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Age Distribution and Serological Reactivity of Viral Antigen in Brains of Mice Infected Congenitally with LMC Virus

By

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With 2 figures and 3 tables

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In a previous publication (10) it was pointed out that the central nervous system (CNS) seems to be involved to a minor extent in "late onset disease" (3) occurring in NMRI mice infected congenitally with LCM virus. Since persistent virus infections are gaining more and more importance in general neuropathology, it was decided to investigate the role of the CNS in mice with chronic LCM infection in more detail.

The present paper reports results of titrations of viral antigen in brains of congenital carriers of different age and of quantitative complement fixation tests indicating a difference in reactivity between brain extracts and those from spleen and kidneys. Furthermore, an "early onset disease" observed in a small percentage of infected young mice will be described and the pathogenesis of this condition discussed.

Material and Methods

Mice and virus strains: Outbred white NMRI mice congenitally infected with virus strains "W" or "WCC" were used as in earlier work (10). The animals came from the 12th to 20th generation after establishment of the persistent infection in the respective small colonies.

Immune sera (see Table 3): Normal control serum (omitted in Table 3 since it reacted uniformly negative in a dilution of 1 : 10 with all antigens under test) was obtained by bleeding from the heart (0.6 ml. per mouse) in deep ether anesthesia from 33 six week-old females purchased from Ivanovas & Co., Kisslegg, Allgäu.

Immune serum 1 was furnished by 32 of the same animals bled 40 days after subcutaneous (sc) inoculation with 0.2 ml. 1 % brain extract from intra-

cerebral (ic) passage 380 of LCM strain "WCC". The infection had been symptomless in all mice.

Immune serum 2 came from the survivors of this second bleeding, which were given an ic inoculation with 0.05 ml. 10 % brain extract from ic passage 381 of "WCC". Starting 24 hours later, all mice showed a very marked "accelerated reaction" (8, 5) and 4 animals died in typical convulsions 2 to 4 days after the ic injection. The remaining 26 mice recovered slowly and were bled 21 days after the ic test inoculation.

Immune serum 3 was obtained from 24 animals surviving this operation after they had received 3 intraperitoneal (ip) inoculations with 0.2 ml. 10 % brain extract made at 4-day intervals. They showed no reaction to this treatment and were exsanguinated 10 days after the last injection.

Immune sera 4 and 6 were supplied by two different batches of mice which received 1 sc inoculation with 10 % kidney extract from 8 months-old carriers of strain "W" suffering from severe glomerulonephritis. They showed no visible reaction and were hyperimmunized one month later by 5 ip inoculations with 0.2 ml. of the same, in the meantime deep-frozen, extract given at 4 to 6-day intervals. The animals were exsanguinated 7 days after the last inoculation.

Immune serum 5 originated from guinea pigs injected sc into the foot pads with 0.5 ml. 10 % organ extract (prepared from about equal parts of spleen, liver, kidneys and lung, not brain) from the first guinea pig passage after 372 sc mouse passages of strain "WCC", whose pathogenicity for guinea pigs is very low. 32 days later the animals received sc into each foot pad 1 ml. of the same, in the meantime deep-frozen, extract to which 0.75 mg./ml. saponin was added. This treatment was repeated 3 days later. Serum was obtained 14 days after the last injection.

Titration of complement-fixing (CF) antigen in brain extracts: Donors of brain tissue originated from the two breeding stocks persistently infected with strains "W" and "WCC", respectively. They had not received any inoculations. Brain suspensions from individual mice to be titrated contained 20 % (w/v) instead of 10 % tissue. Otherwise the same procedure was followed as described elsewhere (10). AD_{50} titers determined graphically are the highest dilutions of infectious brain tissue giving 50 % complement (C′) fixation at 37 °C for 30 min. Mean AD_{50}'s of brains from individual mice falling into certain age groups as indicated in Fig. 1 were calculated later. These groups averaged 19 animals carrying strain "W" and 13 mice infected with "WCC". Due to marked differences between individual brain titers even in litter mates, it was necessary to include large enough numbers of animals in every group in order to avoid errors.

The tests shown graphically in Fig. 1 extended over more than one year and could therefore not be carried out with exactly the same reagents. We tried to keep experimental conditions as constant as possible. Preserved C′ (obtained from Behringwerke, Marburg/Lahn) was titrated before each series of simultaneous tests and the C′ dose adjusted accordingly. A change was rarely necessary. Above all, the same hyperimmune serum from guinea pigs (1 : 40) was used throughout.

"Checkerboard" CF tests: Serum was diluted in phosphate-buffered saline in two-fold steps starting from a 1 : 10 dilution. Serial antigen dilutions also were two-fold beginning with 20 % organ extracts (brain, spleen or kidneys) from 4 to 8 months-old carriers of "WCC". These extracts were considered as 1 : 5 dilutions of the respective tissues. Different antigens were used in the 3 tests recorded in Table 3.

In order to overcome the anticomplementary (a. c.) effect of tissue extracts in lower dilutions, which is particularly disturbing with kidney extracts, 8 % C′ was uniformly employed in all tests. This amount corresponded to about $2^1/_2$ lytic units when titrated in presence of buffered saline under the same conditions (time and temperature) as in the test proper. It caused complete hemolysis in all antigen controls (without serum) containing brain or spleen extract, undiluted and in serial dilutions. The a. c. activity of these extracts was about equally low. Kidney antigens, on the other hand, had such a strong a. c. effect that the antigen controls with 20 % extract and serial dilutions down to 1 : 16 were only partially lysed, the a. c. activity decreasing with dilution. Before calculating the SD_{50}'s and AD_{50}'s, which was always done in the zone of equivalence, we therefore subtracted the estimated percentages of unlysed cells in the antigen controls from those for the corresponding tubes containing antigen plus serum. None of the sera used were a. c. in dilutions of 1 : 10 or higher.

Cold fixation for 21 hours at + 3 °C and lysis at 37° for 30 min. were practised in all tests. For further technical details we refer to a previous description of direct CF tests (10).

Indirect CF (ICF) tests: A technique described previously (10) was employed. Due to the small amount of serum available in some cases, the number of C′ doses was reduced to 2. Usually, 6 and 7 % C′ was used, corresponding to about 1.5 and 1.75 lytic units when titrated at 37 °C for 30 min.

Sera from congenital carriers of different age, infected with strain "W" or with "WCC" (ratio about 3 : 2), were tested individually. The donors were divided into various age groups (see Fig. 1) averaging 24 mice of similar age, and the percentage of positive reactors was calculated for each group.

Experiments and Results

CF antigen content of the brain in congenital carriers of different age. The curves presented in Fig. 1 for mice infected either with strain "W" or with "WCC" are not alike during the first 3 weeks of life but, on the whole, a common principle can be recognized. A fall of the antigen titer during the first 3 months was followed by a more or less rapid rise at older age. A higher degree of neurotropism of "WCC" is evident as in earlier tests (10).

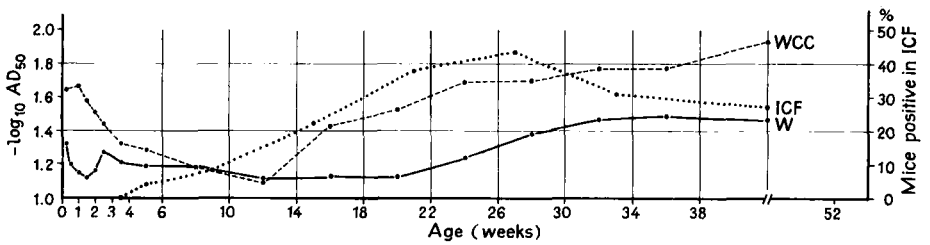

Fig. 1. Antigen titers of the brains in mice of different age infected congenitally with virus strain "W" (————) or with "WCC" (— — — — —), and percentages of mice of various age groups carrying antibodies demonstrable by ICF (.....).

A curve from another experiment indicating the formation of LCM-specific antibodies by congenitally infected mice of various age groups was reproduced in Fig. 1 in order to facilitate a comparison with the curves for cerebral antigen titers. The dotted line shows that antibodies demonstrable by ICF first appeared in a small percentage of animals 4—6 weeks old with a

maximum at about 27 weeks, followed by a gradual decrease at higher age. This result confirms and extends earlier observations made in our laboratory (1).

Weight increase of the spleen in young mice infected congenitally in comparison with normal NMRI mice of the same age. The infected animals were carriers either of strain "W" or of "WCC" with a ratio of 18 : 13. Spleen weights in mgms. are recorded in Table 1 in which it can be seen that during the first 3 weeks of life the mean spleen weight was slightly lower in infected mice than in their normal counterparts. This situation was reversed at 4 weeks. Further reversals occurred at 6 and 8 weeks. The latest increase in congenital carriers lasted until the 10th week, when the weighings were discontinued.

Table 1

Spleen growth during the first 10 weeks of life in congenitally infected and normal NMRI mice

Age (days)	Congenital carriers		Normal mice		Infected minus normal (mgms.)
	No.	Mean spleen weight (mgms.)	No.	Mean spleen weight (mgms.)	
1	35	2.5	21	2.8	- 0.3
2	61	4.8	15	6.0	- 1.2
4	4	12.5			
7	41	19.0	26	21.9	- 2.9
9 - 11	4	25.0			
14	14	49.0	14	55.0	- 6.0
16	3	60.0			
21	17	110.6	14	139.3	- 29.3
23 - 26	4	140.0			
28	16	174.4	16	147.5	+ 26.9
32 - 33	5	208.0			
35	4	187.5	10	165.0	+ 22.5
36 - 40	7	147.1			
42	4	152.5	10	164.0	- 11.5
43 - 48	6	163.3			
49	3	150.0	10	161.0	- 11.0
53 - 55	4	160.0			
56	7	171.4	8	156.2	+ 15.2
57 - 60	9	180.0			
63	4	180.0	6	148.3	+ 31.7
70	7	181.4	3	150.0	+ 31.4

Artificial production of disease in newborn mice infected congenitally. When newborn congenital carriers were inoculated ic with mouse brain virus from a high serial passage of strain "WCC" which, in contrast to "W", is highly pathogenic for normal baby mice infected by this route, some animals became ill. Such inoculations were practised for some time to confirm the congenital infection before these mice were taken into experiments. Our records show that 124 (= 11.6 %) of 1073 baby mice tested in this manner presented signs of LCM-disease 1—2 weeks later. Some of them died, some became

runts and others recovered quickly. As a rule, not all baby mice of an inoculated litter showed signs of disease. Virus isolated from sick or moribund animals usually had the pathogenic properties of the strain carried and transmitted vertically by the mother, not those of the inoculated virus. This agrees with previous observations (9).

There was a striking numerical discrepancy between the incidence of disease in inoculated newborn carriers and the results of tests for CF antigen in embryos or newborn baby mice from congenitally infected females: All of 170 embryos from 20 litters and 98 newborns tested individually contained specific antigen. As a rule, only 2 baby mice from a newborn litter were sacrificed so that these tests covered a large spectrum of litters infected either with strain "W" or with "WCC".

Spontaneous cases of "early onset disease" observed in young congenital carriers. Spontaneous disease with ill-defined spastic nervous symptoms was occasionally seen during the last few years in uninoculated 1—2 week old baby mice from our persistently infected breeding stocks including CBA/J mice. In every case, not all baby mice of the litter were affected. The symptomatology reminded strongly of LCM, but we did not see those typical convulsions with outstretched hind legs which characterize the acute disease in adult mice. Sick animals usually died or were eaten by the mother, especially since cannibalism has increased in our animals lately. Rarely, a diseased mouse survived and became a runt. Such acute cases in infantile mice may remain unrecognized if the animals are not checked daily and taken out of their nests for inspection.

We are unable to give precise figures on the incidence of this disease because we do not exactly know how many baby mice were sick before they were eaten by the mothers. At any rate, the percentage of spontaneous cases was considerably lower than that in newborn carriers inoculated with "WCC" (11.6 %). It may perhaps lie between 1 and 2 %.

Recently, a number of 1—4 months-old congenital carriers, mostly infected with "WCC", from batches of NMRI mice kept under observation for other purposes died unexpectedly without forewarning (see Table 2). Most of these animals succumbed overnight or over weekends and were more or less decomposed when found in the morning. One mouse, however, was seen dying (F. K.) in a typical spontaneous convulsion at the age of 57 days. Several

Table 2

Unexpected sudden deaths among congenital carriers 1—4 months old

Virus strain	Age (days)	Autopsy findings	AD_{50} of brain
W (118)*	35	slightly decomposed, marked general congestion	1.11
	38	decomposed, discarded	
WCC (93)*	45	decomposed, tongue hanging out, discarded	
	46	typically stretched out, still in rigor mortis, congested	1.14
	47	moderately decomposed, no definite changes, discarded	
	48	decomposed, partly eaten up by cage mates, discarded	
	57	slightly decomposed, tongue hanging out	0.90
	57	seen dying in typical convulsion, general congestion	0.91
	100	decomposed, discarded	
	122	moderately decomposed, no definite changes, discarded	

* No. of mice under observation

hours before, a 46 day-old mouse had been found dead in another cage. It was still in rigor mortis and stretched out in typical posture, apparently another victim of an acute LCM attack. This is also likely for 2 other mice found dead when 45 and 57 days old with their congested tongues hanging out of their mouths, a picture occasionally seen before in mice succumbing to the acute disease. There was no evidence of kidney involvement in any animal.

CF antigen titers of the brains, still measurable in 4 mice (see Table 2), were low and therefore in accordance with mean titers recorded in Fig. 1 for the respective age span.

Other animals of the second group in Table 2 carrying "WCC" virus did not show tremors or convulsions, but remained exceptionally slender for a long time. This was also noticed in young breeders which, in addition, either failed to become pregnant or conceived only at higher age when they had recovered from a probable non-fatal attack of "early onset disease".

In a comparable batch of 49 normal NMRI mice there was no mortality in the age span between 1 and 4 months and the animals developed normally.

Mode of reaction of mouse brain antigen in comparison with spleen and kidney antigens in quantitative direct CF tests. Results of 3 "checkerboard" tests are recorded in Table 3 in which it can be seen that all immune sera reacted better with brain antigens than with those from spleens and kidneys as shown by the SD_{50}'s in the respective columns. That this is not due to a higher antigen content of brain tissue is evident from the AD_{50}'s which invariably indicate the opposite.

Table 3

Results of "checkerboard" tests with 6 immune sera and brain, spleen and kidney antigens from congenitally infected mice

Test No.	Immune sera		Antigens					
	No.	Origin	Brain		Spleen		Kidney	
			SD_{50}*	AD_{50}*	SD_{50}	AD_{50}	SD_{50}	AD_{50}
1	1	mice convalescent from sc infection with mouse brain virus ("WCC")	1.57 1 : 37	1.27 1 : 19	1.12 1 : 13	2.20 1 : 158	1.19 1 : 15	2.46 1 : 288
	2	same mice, hyperimmunized once with mouse brain virus ("WCC")	2.62 1 : 417	1.41 1 : 26	2.27 1 : 186	2.60 1 : 398	2.38 1 : 240	2.43 1 : 269
	3	same mice, h. i. 4x with mouse brain virus ("WCC")	2.58 1 : 380	1.42 1 : 26	2.17 1 : 148	2.37 1 : 234	2.25 1 : 178	2.41 1 : 257
	4	mice h. i. with mouse kidney virus ("W")	2.15 1 : 141	1.40 1 : 25	1.79 1 : 62	2.50 1 : 316	2.02 1 : 105	2.63 1 : 427
	5	guinea pigs h. i. with virus from g. pig organs ("WCC")	2.66 1 : 457	1.72 1 : 52	2.53 1 : 339	2.65 1 : 447	2.61 1 : 407	2.64 1 : 436
2	1	see above	1.60 1 : 40	1.95 1 : 89	1.08 1 : 12	2.10 1 : 126		
	3	see above	2.86 1 : 724	2.07 1 : 117	2.36 1 : 229	2.36 1 : 229		
3	2	see above	2.92 1 : 832	2.01 1 : 102	2.31 1 : 204	2.62 1 : 417	2.46 1 : 228	2.36 1 : 229
	6	mice h. i. with mouse kidney virus ("W")	2.36 1 : 229	1.95 1 : 89	1.93 1 : 85	2.64 1 : 436	1.98 1 : 95	2.68 1 : 479
	5	see above	2.78 1 : 603	2.12 1 : 132	2.65 1 : 447	2.73 1 : 537	2.66 1 : 457	2.55 1 : 355

* negative $\log_{10}$ with antilog

However, the differences between the SD_{50}'s in presence of the various antigens were not strictly the same with all sera. According to a calculation of arithmetical means from tests 1 and 3 in Table 3, the mean SD_{50} of sera 1—3 (from mice immunized with mouse brain virus) versus brain antigen differed by 0.455 log (= 2.8-fold) from that vs. spleen antigen, and by 0.352 log (= 2.2-fold) from that vs. kidney antigen. The corresponding values for sera 4 and 6 from mice immunized with mouse kidney virus are 0.395 log (= 2.5-fold) and 0.255 log (= 1.8-fold). With immune serum 5 from guinea pigs the differences between the mean SD_{50}'s in presence of the 3 kinds of antigen were smaller: 1.13 log (= 1.3-fold) and 0.085 log (= 1.2-fold), respectively.

SD_{50}'s of immune sera 2 and 5 in presence of serial dilutions of mouse brain and spleen antigens are shown graphically in Fig. 2. It can be seen there that both sera reacted in higher dilutions with brain than with spleen extract in spite of the higher antigen content of the latter. The curves also show that guinea pig immune serum differentiated the two antigens less well than murine immune serum.

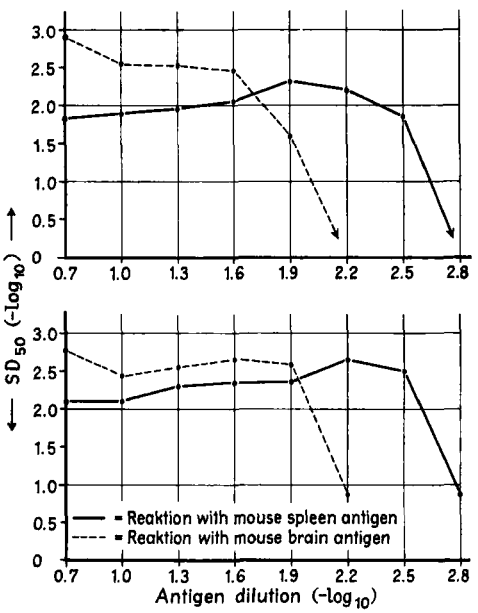

Fig. 2. Titration by direct CF of mouse immune serum No. 2 (top curves) and of guinea pig immune serum No. 5 (bottom curves) against serial dilutions of mouse spleen and brain antigens

A comparison of the potency of immune sera 2 and 3 in Table 3 indicates that 3 additional ip inoculations after the ic immunity test failed to increase the amount of specific CF antibodies in the serum of the recipients.

Discussion

The previously described decrease of the antigen titer of the kidneys during the first few weeks of life (10, 11) and that of the brain titer reported here undoubtedly have a common cause. It becomes more and more likely that this phenomenon is due to an abortive immune reaction of the host. The following observations support this concept:

(a) the occurrence of acute cases of "early onset disease" involving the CNS as described above. Nervous symptoms and other signs of acute disease except glomerulonephritis are generally ascribed to a cellular immune response of the host (2).

(b) fluctuation of the spleen weight (Table 1) during the age span in which such cases occur. At the height of the acute disease in adult NMRI mice

injected ip with "WCC" the spleen is considerably enlarged, sometimes reaching 2 to 3 times its normal weight (6). A weight increase of the lymph nodes goes roughly parallel with that of the spleen. Infectious virus and viral antigen are eliminated rapidly in such animals (6). On the other hand, spleen and lymph nodes are often small in mice very sick from infection with an "aggressive" strain, and the virus persists much longer in survivors (8). In congenital carriers the fluctuating spleen weight may indicate repeated futile attempts of the host to get rid of the infection. This fluctuation may also be correlated with irregularities in the antigen curves of brain (Fig. 1) and kidneys (10) from NMRI baby mice during the first three weeks of life.

(c) formation of LCM-specific antibodies demonstrable by ICF by congenitally infected mice as shown in Fig. 1. It starts at an age level where cases of "early onset disease" occur but is apparently preceded by a cellular immune response leading to illness in baby mice as young as 1—2 weeks.

Both the hypothetical cellular reaction and the antibody response are weak compared with those in non-tolerant mature mice. Except in one very rare case (10), they never accomplished complete elimination of viral antigen. On the contrary, both the kidney titer (10) and the cerebral antigen titer (Fig. 1) rose with increasing age.

In previous experiments (10), a temporary low of the kidney titer with a minimum at 4—5 weeks could be demonstrated in litter mates from all of 16 litters borne by congenitally infected NMRI females. From this result it may be tentatively concluded that a cellular immune reaction is much more frequent in young congenital carriers than is indicated by the low percentages of clinical cases observed. In other words, the immune response does not seem to lead to overt disease in the majority of such animals.

The question of why the antigen titer of the spleen did not show a significant decrease at any age level (10, 11) cannot be answered at present.

The higher morbidity rate in newborn carriers inoculated ic with "WCC" virus compared with that in uninoculated baby mice is probably not due to missing resistance to superinfection in the former animals, since virus isolated from their brains usually had the pathogenic properties of the strain carried by the mother. It rather seems that the cerebral trauma somehow effected a localization of the disease process in the brain. This reminds one of the first isolation of LCM virus from mice (7), namely, from presumptive carriers inoculated ic with inert material.

Nervous symptoms shown by young congenital carriers differed from those in old mice. In these we never saw spasms but frequently noticed changed behavior, nervousness, sometimes a wobbly gait and, in one case, circling movements. Some animals appeared more or less emaciated. Clinical signs of CNS involvement were more pronounced and much more frequent in old carriers of "WCC" than in those of strain "W". This may be correlated with the higher content of viral antigen (and probably of infectious virus as well) in brains of the former mice. All animals which had shown symptoms invariably had relatively high cerebral antigen titers, thus reminding one of the high antigen content of the kidneys in congenital carriers showing glomerulonephritis (10). The absence of more severe nervous symptoms in such mice is nevertheless surprising and further proof for the low pathogenicity of the virus itself, even for the CNS.

Results reported here may be interpreted as indicating that mice infected congenitally have only a partial tolerance towards LCM virus, thus lending support to the view of OLDSTONE and DIXON (4) who denied the existence of tolerance in such animals altogether. It seems, however, that the possibility has

thus far not been excluded that the immune reaction described above may be directed against "new antigens" (virus-host-complexes) more or less organ-specific and not fully covered by the tolerance of the host for LCM virus per se. Results of a serological study to be published shortly point in this direction.

The tests recorded in Table 3 and Fig. 2 may indicate a qualitative difference between brain antigen on one hand and spleen and kidney antigens on the other, but they do not completely rule out an effect of inhibitors of some sort, possibly present in spleen and kidney extracts. These antigens were differentiated more clearly by murine immune sera than by immune serum from guinea pigs.

Summary

In continuation of previous studies on the age distribution of viral antigen in spleen and kidneys of mice infected congenitally with LCM virus, the brain was subjected to a similar investigation since it participates to some extent in "late onset disease" in such animals.

The antigen curve obtained differed from that for the spleen but resembled the curve for the kidneys in that an initial decrease of the antigen content in young mice was followed by a continuous rise later in life. Cerebral antigen titers in newborns and in old mice were considerably higher in carriers of the "neurotropic" strain "WCC" than in those infected with the "natural" strain "W". In old carriers of these strains there was a clear correlation between the cerebral antigen titer and the frequency and intensity of nervous symptoms which, in general, were not very striking but clearly recognizable to an experienced observer.

In young congenital carriers cases of "early onset disease" characterized by spastic symptoms were observed. The latter were missing in old mice.

"Early onset disease" and the temporary decrease of the cerebral and renal antigen titers in young animals are attributed to an abortive cellular immune response not strong enough to eliminate viral antigen completely from the organs. Specific antibodies, demonstrable by indirect CF in 43 % of the mice reaching the age of 6—7 months, were likewise ineffective in this respect.

In quantitative direct CF tests, immune sera from non-tolerant mice and, to a lesser degree, an immune serum from guinea pigs reacted better with brain extracts from congenitally infected mice than with extracts of spleens or kidneys in spite of the lower antigen content of the brain. This result seems to indicate a qualitative difference between the respective antigens.

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Zusammenfassung

Altersverteilung und serologische Reaktivität von viralem Antigen in Gehirnen von kongenital mit LCM-Virus infizierten Mäusen

Anknüpfend an frühere Untersuchungen über die Altersverteilung von Virusantigen in Milz und Nieren von congenital infizierten Mäusen wurde in ähnlicher Weise das Gehirn geprüft, da es in gewissem Maße an Späterkrankungen bei solchen Tieren beteiligt ist.

Die erzielte Antigenkurve unterschied sich von der für die Milz, ähnelte jedoch der Nierenkurve insofern, als jeweils einem Abfall des Antigengehaltes bei jungen Mäusen ein kontinuierlicher Anstieg im späteren Alter folgte. Die cerebralen Antigentiter bei neugeborenen und alten Mäusen lagen bei den mit dem „neurotropen" Virusstamm „WCC" infizierten Virusträgern wesentlich höher als bei den mit dem Naturvirus „W" infizierten Tieren. Bei älteren Virusträgern bestand eine eindeutige Beziehung zwischen dem Antigengehalt des Gehirns und der Häufigkeit und Stärke nervöser Symptome, die zwar im allgemeinen nicht sehr auffällig, für den erfahrenen Beobachter aber klar zu erkennen waren.

Bei jungen congenital infizierten Virusträgern wurden Früherkrankungen mit spastischen Symptomen beobachtet, die bei spät erkrankten älteren Mäusen fehlten.

Diese Früherkrankungen und das Absinken des Antigengehaltes von Gehirn und Nieren bei Jungmäusen werden als Folgen einer abortiven zellulären Immunreaktion angesehen, die jedoch nicht stark genug war, um das Virusantigen aus den Organen vollständig zu eliminieren. Ebenfalls wirkungslos in dieser Hinsicht waren spezifische Antikörper, die durch indirekte Komplementbindung bei 43 % der Mäuse nachgewiesen wurden, welche das Alter von 6—7 Monaten erreichten.

In quantitativen direkten KB-Testen reagierten Immunseren von nicht toleranten Mäusen und, in geringerem Maße, ein solches von Meerschweinchen stärker mit Gehirnextrakten von congenital infizierten Mäusen als mit Milz- und Nierenextrakten, obwohl die ersteren weniger Virusantigen enthielten. Dieses Ergebnis deutet auf einen qualitativen Unterschied zwischen den betreffenden Antigenen hin.

Résumé

Répartition selon l'âge et réaction sérologique d'un antigène viral dans des cerveaux de souris infectées congénitalement avec un virus LCM

En relation avec des recherches antérieures sur la répartition selon l'âge d'un antigène viral dans la rate et les reins de souris infectées congénitalement, on a examiné de la même façon le cerveau étant donné sa participation dans certaines circonstances à des maladies retardées chez quelques animaux. La courbe antigénique obtenue s'est différenciée de celle de la rate et ressemblait à celle des reins alors que de temps en temps une augmentation continue à un âge plus avancé a fait suite à une chute du taux d'antigène chez des jeunes souris. Le taux antigénique cérébral des nouveaux-nés et des souris âgées se situait à un niveau nettement plus élevé chez les porteurs du virus infectés avec une souche «WCC» neurotrope que chez les animaux infectés avec un virus sauvage «W». Il a existé un rapport significatif chez des porteurs plus âgés entre le taux antigénique du cerveau et la fréquence et l'intensité des symptômes nerveux qui n'étaient en général pas très apparents mais toutefois nettement reconnaissables pour l'observateur expérimenté.

Des signes de maladie précoce avec des symptômes spastiques ont été observées chez des jeunes porteurs du virus infectés et ont fait défaut chez des souris plus âgées tombées malades tardivement. Ces signes de maladie précoces et la chute du taux des antigènes du cerveau et des reins chez des jeunes souris ont été attribués à une réaction immunitaire cellulaire abortives qui ne fut toutefois pas assez forte pour éliminer complètement l'antigène viral des organes. Des anticorps spécifiques, mis en évidence par la

réaction de fixation du complément indirect chez 43 % des souris, atteignant l'âge de 6—7 mois, furent également sans effet de ce point de vue.

Les immunsérums des souris non tolérantes et dans une moindre mesure un sérum de cobaye ont réagi plus fortement dans des tests quantitatifs de fixation du complément direct avec des extraits de cerveau des souris infectées congénitalement qu'avec des extraits de rate et des reins, bien que les premiers contenaient moins d'antigène viral. Ce résultat montre une différence qualitative entre les antigènes concernés.

Resumen

Distribución con arreglo a la edad y reactividad serológica de antígeno viral en cerebros de ratones infectados con virus LCM por vía congénita

Enlazando con investigaciones anteriores sobre la distribución con arreglo a la edad de antígeno viral en bazo y riñones de ratones infectados por vía congénita, se contrastó de manera semejante el cerebro, ya que participa en cierta medida en las enfermedades tardías en tales animales.

La curva antigénica conseguida se diferenciaba de la esplénica, aunque se asemejaba a la curva renal en cuanto que cada vez seguía a un descenso del contenido antigénico en ratones jóvenes un aumento continuo en la edad posterior. Los títulos antigénicos cerebrales en ratones recién nacidos y viejos se hallaban en los portadores de virus infectados con la estirpe virósica «neurotropa» «WCC» bastante más elevados que en los animales infectados con el virus natural «W». En los portadores mayores de virus existía cierta relación inequívoca entre el contenido antigénico cerebral y la frecuencia e intensidad de los síntomas nerviosos, los cuales, aun sin ser muy llamativos, podían ser reconocidos con claridad por un observador experto.

En portadores jóvenes de virus, infectados congénitamente, se observaron enfermedades precoces con síntomas espásticos, los cuales faltaban en los ratones mayores que enfermaban más tarde.

Estas enfermedades precoces y el descenso del contenido antigénico en cerebro y riñones en ratones jóvenes se conceptuan como secuelas de una reacción inmunológica celular abortiva, la cual, sin embargo, no era tan fuerte como para eliminar por completo el antígeno viral de los órganos. Tampoco tenían efecto en este aspecto los anticuerpos específicos, los cuales se identificaban mediante fijación indirecta del complemento en el 43 % de los ratones que alcanzaron los 6—7 meses de edad.

En tests cuantitativos directos de FC reaccionaron los sueros inmunes de ratones no tolerantes y, en medida más reducida, uno semejante de cobayas más fuerte con extractos cerebrales de ratones infectados por vía congénita que con extractos esplénicos y renales, a pesar de que los primeros contenían menos antígeno viral. Este resultado indica hacia una diferencia cualitativa entre los antígenos correspondientes.

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