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Anti-interferon globulin inhibits the development of glomerulonephritis in mice infected at birth with lymphocytic choriomeningitis virus*

(interferon/late lymphocytic choriomeningitis virus disease)

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ABSTRACT **Swiss mice infected at birth with lymphocytic choriomeningitis virus develop glomerulonephritis. Injection of potent anti-mouse interferon globulin at the time of viral infection inhibited the development of these renal lesions. We conclude that the production of endogenous interferon by this virus in the first few days of life plays an important role in the pathogenesis of this glomerulonephritis.**

Interferon has usually been considered to be only beneficial to the host. It appears early in response to viral infection and has been shown to play an important role in host defense (for review see refs. 1–3). Likewise, administration of interferon to virus infected animals affords considerable therapeutic benefit (see ref. 4 for review) (5). Nevertheless, it has been emphasized that, in addition to its pronounced antiviral action, interferon can also inhibit normal cell division (6, 7) and influence cell function (8, 9). That interferon can at times even be injurious to the host is supported by the observation (10) that injection of potent interferon preparations into various strains of neonatal mice—an age at which there is rapid cell division and maturation—resulted in impaired growth, extensive liver cell degeneration, and death in the 2nd week of life. When daily interferon treatment begun at birth was stopped after the first week, most mice recovered but subsequently developed a progressive and lethal glomerulonephritis (11).

Some strains of newborn mice infected at birth with lymphocytic choriomeningitis (LCM) virus exhibit an identical syndrome [i.e., impaired growth, liver cell degeneration, and death in the first few weeks of life (12–16) and glomerulonephritis later in the life of surviving mice (14, 17, 18)]. Although it has often been stated that LCM virus does not induce interferon (19–22), it has recently been shown that this virus is an excellent interferon inducer in suckling and adult mice (23–26). We postulated, therefore, that the endogenous interferon induced by LCM virus in the neonatal period (26) was in part responsible for this syndrome. To test this hypothesis we injected newborn Swiss mice with LCM virus and potent sheep anti-mouse interferon antibody. Injection of this immunoglobulin neutralized the endogenous interferon induced and resulted in a 100-fold increase in the serum LCM virus titer on day 3 of life (26). Despite this marked increase in viral titer, anti-interferon globulin-treated mice grew almost normally and showed no liver lesions, and the incidence of death was much decreased (26).

We show in this paper that injection of anti-interferon globulin into newborn mice infected with LCM virus also markedly inhibits the appearance of glomerulonephritis characteristic of *late* LCM virus disease. Thus, the production of endogenous interferon in the critical period after birth not only is harmful to the suckling mouse in the acute period of infection but also plays an important role in the development of a disease that becomes apparent later in life.

MATERIALS AND METHODS

Experimental Design. In the three experiments, newborn Swiss mice from a pathogen-free colony at the Institut du Cancer were inoculated subcutaneously in the interscapular region with 0.05 ml of sheep anti-interferon globulin or with control sheep globulins or were left untreated. Six hours later they were injected subcutaneously with 10^4 LD_{50} of LCM virus CIPV 76001 of the Pasteur Institute. (Because this virus was maintained by intracerebral inoculation of 3-week-old Swiss mice, as a further control, one group in exp. 1 was injected with an extract of brain from normal Swiss mice.) Three days later, globulin-treated mice were reinjected subcutaneously with 0.05 ml of the different globulin preparations.

Immunoglobulins. The immunization procedures used to obtain potent sheep antiserum to mouse interferon, the techniques for partial purification of normal sheep globulin and immunoglobulin, and the assay for interferon neutralizing activity have been described (2). The sheep anti-mouse interferon globulin was diluted 1:2 or 1:3 in phosphate-buffered saline to give a titer of 8×10^{-5} or 5.3×10^{-5}. Other sheep serum globulins were used as controls: (*i*) normal sheep serum globulin, (*ii*) serum globulin from a sheep (no. 4) partially immunized to mouse interferon and having a very low anti-mouse interferon neutralizing titer (2.5×10^{-1}), and (*iii*) serum globulin from a sheep immunized with human leukocyte interferon (2). (The anti-human interferon neutralizing titer was 1×10^{-5} and the anti-mouse interferon neutralizing liter was 2×10^{-1}.)

RESULTS

Three experiments were undertaken to determine the effect of sheep anti-mouse interferon globulin on the early phase of LCM virus disease and on the subsequent development of glomerulonephritis. The results of the inhibitory effect of sheep anti-mouse globulin on the manifestations of early LCM virus

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Abbreviation: LCM virus, lymphocytic choriomeningitis virus.

* This article is no. 4 of the series "Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum." Article no. 3 is ref. 26.

Table 1. Glomerulonephritis in mice injected at birth with LCM virus

Experimental group		Mice alive at 21 days/ mice at birth,*	Evidence of glomerulonephritis at various times after inoculation of LCM virus†					
Virus	Treatment‡	no./no.	15 days	38 days	65 days	125–129 days	150 days	195 days
			Experiment 1					
None	None	19/19	NT	0/4	0/5	0/5		1/5 (0.2)
None§	None	20/20	0/2	0/4	0/5	0/5		0/6
LCM	None	25/34	NT	4/4 (2.3)	5/5 (1.6)	8/8 (2.1)		8/8 (2.0)
LCM	Normal sheep globulin	23/41	3/3 (1.0)	4/4 (1.8)	5/5 (2.2)	8/8 (2.3)		6/6 (2.5)
LCM	Sheep no. 4 globulin	26/33	NT	4/4 (1.5)	5/5 (1.6)	8/8 (2.0)		9/9 (2.3)
LCM	Anti-mouse IF globulin	39/43	0/3	4/10 (0.5)	3/10 (0.3)	7/10 (1.1)		9/9 (2.1)
			Experiment 2					
LCM	None	9/18				9/9 (2.7)		
LCM	Normal sheep globulin	3/10				3/3 (2.7)		
LCM	Anti-human IF globulin	4/20				4/4 (2.3)		
LCM	Anti-mouse IF globulin	20/28				15/20 (1.4)		
			Experiment 3					
LCM	Normal sheep globulin	4/20					4/4 (2.8)	
LCM	Anti-mouse IF globulin	13/22					13/13 (1.4)	

* Mice that were sacrificed in the period 0–21 days for experiments reported in ref. 26 have been excluded.

† Numbers of mice with histological confirmation of glomerulonephritis per total number of mice killed. Kidneys were examined by light microscopy by three observers and the severity of lesions was graded on a scale from 0 to 4. The numbers in parentheses indicate the mean index of severity of kidney lesions for each series. All kidneys were also examined by immunofluorescence with anti-mouse IgG, anti-C3, anti-fibrinogen, and anti-LCM virus antisera. Because there was a direct correlation between the degree of renal lesions observed by light microscopy and the extent of deposits of IgG and C3, we have only presented the results of light microscopy. There was no difference in the severity of glomerulonephritis between male and female mice. NT, not tested.

‡ IF, interferon.

§ Normal brain.

disease in suckling mice have been reported (26). It is relevant to recall from those experiments that, on day 3 of life, the virus titers in pools of sera from suckling mice injected with normal sheep globulin were $10^{4.5}$ and $10^{4.7}$ LD_{50}/ml and the interferon titers were 1:68 and 1:74 whereas the serum virus titers in suckling mice injected with anti-mouse interferon globulin were $10^{6.6}$ and $10^{6.6}$ LD_{50}/ml and interferon was not detected in the sera (<1:17) (26) (see exp. 1, Table 1). Male and female mice from these three experiments surviving beyond 2–3 weeks of life were sacrificed at intervals to determine the presence of renal lesions.

Development of Glomerulonephritis. Renal lesions characteristic of LCM virus-induced glomerulonephritis (17, 18, 27, 28) appeared in *all* virus-infected mice, whether treated or not with control globulin preparations (Table 1). Minimal lesions were first observed at 15 days, and were more pronounced at 38 days (exp. 1). In contrast, at 15, 38, and 65 days, only 0/3, 4/10, and 3/10 LCM virus-infected mice treated with anti-mouse interferon globulin showed renal lesions and the lesions in these mice were less marked than in LCM virus-infected mice untreated or treated with control serum globulins (see mean lesion scores). The typical lesion of advanced glomerulonephritis at age 65 days in a mouse injected at birth with LCM virus and normal sheep globulin is shown in Fig. 1*A*. In contrast, Fig. 1*B* shows the absence of lesions in a virus-infected mouse injected with anti-mouse interferon globulin. Heavy granular and coarse deposits of IgG and C3 were present *only* when renal lesions were observed by light microscopy (Fig. 1*C*).

With time (i.e., 125–129 days), of these mice infected with LCM virus and treated with anti-mouse interferon globulin, the number showing glomerulonephritis increased (exps. 1 and 2, Table 1), although the severity of lesions was still less than that observed in LCM virus-infected mice in the control groups. At 195 days there was no longer any difference in the incidence and severity of glomerulonephritis between LCM virus-infected mice injected with anti-interferon globulin and the various control groups.

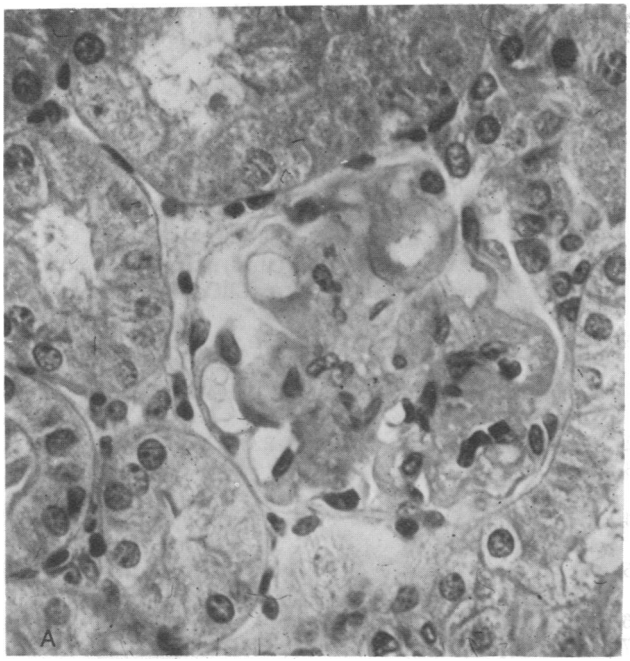
No difference was observed in these experiments in the severity of glomerulonephritis between male and female mice.

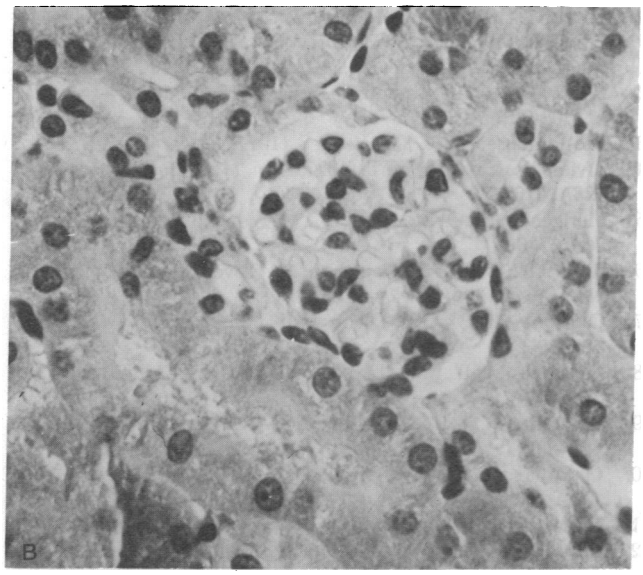
Serum Virus Titers in Adult Mice Infected at Birth with LCM Virus. The inhibition of the development of glomerulonephritis in mice treated at birth with anti-mouse interferon globulin prompted us to determine whether a difference existed in the serum virus titers between the different experimental groups. Accordingly, the sera from some LCM virus-infected mice in the different groups in exp. 1 taken at 65 and 125 days were assayed for LCM virus. No significant difference was observed between the amount of infectious LCM virus in the sera of mice with normal kidneys (mice treated with anti-mouse interferon globulin) and mice with minimal, moderate, or extensive renal lesions (Fig. 2). These results show therefore that inoculation with anti-interferon globulin did not interfere with the establishment of the "carrier state" and that the development of early renal lesions was not related to the amount of circulating infectious virus.

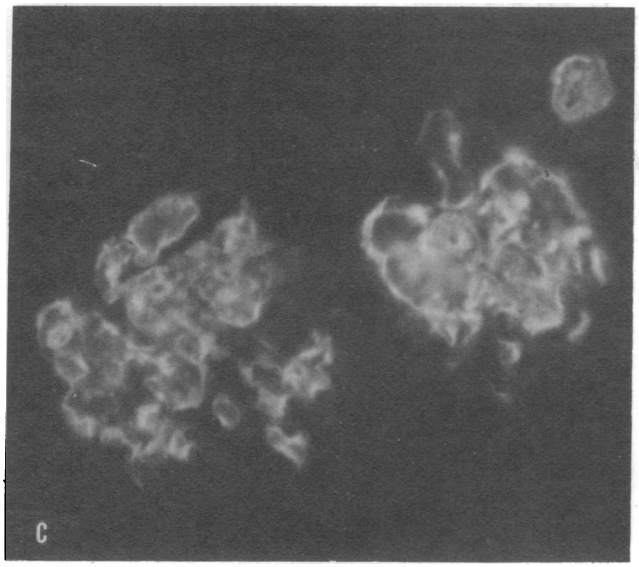
LCM viral antigen was also detected (by using a fluorescein-conjugated guinea pig anti-LCM virus antiserum) mostly in the tubular epithelium and, to a lesser degree, in the glomerular tufts in all mice with renal lesions and deposits of Ig and C3. In the absence of lesions (mice treated with anti-mouse interferon globulin), IgG and C3 were not present but viral antigen was detected nevertheless in the tubular epithelium and in some glomeruli.

DISCUSSION

The immune complex type of glomerulonephritis induced by LCM virus has been studied intensively for a number of years







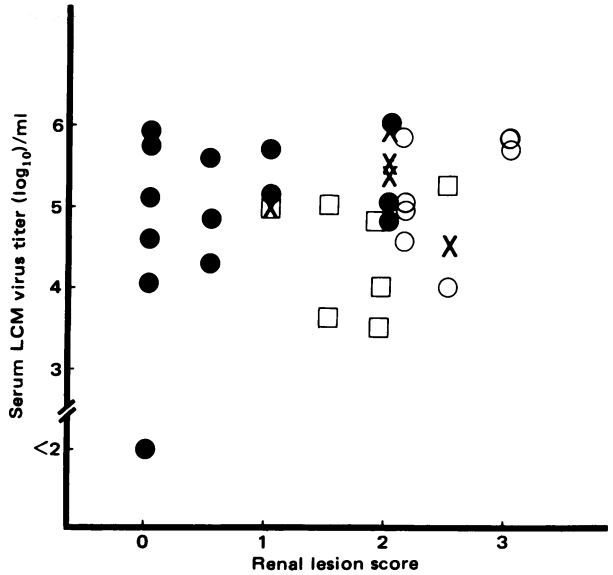


FIG. 2. Serum LCM virus titers (LD_{50} log_{10})/ml at 65 and 125 days in 21 individual mice or 12 pools of two sera from mice injected at birth with LCM virus and left untreated (×) or treated with normal sheep globulin (○), sheep no. 4 globulin (□), or sheep anti-mouse interferon globulin (●) (exp. 1, Table 1). LCM virus was titered by intracerebral inoculation of 3-week-old Swiss mice.

and has served as a model for virus-associated nephritides (27–29). It is believed that complexes of virus, antibody to virus, and complement are deposited in the glomeruli and lead to the development of renal lesions (28–31). Interferon has never been considered to be a contributing factor. The results presented herein indicate that interferon induced by LCM virus in suckling mice does play an important role in the pathogenesis of the early renal lesions. First, it is relevant that exogenous interferon administered during the first week of life resulted in the development of a glomerulonephritis comparable to that observed in LCM virus-infected mice (11). This interferon-induced glomerulonephritis had all the characteristics of an "immune complex type" nephritis with granular deposits of IgG and C3 in the glomeruli (11). Second, injection of LCM virus-infected newborn mice with potent anti-mouse interferon globulin neutralized the endogenous interferon induced by LCM virus (26) and, as shown herein, significantly delayed the appearance of glomerulonephritis. Furthermore, in mice treated with anti-mouse interferon globulin, the extent of renal lesions was much less for the first 5 mo than in control mice (Table 1).

All mice injected with anti-interferon globulin eventually developed glomerulonephritis, and differences in the severity of renal lesions in mice in the different experimental groups were no longer detected at 195 days (Table 1). Because mice received only two injections of anti-mouse interferon globulin, it is possible that all the interferon induced by LCM virus in the

FIG. 1. Kidney of 65-day-old Swiss mice injected at birth with LCM virus. (*A*) Given normal sheep globulin (exp. 1, Table 1). Note enlarged glomerular tuft with conspicuous thickening of glomerular basement membranes and foci of cellular proliferation reducing capillary lumina. (Mallory trichrome stain; ×300.) (*B*) Given anti-mouse interferon globulin (exp. 1, Table 1). Note normal appearance of glomerulus. (Mallory trichrome stain; ×288.) (*C*) Given normal sheep globulin (exp. 1, Table 1). Note enlarged glomerular tufts with conspicuous occlusive subendothelial deposits of immunoglobulins invading capillary lumina. (Fluorescein-conjugated sheep anti-mouse IgG; ×177.5.)

first week of life was not neutralized and that a greater degree of inhibition of glomerulonephritis might have been observed had these mice received more anti-interferon globulin and in repeated injections. On the other hand, it is possible that interferon is only one of multiple factors important in the development of glomerulonephritis and that maximal delay was achieved.

The pertinent question then is How does exogenous or endogenous interferon in neonatal mice contribute to the subsequent development of glomerulonephritis? Interferon has been shown to modify the surface of cells both *in vitro* (32–35) and *in vivo* (36). The preliminary results of an ultrastructural study (J. Sloper, personal communication) of the kidneys of suckling mice either treated with exogenous interferon or infected at birth with LCM virus show a gross thickening of the glomerular basement membrane, which could be of considerable importance in the development of glomerulonephritis. Perhaps circulating antigen–antibody complexes can no longer be filtered efficiently through an altered basement membrane and these trapped complexes may then lead to kidney damage.

In previous experiments, interferon treatment begun after the first week of life did not lead to glomerulonephritis (11). It is possible that the effect of interferon (direct or indirect) on the kidney also depends on the degree of maturation of the kidney at the time of birth. If one can extrapolate our findings in mice to man, it seems possible that some instances of nephritis of unknown etiology in young patients may be the delayed result of interferon induced during a viral infection. The point we should like to emphasize, however, is the more general principle that endogenous interferon induced for only a few days (i.e., as a result of a virus infection) at a crucial stage of development can be an important factor in the development of a disease that only becomes manifest later in life. Therefore, it seems important to consider the possibility that the pathologic manifestations of some virus infections can also be due to virus-induced cell products, such as interferon, rather than to direct virus cytopathic effects *per se*.

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