifice. 10 % spleen and 20 % brain extracts were titrated individually against GPHIS 1 : 80 using cold fixation as described elsewhere (8).

Individual AD_{50} titers listed in Table 2 (referring to tissue, not extracts) varied more or less. The arithmetical means recorded at the foot of the table

Table 2

Titrations of CF antigen in spleens and brains of NMRI mice infected neonatally (ip) with strain "W" or "WCC"

Mice carrying strain "W"				Mice carrying strain "WCC"			
No.	Age (days)	AD_{50} of spleen	AD_{50} of brain	No.	Age (days)	AD_{50} of spleen	AD_{50} of brain
1	70	2.055*	1.14*	1	71	1.21*	1.45*
2	70	2.01	1.07	2	71	1.00	1.215
3	70	2.355	1.085	3	70	1.42	1.27
4	70	2.35	1.03	4	70	1.76	1.185
5	68	2.61	1.165	5	70	1.30	1.215
6	68	2.47	0.97	6	70	1.57	1.17
7	68	2.155	1.00	7	70	1.815	1.23
8	67	2.31	0.935	8	70	1.45	1.33
9	67	2.37	1.00	9	70	0.93	1.235
10	67	2.36	0.95	10	70	2.145	1.395
				11	70	0.85	1.20
Arithmetical means		2.3045* (1:202)	1.0345* (1:11)	Arithmetical means		1.4045* (1:25)	1.2632* (1:18)

* $= \log_{10}$.

show that the average Ag content of the spleen was about 8 times higher in "W"-infected animals than in carriers of "WCC", whereas the brain contained about 1.7 times more viral Ag in "WCC"-infected mice than in carriers of "W". This difference was to be expected on the basis of previous titrations (9).

Comparison of ICF and DCF results obtained with sera from individual carriers

In previous work the reactions of sera from individual NMRI carriers often varied considerably in parallel ICF and DCF tests. More or less striking differences were again observed in the tests summarized in Fig. 2. Many of these sera were either positive or negative with both methods. In some cases, however, the results disagreed considerably. ICF and DCF indices of 24 selected sera showing various degrees of discrepancy are listed in Table 3.

The results of both kinds of test are not easy to compare since ICF indices are specific, whereas reactions obtained with carrier brain Ag in DCFT are often complicated by non-specific cross reactions with normal brain extract. In order to arrive at roughly comparable figures, the DCF index with normal brain Ag (b) was subtracted from the index obtained with carrier brain extract (a), the difference representing the specific DCF index.

Table 3
Reactions of selected sera from individual carriers (NMRI) in ICFT and DCFT

Serum No.	Indices (%)				Serum No.	Indices (%)			
	ICF	DCF				ICF	DCF		
		a carrier brain Ag	b normal brain Ag	Diff. a - b			a carrier brain Ag	b normal brain Ag	Diff. a - b
1	5	9	7	2	13	68	91	90	1
2	5	97	39	58	14	81	100	13	87
3	16	95	28	67	15	82	96	5	91
4	18	2	2	0	16	83	99	25	74
5	21	43	16	27	17	93	100	37	63
6	22	31	6	25	18	93	5	3	2
7	31	100	5	95	19	96	0	4	-4
8	34	96	19	77	20	98	72	21	51
9	35	2	1	1	21	102	5	2	3
10	57	100	8	92	22	103	10	5	5
11	62	83	9	74	23	105	68	1	67
12	68	97	34	63	24	110	81	20	61

It can be seen in Table 3 that ICF and specific DCF indices did not agree in many cases. There were sera with relatively much ICF antibody and little or no DCF antibody (Nos. 1, 4 and 9) and vice versa (Nos. 14—17). In some other cases a clear parallelism between ICF and DCF results was missing also. Sera 23 and 24, negative in ICF, nevertheless gave positive reactions in DCF, a result explicable by the higher sensitivity of the latter test (see SD_{50} titers listed in Table 1).

When sera are grouped, for instance, according to donor age as in Fig. 2, calculating arithmetical means from results of single-serum tests, individual

discrepancies are obscured, but general trends are still evident provided that the groups comprise sufficient numbers of individual sera.

Absorption of carrier sera with strong non-specific components using normal mouse brain

Most of the absorption tests mentioned in Table 4 were carried out with extracted, dry normal mouse brain as described above. It gave slightly better results than untreated fresh brain, deep-frozen and thawed. In general, the experiments were not fully satisfactory because normal mouse brain failed to absorb all or nearly all of the non-specific Ab present in the serum pools. Nevertheless, certain conclusions can be drawn from the results. Since the outcome of individual tests varied to some extent, arithmetical means of the percentage of Ab absorbed in all tests, were computed. They are shown in Table 4.

Table 4

Attempts to eliminate LCM-specific and anti-host antibodies from murine sera by absorption with normal mouse brain

Serum donors	No. of serum pools used	Calculated mean percentage of antibody absorbed	
		LCM - specific	anti-host
Congenitally infected adult NMRI mice	11	6 %	32 %
Adult NMRI mice infected neonatally	2	10 %	64 %
Congenitally infected adult CBA / J mice	5	-*	54 %

* These pools did not contain specific antibodies.

A phenomenon common to all tests was a more or less marked, sometimes very strong, ac effect of absorbed sera, contrasting with missing or minimal ac activity of the unabsorbed control sera and of the Ag used. This indicates that part of the C dose was fixed by the mixture of Ab and normal brain Ag present in absorbed sera.

It can be seen in Table 4 that the mean percentage of absorbed non-specific Ab varied between 32 and 64 %, whereas only minimal amounts of specific Ab were eliminated from the serum pools furnished by NMRI carriers. Sera obtained from congenitally infected CBA/J mice did not contain any specific Ab to begin with. The amounts of non-specific Ab absorbed in them corresponded roughly to the quantities eliminated from sera of NMRI mice.

Discussion

As reported elsewhere (8), antigenic variation appears to trigger limited formation of specific CF antibodies in NMRI mice infected congenitally with LCMV. Other factors influencing the immune response, such as specific immunosuppression, genetic constitution of the host and the virus strain carried will be discussed here.

The immunosuppressive effect of carried LCMV, known for a long time, was again confirmed by the data presented in Fig. 1 and Table 1. They show further that it was possible to boost the production of specific Ab slightly in congenital carriers by inoculating them repeatedly with fresh extracts of

carrier organs. The latter undoubtedly contained altered Ag (8) besides infectious virus, towards which such animals are completely tolerant (10). The weak humoral immune response in carriers, contrasting sharply with high serum titers in normal adults treated in the same way (Table 1), agrees with the hypothesis (8) that the altered Ag may be an integral part of the complete virion, e. g., defective interfering (DI) virus particles. These, especially when present in relatively large quantities, may not be fully covered by the immunological tolerance of the host towards complete LCMV.

The superior reactivity of carrier brain Ag compared with spleen and kidney extracts (9) seems to account for the fact that in the DCFT shown in Fig. 1 small amounts of specific Ab could only be demonstrated in carrier sera by the use of brain Ag.

The curves concerning outbred NMRI mice and inbred CBA/J animals in Fig. 2 indicate that the genetic constitution of the host can profoundly influence the production of specific CF antibodies in congenital carriers. Whereas such Ab were missing in CBA/J mice, they could be detected in adult NMRI animals by both direct and indirect CF. OLDSTONE et al. (5), using other methods, recently reported similar observations. They found different amounts of precipitating Ab and of C 1 q binding materials in sera from virus carrier mice of various murine strains, although they were unable to demonstrate CF antibodies in such sera. With regard to formation of specific CF antibodies, CBA/J carriers appear to be completely tolerant towards both complete and altered viral Ag. As mentioned elsewhere (7), the immune responsiveness to LCMV differs in CBA/J and NMRI mice in certain other respects as well.

A correlation between the virus strain carried and formation of specific Ab is clearly evident from the results obtained with sera from NMRI carriers of strain "W" and "WCC" (see Fig. 2). Carriers of "WCC" produced both specific DCF and ICF antibodies earlier and to higher titers than mice carrying strain "W". In each group of animals the formation of both kinds of Ab started about simultaneously. Since ICF antibodies are strictly virus-specific, this result provides further evidence against involvement of a foreign virus in DCFT reported previously (8) and here.

The weaker humoral immune response in NMRI mice carrying strain "W" compared with that in carriers of "WCC" can be explained by assuming a greater immunodepressive effect in the former animals due to a higher viral Ag content of organs of the lympho-reticular system, e. g. the spleen (see Table 2). The higher Ag titer of the brain in carriers of "WCC" had no apparent influence on specific immunodepression.

It is now unlikely that non-specific reactions of carrier sera with normal brain extract indicate formation of virus-host Ag complexes in the course of the persisting infection. A missing parallelism of the DCF curves in Fig. 2 obtained with sera from NMRI mice in tests with carrier and normal brain Ag as well as the result of the absorption tests shown in Table 4 do not favour this possibility.

Some normal sera from adult NMRI and CBA/J mice also gave positive reactions with normal brain Ag, the DCF indices being as high as 50 % in exceptional cases. In pools of normal serum obtained from large batches of mice non-specific Ab in individual sera are diluted out, reducing the non-specific index of the pool to a low level.

In unpublished own experiments the formation of non-specific Ab was markedly enhanced by LCM infection. This may also have happened in the experiment recorded in Fig. 2, especially in 3—5 months old CBA/J mice. The

mean DCF indices of their sera far exceeded those to be expected in normal animals of the same breed and age. It even seems possible that in this case inverse immunosuppression took place, namely, by normal brain Ag vs. viral Ag.

The curves in Fig. 2 obtained with sera from NMRI mice carrying strain "WCC" indicate a rough parallelism between DCF and ICF antibody formation. However, marked differences between DCF and ICF titers of sera from individual carriers were frequently noted as some examples presented in Table 3 show. It is not known whether different Ab or different Ag are the cause of these reactions. Further research with different Ig fractions and the three main structural polypeptides of LCMV (2) is indicated.

Summary

Besides serologically demonstrable antigenic variation (8), which seems to elicit low-level formation of specific CF antibodies in congenital carriers of LCM virus descending from the outbred NMRI stock, other factors were found to influence antibody production in such animals.

An important role is played by the long known immunosuppressive effect of persisting LCM virus which, however, only partially covers the altered antigen.

The genetic constitution of the carrier host also affects antibody synthesis decisively. Thus, it has not been possible to detect specific antibodies either by direct or by indirect CF in congenitally infected inbred CBA/J mice.

A further factor is the virus strain present in carriers. NMRI mice harboring the wild mouse strain "W" produced considerably less antibody than carriers of the old laboratory strain "WCC". This difference has been tentatively correlated with a greater affinity of the former strain for the lymphoreticular cell system and a resulting stronger immunodepressive effect.

No evidence for complex formation between viral and non-specific host antigens has been obtained.

Since the results of direct and indirect CF tests with sera from individual carriers from the NMRI stock often disagreed, one may suspect that either different classes of antibodies or different antigens are operative in these reactions.

Acknowledgements

The author is grateful to Professor A. MAYR for kindly providing laboratory facilities and materials for these studies, and to Miss F. KESTING for excellent technical collaboration.

Zusammenfassung

Einfluß verschiedener Faktoren auf die Bildung spezifischer Antikörper bei persistent mit dem LCM-Virus infizierten Mäusen

Neben einer serologisch nachweisbaren Antigenveränderung (8), die bei congenital infizierten Viruströgern des nicht ingezüchteten NMRI-Stammes die Bildung geringer Mengen von spezifischen komplementbindenden Antikörpern auszulösen scheint, beeinflussen weitere Faktoren die Antikörpersynthese bei solchen Tieren.

Eine wichtige Rolle spielt hierbei die seit langem bekannte immunsuppressive Wirkung von persistierendem LCM-Virus, die jedoch das veränderte Virusantigen nicht vollständig abdeckt.

Auch die genetische Konstitution der Trägertiere beeinflußt die Antikörperbildung entscheidend. So war es nicht möglich, weder durch direkte

noch durch indirekte Komplementbindung spezifische Antikörper bei Virusträgern des ingezüchteten CBA/J-Stammes nachzuweisen.

Ein weiterer Faktor ist der im Trägertier heimische Virusstamm. Mit dem Wildmausstamm „W" infizierte NMRI-Mäuse bildeten wesentlich weniger Antikörper als Träger des alten Laborstammes „WCC". Dieses unterschiedliche Verhalten wird spekulativ mit der stärkeren Affinität des erstgenannten Stammes zum lymphoretikulären Zellsystem und einem hierauf beruhenden, gesteigerten immunsuppressiven Effekt in Zusammenhang gebracht.

Für Komplexbildung zwischen Virusantigen und unspezifischen Wirtsantigen wurden keine sicheren Anhaltspunkte gewonnen.

Da die Ergebnisse der direkten und indirekten Komplementbindung bei Einzelseren von Virusträgern des NMRI-Stammes oft nicht übereinstimmten, kann man vermuten, daß bei beiden Reaktionen entweder verschiedene Antikörperklassen oder unterschiedliche Antigene im Spiele sind.

Résumé

Influence de différents facteurs sur la formation d'anticorps spécifiques chez des souris infectées de façon persistante par le virus LCM

A côté d'une modification de l'antigène sérologiquement mise en évidence qui semble déclencher la formation d'une très faible quantité d'anticorps spécifiques fixant le complément chez des porteurs infectés congénitalement de la souche NMRI non consanguine, d'autres facteurs influencent la synthèse des anticorps chez de tels animaux. L'action immunosuppressive connue depuis longtemps du virus LCM persistant joue un rôle important mais ne couvre pas complètement l'antigène viral modifié.

La constitution génétique du porteur influence également la formation des anticorps de façon déterminante. Il n'a pas été possible ainsi de mettre en évidence des anticorps spécifiques chez des porteurs de virus de la souche consanguine CBA/J par la fixation du complément directe ou indirecte.

La souche virale locale chez l'animal porteur est un autre facteur. Des souris NMRI infectées avec la souche «W» de souris sauvages ont formé nettement moins d'anticorps que des porteurs de la vieille souche de laboratoire «WCC». Ce comportement différent est attribué spéculativement à la plus forte affinité de la première souche mentionnée pour le système cellulaire lymphoréticulaire et en rapport à un effet immunosuppressif augmenté.

On n'a pas trouvé de point de repère concernant une formation d'un complexe entre l'antigène viral et l'antigène non spécifique de l'hôte.

Etant donné que les résultats de la fixation directe ou indirecte du complément n'ont souvent pas correspondu avec les sérums individuels des porteurs du virus de la souche NMRI, on peut supposer qu'il existe dans les deux réactions soit des classes d'anticorps distinctes soit des antigènes différents.

Resumen

El influjo de varios factores sobre la formación de anticuerpos específicos en ratones infectados persistentemente con el virus de la LCM

Al lado de una modificación antigénica (8), demostrable serológicamente, la cual parece desencadenar en los portadores de virus infectados congenitalmente de la estirpe NMRI, no consanguínea, la formación de cantidades escasas de anticuerpos específicos fijadores del complemento, influyen otros factores más sobre la síntesis de anticuerpos en los animales mencionados.

Juega aquí un papel importante la acción inmunosupresiva, conocida desde hace tiempo, del virus persistente LCM, la cual, sin embargo, no cubre por completo el antígeno virósico modificado.

También la constitución genética de los animales portadores influye decisivamente sobre la anticuerpogénesis. Así es que no fué posible el identificar anticuerpos específicos en los portadores de virus de la estirpe consanguínea CBA/J, ni mediante la fijación directa ni indirecta del complemento.

Otro factor más es la estirpe de virus aclimatizada en el animal portador. Los ratones NMRI infectados con la estirpe de ratón salvajino «W» formaban bastante menos anticuerpos que los portadores de la cepa vieja de laboratorio «WCC». Este comportamiento diferente se conecta especulativamente con la afinidad más fuerte de la cepa mencionada en lugar primero para con el sistema celular linforreticular y un efecto inmunosupresivo intensificado que se basa en ella.

No se lograron criterios seguros sobre la formación de complejos entre antígeno virósico y antígeno anfitrión inespecífico.

Puesto que los resultados de la fijación directa e indirecta del complemento en sueros individuales de portadores de virus de la estirpe NMRI no coinciden en muchas ocasiones, se puede sospechar que en ambas reacciones intervienen o clases diferentes de anticuerpos o antígenos diversos.

References

1. Albrecht, H., 1975: Versuche zum Antikörpernachweis durch indirekte Komplementbindung bei der lymphocytären Choriomeningitis der Mäuse. Vet. Med. Dissertation, University of Munich.
2. Buchmeier, M. J., J. H. Elder, and M. B. A. Oldstone, 1978: Protein structure of LCM virus: Identification of the virus structural and cell associated polypeptides. Virology *89*, 133—145.
3. Casals, J., and L. V. Brown, 1954: J. exp. Med. *99*, 421.
4. Oldstone, M. B. A., and F. J. Dixon, 1967: Lymphocytic choriomeningitis: Production of antibody by "tolerant" infected mice. Science *158*, 1193—1195.
5. Oldstone, M. B. A., M. J. Buchmeier, M. V. Doyle, and A. Tishon, 1980: Virus-induced immune complex disease: Specific anti-viral antibody and Clq binding material in the circulation during persistent lymphocytic choriomeningitis virus infection. J. Immunology *124*, 831—838.
6. Traub, E., 1975: Observations on "late onset disease" and tumor incidence in different strains of laboratory mice infected congenitally with LCM virus. I. Experiments with random-bred NMRI mice. Zbl. Vet. Med. B, *22*, 764—782.
7. Traub, E., 1975: Observations on "late onset disease" and tumor incidence in different strains of laboratory mice infected congenitally with LCM virus. II. Experiments with inbred CBA/J mice. Zbl. Vet. Med. B, *22*, 783—792.
8. Traub, E., 1980: Serological evidence for antigenic variation in brains of mice infected persistently with the virus of lymphocytic choriomeningitis. Zbl. Vet. Med. B, *27*, 806—822.
9. Traub, E., and F. Kesting, 1977: Age distribution and serological reactivity of viral antigen in brains of mice infected congenitally with LCM virus. Zbl. Vet. Med. B, *24*, 548—559.
10. Volkert, M., K. Bro-Jørgensen, and O. Marker, 1975: Persistent LCM infection in the mouse. Immunity and tolerance. Bull. of the WHO *52*, 471—478.

Author's address: Prof. Dr. Erich Traub, Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin, Ludwig-Maximilians-Universität, Veterinärstraße 13, D-8000 München 22.