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Interferon induces pulmonary cysts in A2G mice

DAVID WOODROW*, JILL MOSS*, AND ION GRESSER†‡

*Laboratory of Experimental Pathology, Charing Cross Hospital Medical School, London W6 8RF, England; and †Laboratory of Viral Oncology, Institut de Recherches Scientifiques sur le Cancer, BP 8, 94800 Villejuif, France

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ABSTRACT **Suckling A2G mice were treated for the first week of life with either partially purified or electrophoretically pure mouse α/β interferon. All mice subsequently developed multiple large pulmonary cysts. The lesions were never found in interferon-treated Swiss mice and only very infrequently in interferon-treated BALB/c or F_1 Swiss/A2G mice. These observations show that a brief exposure of suckling A2G mice to α/β interferon can result in a severe disease that is determined by the mouse genotype.**

We previously have shown that daily inoculation of newborn mice with partially purified or electrophoretically pure mouse α/β interferon resulted in stunted growth, destruction of the liver, and death (1–3). When interferon treatment was discontinued at week 1 of life, surviving mice developed a severe glomerulonephritis (2–4). Likewise, inoculation of newborn rats with potent rat interferon preparations resulted in stunted growth and the late development of glomerulonephritis (5). In the course of these studies, we noted that, although A2G mice treated with interferon at birth grew poorly and showed severe liver cell necrosis, they did not develop the characteristic glomerulonephritis seen in other strains of mice (3). We now report that A2G mice treated with mouse α/β interferon for the first week of life subsequently develop large pulmonary cysts.

MATERIALS AND METHODS

Animals. Newborn A2G, Swiss, BALB/c, or F_1 Swiss/A2G mice were obtained from the breeding colonies of the Charing Cross Hospital Medical School.

Mouse Interferon and Control Preparations. Mouse α/β interferon was prepared from Swiss mouse C-243 cells induced with Newcastle disease virus, concentrated, partially purified, and assayed as described before (6). Mock interferon was prepared in a manner identical to that used in the preparation of interferon with the exception that the interferon inducer, Newcastle disease virus, was either omitted or added for 1 hr immediately before collection of the culture supernatant. All interferon and control preparations were dialyzed further for 24 hr at 4°C against 0.01 M perchloric acid before testing for toxicity on a line of L1210 cells resistant to interferon (7). The specific activity of mouse C-243 cell interferon preparations was in the range of 2×10^7 units per mg of protein. Electrophoretically pure mouse interferon was prepared by sequential chromatography on a sheep anti-mouse interferon antibody-agarose column and a carboxymethyl Sepharose column (8). When the purified interferon was subjected to electrophoresis in polyacrylamide gels containing $NaDodSO_4$, only three bands corresponding to apparent molecular sizes of 30, 24, and 19 kDa were observed after staining with Coomassie blue. The 30- and 24-kDa interferons have been shown serologically to correspond to β interferon, and the 19-kDa interferon, to α interferon (9). Biologic activity of interferon was found only in eluates of gel slices corresponding to these bands. The specific activity of electrophoretically pure interferon was $0.5–1 \times 10^9$ reference units/mg of protein. Mouse interferon units quoted are as measured in our laboratory and 1 of our units equals 4 mouse interferon reference units.

Light and Electron Microscopy. Both lungs were fixed in 4% buffered formalin and embedded in paraffin wax. Sections (5 μm) were cut and stained with hematoxylin and eosin. For the ultrastructural examination of the lungs, small pieces of tissue were cut into 1 mm^3 and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C for 1 hr. Samples were postfixed in 1% osmium tetroxide in Millonig's phosphate buffer for 1 hr at 4°C, washed in distilled water, immersed in 1% aqueous uranyl acetate for 1.5 hr at room temperature, dehydrated through ascending grades of ethanol, and embedded in Spurr resin. Sections were cut on a Reichert Om U3 ultramicrotome. Semi-thin sections (0.5–1.0 μm) were stained with toluidine blue/pyronin Y in borax and examined by light microscopy. Ultrathin sections (60–80 nm) were stained in 1% aqueous uranyl acetate and Reynolds lead citrate and examined with a Philips EM 300 electron microscope.

Cryostat Sections. Tissue containing cysts was snap-frozen in isopentane and cooled in liquid nitrogen. Sections (6 μm) were air-dried, acetone-fixed, and incubated with fluorescein isothiocyanate-labeled anti-mouse IgA, IgG, IgM, and fibrinogen (Nordic Laboratories, Tilburg, Netherlands) for 30 min. Some sections were air-dried and stained with Oil-red O to demonstrate fat.

Enumeration of Cysts. A longitudinal section of each lung passing through all lobes was examined. The total number of cysts in this 5-μm section of both lungs was counted.

RESULTS

Newborn A2G mice were injected subcutaneously with potent partially purified α/β interferon daily for 1 week beginning on the day of birth. Interferon treatment resulted in a severe inhibition of growth and liver cell necrosis as described (2, 3). When interferon treatment was discontinued at 1 week, mice appeared to recover and gain weight but subsequently developed large pulmonary cysts (Fig. 1). These cysts were present in all lobes of the lungs and were invariably seen in the lower segments of affected lobes (Fig. 1*A*). The cysts measuring 200–300 μm in diameter were thin-walled, often distended, and lined by a flattened layer of cells (Fig. 1 *B* and *C*). They were filled with a pale eosinophilic material that did not contain fat, immunoglobulin, or fibrin.

The pooled results of five experiments presented in Table 1 may be summarized as follows: pulmonary cysts were first noted on day 15 in A2G mice treated with interferon and were then present in virtually all interferon-treated A2G mice thereafter (Table 1). There appeared to be some progression in the development of pulmonary cysts as determined by the increasing number of mice with cysts and the increasing total number of cysts per lung section per mouse

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‡To whom reprint requests should be addressed.

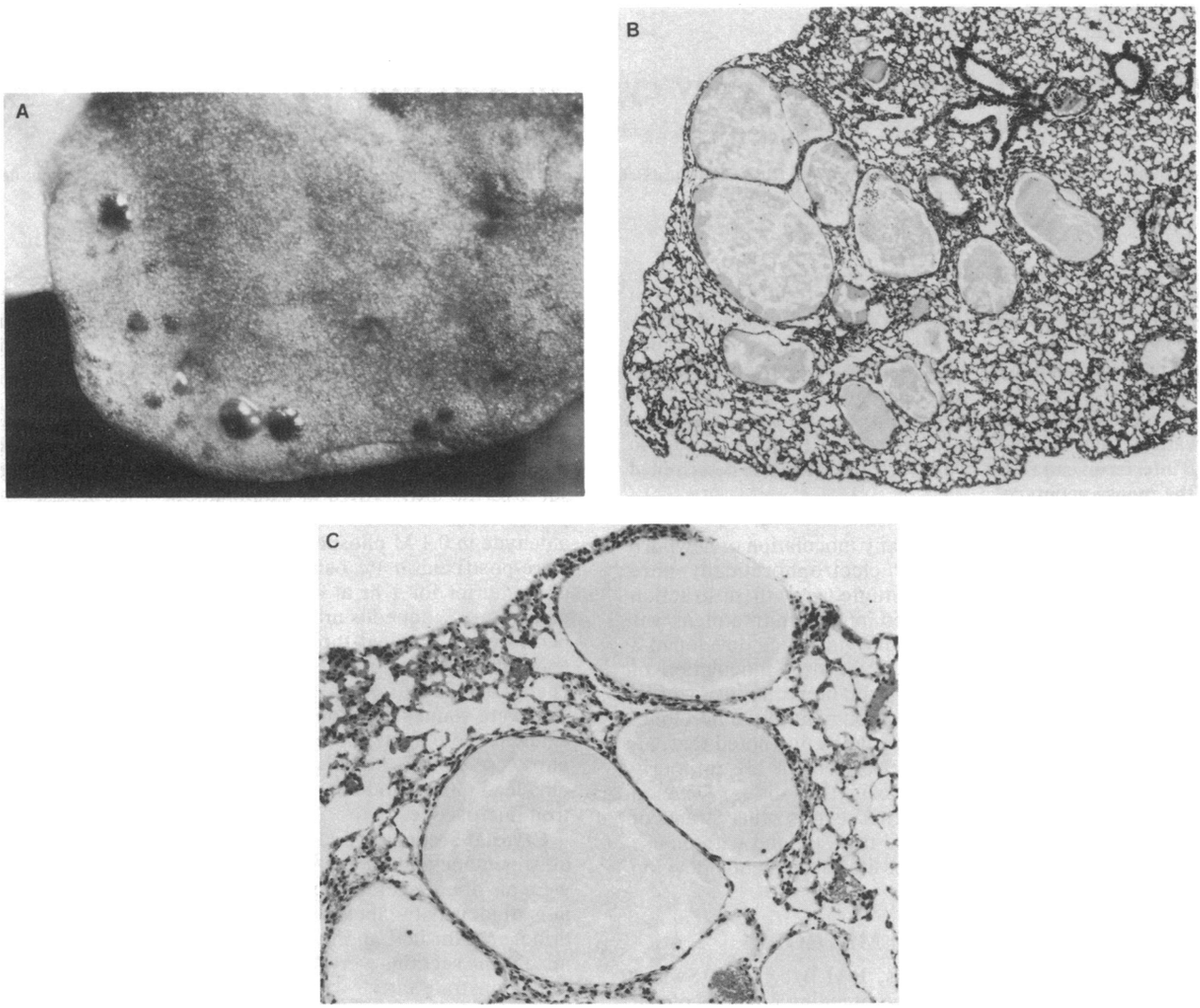

FIG. 1. Presence of cysts in the lungs of an A2G mouse injected subcutaneously daily from birth for 8 days with 0.05 ml of a partially purified mouse interferon preparation having a titer of 10^{-6}. The mouse was killed on day 56. (*A*) Macroscopic view of underside of lower lobe of lung to show numerous cysts projecting from the surface. (*B*) Section of lung to show the distribution of cysts within the lobe. (×40.) (*C*) Higher power view of thin-walled cysts that contain amorphous material together with occasional mononuclear cells. (×256.)

in older mice. Presence of pulmonary cysts did not appear to result in premature deaths. In longevity experiments, interferon-treated A2G mice have survived to date more than 200 days. One control A2G mouse treated with phosphate-buffered saline (P_i/NaCl) had a single lung cyst indistinguishable from those observed in interferon-treated mice. Comparable cysts were not seen in either interferon-treated or P_i/NaCl-treated Swiss mice. Three of 11 interferon-treated BALB/c mice had single cysts, as did one of eight interferon-treated F_1 Swiss/A2G mice (Table 1).

Multiple pulmonary cysts were present at 56 days in all A2G mice injected in the neonatal period with electrophoretically pure mouse interferon as well as with potent partially purified interferon (Table 2). Only single cysts were observed in 3 of 24 A2G mice injected with either bovine serum albumin or a mock interferon preparation, and no cysts were

Table 1. Presence of pulmonary cysts in mice treated in the neonatal period with partially purified mouse interferon

Age of mice, days	Incidence of cysts in mouse strains, no. of mice with cysts/total no. of mice							
	A2G		Swiss		BALB/c		Swiss ♀/A2G/♂ F_1	
	IFN	P_i/NaCl	IFN	P_i/NaCl	IFN	P_i/NaCl	IFN	P_i/NaCl
8	0/9	0/9	0/5	0/6				
15	3/6 (4.7 ± 0.33)	0/6	0/3					
22	2/3 (10 ± 5.0)	0/3	0/3					
45	3/3 (20.7 ± 5.8)	0/3	0/3					
56	8/8 (17.5 ± 4.4)	1/9 (1)	0/6	0/10	2/6 (1)	0/8	0/3	0/3
100	12/12 (11.0 ± 3.2)	0/6	0/10	0/7	1/5 (1)	0/4	1/5 (1)	0/3

In these experiments, suckling mice were injected subcutaneously in the interscapular region with 0.05 ml of partially purified interferon (IFN) having a titer of 10^{-6} or with P_i/NaCl. Mice were injected daily from birth for 8 days. The numbers in parentheses represent the mean no. of lung cysts ± SEM per section per mouse, calculated only for mice with cysts.

Table 2. Pulmonary cysts at day 56 in A2G mice treated in the neonatal period with electrophoretically pure (EP) mouse interferon (IFN) or dilutions of partially purified (PP) mouse IFN

	Incidence of cysts in A2G mice in two experiments									
	Exp. 1: EP IFN			Exp. 2: PP IFN						
Titer of IFN injected*	1.6×10^6	4.0×10^5	Albumin	1.2×10^6	4.8×10^5	4.8×10^4	4.8×10^3	Albumin	Mock IFN	Untreated
No. of mice with cysts/total no. of mice	11/11 (8.1 ± 2.1)	8/8 (16.4 ± 3.4)	1/8 (1)	10/10 (12.5 ± 3.6)	3/6 (1)	0/8	0/8	1/8 (1)	1/8 (1)	0/9

In experiments 1 and 2, A2G mice were injected subcutaneously daily from birth for 8 days with 0.05 ml of dilutions of EP IFN, PP IFN, bovine serum albumin, or mock IFN. IFN was diluted in bovine serum albumin (Sigma) at 100 μg/ml to preserve stability. The numbers in parentheses represent the mean no. of cysts ± SEM per section per mouse, calculated only for mice with cysts.
*Reciprocal of the dilution at the 50% end point.

observed in A2G mice injected with 1:25, 1:250, and 1:2,500 dilutions of partially purified mouse interferon or left untreated. These findings constituted convincing evidence for us that mouse interferon itself was the responsible factor.

The amount of interferon injected and the age of the mice at the time of injection were crucial factors in the induction of pulmonary cysts. Thus, as shown in experiments 1 and 2 of Table 2, it was necessary to inject suckling mice with in-

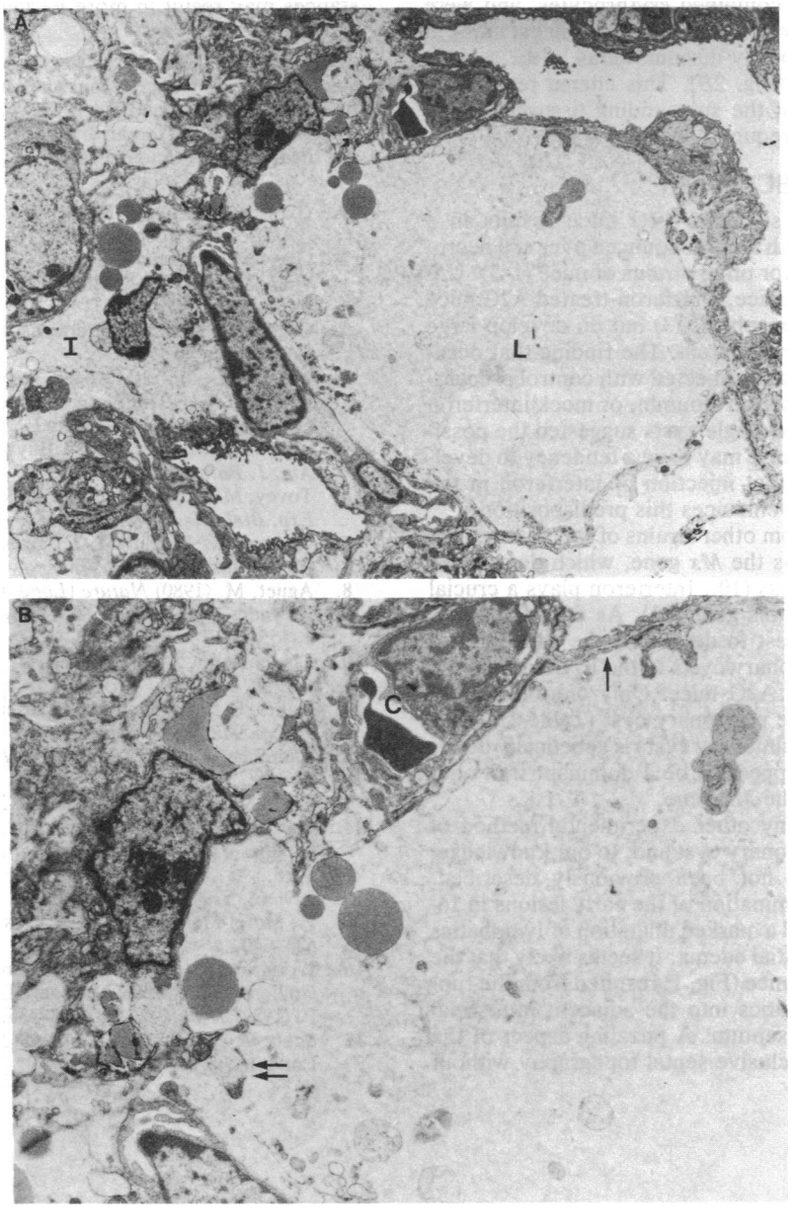

FIG. 2. Electron micrograph of the abnormal interlobular region of lung from an interferon-treated A2G mouse (day 16). (*A*) Dilated lymphatic (L) and extensive interstitial edema (I). (×2,745.) (*B*) Higher magnification of the dilated lymphatic adjacent to an apparently normal capillary (C) to show the lymphatic endothelium (↑) that is discontinuous in the region of the interstitial edema (↑ ↑). (×5,400.)

terferon having a titer of about 4×10^{-5} to induce pulmonary lesions in 50–100% of the mice. This was roughly the amount of interferon that had been required to induce stunted growth, liver cell necrosis, and death (1–3) or glomerulonephritis (3, 4). Initiation of interferon treatment at day 8 of life and continuation daily for 1 week did not result in the development of pulmonary lesions (zero of eight mice) (data not shown). This finding is in accord with previous observations on the failure of interferon to inhibit growth and induce liver necrosis and glomerulonephritis when treatment was initiated after day 8 (1–4).

Ultrastructural examination of the large cysts of adult mice (illustrated in Fig. 1) showed that they contained a finely granular apparently proteinaceous material that did not have the features of amyloid. The cysts were lined by flattened endothelial cells and resembled dilated lymphatics. Ultrastructural examination of early lesions of 16- to 18-day-old suckling mice showed areas of conspicuous interlobular interstitial edema and early cyst formation (Fig. 2*A*). The cysts were lined by endothelial cells that often showed intraluminal projections, rarely contained erythrocytes, and were compatable with dilated lymphatics. The endothelial lining in the distended cyst was focally discontinuous in the regions of the interstitial edema (Fig. 2*B*). This edema resulted in considerable distension of the surrounding tissue in which degenerating cells were frequently seen.

DISCUSSION

Interferon treatment of suckling A2G mice results in a marked inhibition of growth and pronounced liver cell necrosis as has been observed for other strains of mice (1–3). Unlike these other strains of mice, interferon-treated A2G mice do not develop glomerulonephritis (3) but do develop large pulmonary cysts as reported herein. The finding that occasionally some of the A2G mice injected with control preparations of P_i/NaCl, bovine serum albumin, or mock interferon (Tables 1 and 2) developed single cysts suggested the possibility that this strain of mouse may have a tendency to develop pulmonary cysts and that injection of interferon in the neonatal period markedly enhances this predisposition.

A2G mice also differ from other strains of laboratory mice tested in that they express the *Mx* gene, which determines resistance to influenza virus (10). Interferon plays a crucial role in the expression of this gene (11). As the *Mx* gene is dominant, it was of interest to determine the capability of interferon to induce pulmonary cysts in the F_1 offspring of a cross between Swiss and A2G mice. Only one of eight of these F_1 mice had a single pulmonary cyst (Table 1). If induction by interferon of pulmonary cysts is genetically determined, then it does not appear to be a dominant trait and, therefore, not related to the *Mx* gene.

We are not aware of any other experimental method of inducing this type of pulmonary cyst and, to our knowledge, comparable lesions have not been previously described. Electron microscopic examination of the early lesions in 16- to 18-day-old mice showed a marked dilatation of lymphatics and an interlobular interstitial edema. It seems likely that the larger cysts seen in older mice (Fig. 1) resulted from the rupture of the dilated lymphatics into the adjacent interstitial tissue of the interlobular septum. A puzzling aspect of the lesion is its practically exclusive septal topography without involvement of the peribronchial or pleural lymphatics. It is difficult at present to extrapolate our findings to human pathology, as this distribution of lesions appears to be uncommon in man. In congenital lymphangiectasis, for example, there is usually a diffuse involvement of the various lymphatic networks of the lung (12).

Our previous work has emphasized that a brief repeated exposure of immature mice to exogenous or endogenous interferon (13–16) can induce an acute disease characterized by inhibition of growth and severe liver lesions. Even after discontinuation of interferon administration at age 1–2 weeks, mice and rats subsequently developed a severe glomerulonephritis (3). The finding that adult A2G mice developed marked pulmonary lesions emphasizes that many organ systems may be affected by exposure to interferon at a time of rapid maturation and development and that disease may only become manifest later in life. Although interferon has been shown to induce a reversible toxicity in man (17), it seems to us possible that repeated exposure of adult animals (or man) to large amounts of interferon under certain circumstances may result in more permanent pathologic changes.

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