Interaction of Graft-Versus-Host Reaction and Lymphocytic Choriomeningitis Infection in Mice: Histopathological Changes

In an earlier note[1] we reported that in mice simultaneous occurrence of graft-versus-host (GVH) disease and lymphocytic choriomeningitis (LCM) virus-infection results in (a) reduction of neurological manifestations and early mortality due to LCM-infection and, on the other hand, in (b) enhancement of the runting syndrome connected with GVH-reaction. These conclusions were drawn on the basis of clinical criteria. The present paper describes the histopathological findings in these animals, thus giving a morphological basis for our earlier observations.

Material and method. The GVH-reaction was produced in 6–8 week-old (C57Bl × CBA) F_1 hybrid mice[1]; LCM virus was inoculated intracerebrally on the 7th day following cell transfer. Number, grouping and treatment of animals are shown on Table I. From the different groups, altogether 49 animals were studied histologically, using hematoxylin and eosin staining.

Results. Control mice showed no clinical or histological signs of disease. Mice on the Control + LCM group all succumbed between the 6th and 9th day following virus inoculation, presenting typical neurological and histological signs of lymphocytic choriomeningitis (Figure 1a).

Most of the mice of the GVH-group developed overt signs of runting. In this group, starting from the 9th day, a slowly progressing mortality rate was observed, with the bulk of animals succumbing between day 25 and 72 after cell transfer; mice surviving this term were sacrificed. Histological study of the spleens showed with a few exceptions marked reduction of lymphocytes and follicles; in one instance some amyloid deposit was observed. On the basis of histological evidence it could be estimated that – in concordance with clinical findings – GVH disease developed in the overhelming majority of mice of this group.

Mice in the GVH + LCM group presented a mortality rate falling between that of Control + LCM, respectively GVH-group. In most of the animals of GVH + LCM group, the clinical signs of runting appeared earlier and were more pronounced than in the GVH group; the mice all succumbed between day 14 and 45 after cell transfer. Neurological symptoms were seen only in some of the mice, dying in the earlier period of the experiment.

In the spleens of 25 out of 27 animals examined histologically, lymphoid atrophy and hyperplasia of reticular cells as histological expression of GVH-reaction could be demonstrated (Figure 2). In the spleens of 6 of these animals, a different grade of amyloidosis was found (Figure 3, Table II). In 2 out of the 27 mice, no histological signs of GVH disease were found; they died at the same time as LCM-controls and showed previously no clinical signs of runting. In the group as a whole, a clear correlation between clinical signs, spleen-histology and presence of meningeal infiltration could be observed. In

Table I. Number, grouping and treatment of mice

Groups	No. of animals	Treatment	
		Spleen-cells i.v.	LCM-virus i.c.
1. Control	10	50×10^6 ($C_{57}Bl \times CBA)F_1$ (isologous)	–
2. Control + LCM	6	50×10^6 ($C_{57}Bl \times CBA)F_1$ (isologous)	300 DL/50
3. GVH	15	50×10^6 $C_{57}Bl$ (homologous)	–
4. GVH + LCM	31	50×10^6 $C_{57}Bl$ (homologous)	300 DL/50

[1] M. Koltay, I. Virág, Zs. Bános, P. Anderlik and I. Szeri, Experientia *24*, 63 (1968).

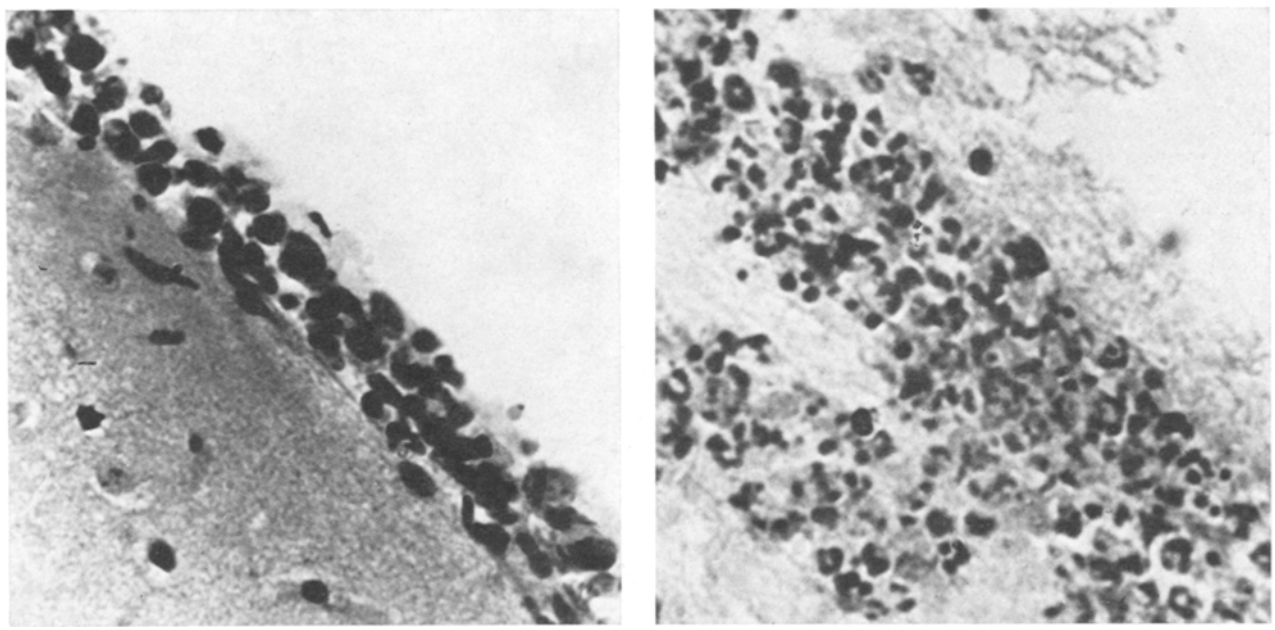

Fig. 1. (a) Meninx of mice infected with LCM virus. Meningeal infiltration consisting of lymphocytes. Haematoxylin and eosin stain. × 480. (b) Meninx of mice undergoing GVH-disease and infected with LCM virus. Polymorphonuclear infiltration of the meninges and adjacent brain tissue. Haematoxylin and eosin stain. × 480.

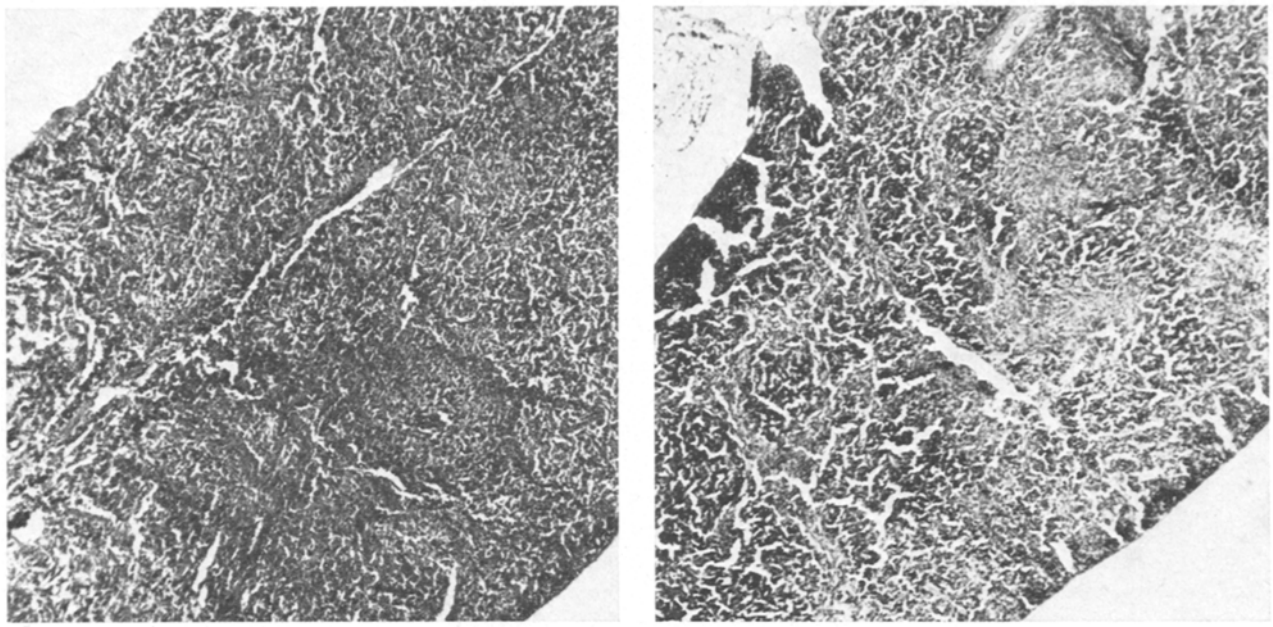

Fig. 2. (a) Spleen of control mice. The pulp is rich in cells, most of them being leukocytes; the follicles are well developed. Haematoxylin and eosin stain. × 120. (b) Spleen of mice undergoing GVH-reaction. The pulp is depleted of cells, the follicles are small or absent. Haematoxylin and eosin stain. × 120.

the 12 mice presenting clean-cut evidence of GVH-disease with a high percentage of amyloid deposits in the spleen, no neurological signs and, correspondingly, no meningeal infiltration was seen. In these animals, a virus-carrier state could be demonstrated. In 13 other mice, presenting less pronounced clinical and histological signs of GVH-disease and succumbing mostly in the earlier phase of the experiment, brain histology showed leukocytic meningo-encephalitis (Figure 1b). Finally, in the 2 mice without clinical and histological signs of runting, i.e. escaping GVH-disease, the course of LCM-infection showed no difference as compared with controls (Table II).

Discussion. It is well established that the course of LCM-infection in mice can be altered, i.e. some of the early ill effects of the virus counteracted by immunosuppressive influences[2-7]. On the other hand, in an earlier study we documented the profound immunosuppression of mice undergoing GVH-reaction[8].

The histological findings presented here substantiate, on a morphological basis, our conclusions drawn from clinical evidence concerning the complexity of interaction between GVH-disease and LCM-infection in mice[1]. They show that runting syndrome connected with GVH-disease is furthered by LCM-infection. Histologically, this

Table II. Histological changes in mice of the GVH-LCM group

Histological changes				Day of succumbing after infection with LCM-virus
Frequency	Spleen	Meninges		
	Lymphocytes and follicles	Lymphocytic infiltration	Leukocytic infiltration	
12/27	Severely decreased or absent (Amyloidosis in 6/12)	– (Virus-carrier state)	–	9–37
13/27	Decreased	±	+++	4–9
2/27	Normal	+++	–	7–8

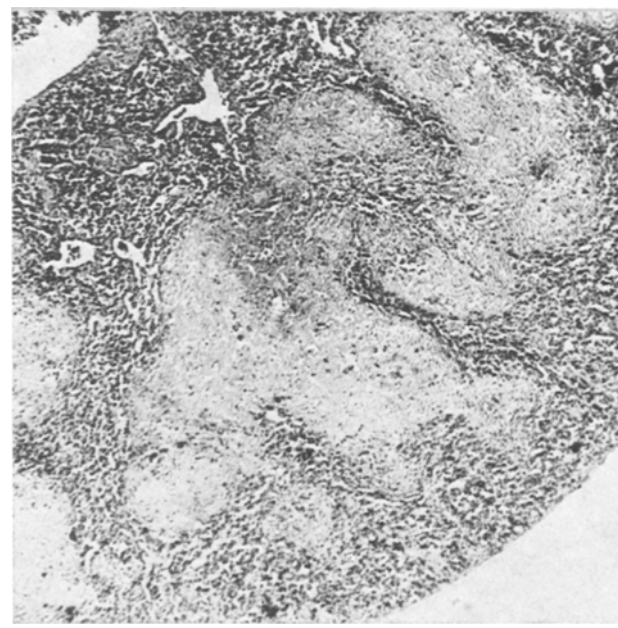

Fig. 3. Spleen of mice undergoing GVH-reaction and infected with LCM virus. Abundant amyloid deposits corresponding to the follicles. Haematoxylin and eosin stain. × 120.

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was demonstrated by the important lymphoid depletion and by the increased frequency of amyloidosis. The latter can be interpreted as a sign of immunological exhaustion[9,10], resulting in our case probably from the joint action of GVH-disease and the antigen-load represented by the LCM-infection.

Further – as morphological basis for the protecting effect of GVH-disease against some ill effects of LCM-infection – an inverse correlation between clinical and histological evidence of runting and typical manifestations of LCM-virus infection was demonstrated. Central nervous system changes typical to LCM-infection appeared only in the few mice escaping GVH-disease, whereas in the others either the central nervous system was not damaged, or, instead of lymphocytic choriomeningitis, a peculiar leukocytic meningoencephalitis developed. Though routine bacteriological examination of the organs of these animals did not show bacterial contamination, infection caused possibly by special microbial agents cannot be ruled out. In this case, the leukocytic infiltration would be merely the result of the increased susceptibility to infections. Notwithstanding, as this finding was observed exclusively in this group of animals, the peculiar meningoencephalitis could also be attributed to the LCM-virus itself, eliciting special and atypical reaction in an organism with impaired immunological functions.

Résumé. Comme conséquence de l'interaction de la maladie homologue produite dans des souris F_1 hybrides adultes et de l'infection avec le virus de la chorioméningite lymphocytique, les signes histologiques de la maladie homologue étaient aggravés. D'autre part, une action protectrice inversement proportionnelle à la gravité des altérations histologiques attribuables à la maladie homologue vis-à-vis des manifestations neurologiques de l'infection avec ce virus était évidente.

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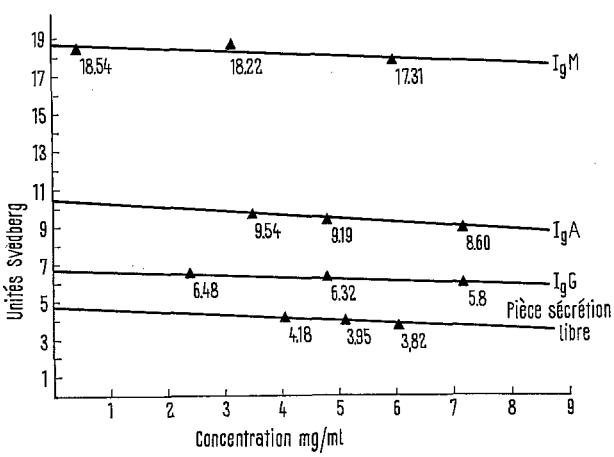
Une IgA à coefficient de sédimentation 11 S dans les sécrétions externes de l'espèce bovine[1]

Depuis que Tomasi[2] et Hanson[3] ont démontré la présence d'une IgA particulière, en concentration relativement élevée, dans la salive et le colostrum humain, ce type d'IgA dites de sécrétion, composée d'un dimère d'IgA sérique plus une pièce de sécrétion a été recherché dans plusieurs espèces. Pour l'instant, seuls Cebra[4] et Sell[5] ont pu isoler, dans le colostrum de lapin, une IgA à coefficient de sédimentation 11 S analogue à celle décrite dans les sécrétions externes humaines. Dans l'espèce bovine, étudiée par les spécialistes des protéines du lait, la situation semblait tout à fait différente. Il existait bien une immunoglobuline très abondante dans le colostrum, isolée par Blanc[6] en 1964, mais cette protéine ne différait pas de son homologue sérique. Il est maintenant admis que cette immunoglobuline, qui ne contient qu'une faible quantité de glucides et dont le coefficient de sédimentation est de 7 S, est une IgG et non pas une IgA (Aalund[7], Hammer[8]). Elle est appelée IgG_1 pour la différencier de la deuxième γ-globuline bovine de mobilité électrophorétique plus lente appelée IgG_2. Le but de ce rapport est de signaler la présence d'une faible quantité d'IgA 11 S dans le colostrum et la salive bovine et de comparer son coefficient de sédimentation avec celui de l'IgG et de l'IgM, isolées aussi à partir du colostrum bovin.

Cette IgA bovine de mobilité β_2 à γ, a été repérée dans le colostrum bovin total en immuno-électrophorèse par un immunsérum dirigé contre les protéines spécifiques au colostrum (immunsérum anticolostrum absorbé par le sérum bovin). Sa purification a été obtenue par un fractionnement sur DEAE cellulose en paliers et par deux filtrations sur gel de Sephadex G 200. Sur DEAE cellulose cette protéine est éluée avec un tampon phosphate pH 7,0, molarité 0,05. Filtrée sur Sephadex G 200, elle sort avant le pic principal d'IgG, prouvant ainsi son poids moléculaire élevé. L'ultracentrifugation analytique de cette fraction, effectuée à trois concentrations diffé-



Coefficient de sédimentation des immunoglobulines et de la pièce de sécrétion libre du colostrum bovin. Valeurs non corrigées par le facteur de viscosité du solvant. (Pour le KCl 0,15 molaire, utilisé ici, le facteur du correction est de 1,033.)

[1] Ce texte est le résumé d'une communication faite à la Société Suisse de Biochimie le 17 mai 1969 (un rapport plus détaillé est sous presse dans «Nature»).
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