INFECTION AND IMMUNITY, Mar. 1976, p. 844-854

Vol. 13, No. 3
Printed in U.S.A.

Genetically Determined Resistance to Infection by Hepatotropic Influenza A Virus in Mice: Effect of Immunosuppression

O. HALLER,[1] H. ARNHEITER, AND J. LINDENMANN*

Division of Experimental Microbiology, Institute for Medical Microbiology, University of Zürich, 8028 Zürich, Switzerland

Received for publication 30 September 1975

Mice carrying the gene *Mx* were resistant to the lethal action of a hepatotropic line of avian influenza A virus. In resistant animals, foci of liver necrosis were self-limiting, and maximal virus titers reached were much below those in susceptible animals. Resistance could not be abrogated by immunosuppressive treatment with cyclophosphamide, methotrexate, or procarbazine, although such treatment prevented cellular infiltration at sites of virus replication and appeared to delay virus clearance. Silica and thorium dioxide, thought to inhibit macrophage function, likewise failed to abolish resistance. Regenerating liver tissue did not support more extensive virus replication than did intact adult liver.

Mechanisms of natural resistance to viruses are not well understood (23). Depending on the systems studied and the preferences of authors, the following possibilities have been contemplated: lack of cell surface receptors, presence of humoral inhibitors, barriers to intercellular spread, induction of interferon, release of noninfectious virus, formation of double-stranded ribonucleic acid, triggering of cellular, humoral, and secretory immune mechanisms, and peculiarities of macrophages (2, 5, 7, 13, 23, 24, 35, 37).

Instances where a single gene determines resistance or susceptibility to a virus would seem to offer the best prospects for elucidating some of the factors involved. In laboratory mice a dominant gene, *Mx*, conferring a high degree of resistance towards neurotropic and pneumotropic influenza virus infection, has been described (8, 16, 17, 19). The course of infection in a third type of organ, profoundly different from both brain and lung, should allow interesting comparisions to be made and might yield additional clues.

Although influenza A viruses of human origin are readily adaptable to mice, only pneumotropic and a few neurotropic variants are known. Certain avian influenza A strains, however, have a tendency to grow in mouse liver, where histological evidence of infection was described long ago (15). We have recently produced a highly hepatotropic variant, starting with a virus originally isolated from turkeys (11). This variant killed ordinary laboratory mice at high dilutions with signs of a fulminant hepatitis. The present paper shows that mice bearing the gene *Mx* were refractory to the lethal action of this virus. No satisfactory explanation for this resistance could be found.

MATERIALS AND METHODS

Animals. Inbred A2G mice (genetically *Mx/Mx*) were raised locally. A/J and C57BL/6J mice were purchased from the Jackson Laboratory, Bar Harbor, Me. Crosses between A/J, C57BL/6J, and A2G mice were arranged at our institute. Random-bred ICR mice were obtained from the Department of Animal Husbandry, Veterinary School, University of Zürich. Adult mice were matched for age and sex before use and were kept on acidified drinking water throughout the investigation period unless otherwise nemtioned. Rabbits were locally purchased.

Viruses. The mouse hepatotropic variant TURH derived from the avian influenza virus strain A/TURKEY/England 63 (Havl, Nav3) was used throughout. This virus had undergone 39 serial passages in ICR liver. It differed from the original virus strain (designated TUR_0) in its capacity to grow in mouse liver and in its high intraperitoneal (i.p.) lethality for normal adult mice (11). Stock virus was prepared in 10-day-old embryonated eggs. The allantoic fluid with a hemagglutination (HA) titer of 16 contained $10^{7.7}$ 50% egg-infective doses (EID_{50}) per ml and was stored in ampoules at −70 C.

Liver-grown TURH virus consisted of infected ICR mouse liver homogenates stored similarly and containing $10^{9.0}$ to $10^{11.0}$ EID_{50}/ml.

Egg-grown stocks of TUR_0 (never passed in mouse liver but antigenically identical with TURH [11]) were used for antibody production in rabbits.

Inoculation of virus. All inoculations of TURH

[1] Present address: Institute for Immunology, Uppsala University, P.O.B. 570, 751 23 Uppsala, Sweden.

virus were done by the i.p. route. Appropriate virus dilutions in phosphate-buffered saline (PBS) containing 100 U of penicillin and 100 μg of streptomycin per ml (PBS + AB) were injected in volumes of 0.1 to 0.5 ml per mouse.

Virus assays. Infectivity of TURH virus was assayed in 10-day-old fertile chick eggs. Three to four eggs were injected allantoically with 0.1 ml of serial 10-fold virus dilutions in PBS + AB. Deaths were recorded during 96 h at 35 C. Allantoic fluids from the dead eggs and from all surviving eggs up to the first dilution where no deaths occurred were tested for HA to determine the EID_{50}.

Standard i.p. lethality titrations were performed in normal adult ICR mice. A 0.1-ml amount of serial decimal dilutions was injected i.p. into groups of four animals. Deaths were recorded for 14 days thereafter, and 50% i.p. lethality end points (i.p. LD_{50}) were calculated. Titrations in mice under experimental conditions, when required by the protocol, were performed similarly.

HA and hemagglutination inhibition titrations were done according to World Health Organization standard procedures. HA titers were recorded as the reciprocal of the highest dilution causing borderline agglutination of 10^7 chick erythrocytes.

Liver homogenates. Pools of three to five livers collected aseptically from exsanguinated mice were homogenized with an equal weight of PBS + AB in a refrigerated Sorvall Omnimix homogenizer, using two successive freeze-thaw cycles. The supernatant after centrifugation (6,000 $\times$ g, 20 min, 4 C) was stored at -70 C until tested.

Histology. Representative liver specimens from infected and control mice were fixed in 10% neutral formalin. Routine paraffin sections were prepared and stained with hematoxylin and eosin at the Institute for Pathological Anatomy, University of Zürich.

Immunofluorescence. Indirect staining using fluorescein isothiocyanate labeled goat anti-rabbit immunoglobulin (Miles Laboratories, Kankakee, Ill.) was used. Mice were bled under ether anesthesia, and individual livers were perfused via the portal vein with PBS before removal from the peritoneal cavity. Small cubes were cut and quick-frozen in liquid nitrogen. Sections (6 μm) made in a cryostat microtome were air-dried and overlaid with a 1:10 dilution of specific rabbit antiserum for 30 min at 37 C in a moist chamber. After two washes in excess PBS for 20 min, the sections were incubated (30 min, 37 C) with the labeled anti-immunoglobulin serum, washed again, and mounted in buffered glycerol (pH 9). The preparations were observed in a Zeiss Ultraphot microscope equipped with a high-pressure mercury lamp (HBO 200 W) providing dark-field illumination. A BG38 excitation filter and appropriate barrier filters were used. Uninfected control specimens prepared and stained in the same manner never showed fluorescence. No specific fluorescence was seen in sections from infected livers treated with normal rabbit serum.

Antisera. Rabbit antiviral antisera were obtained by 5 weekly intramuscular injections of egg-grown TUR_0 virus mixed with equal volumes of complete Freund adjuvant (Difco). Animals were bled 14 days after the last injection and provided sera with hemagglutination inhibition titers of 1:1,280 against both TUR_0 and TURH viruses.

Immunosuppressive treatments. Cyclophosphamide (Cyc; Asta-Werke, Brackwede, German Federal Republic) was used as a freshly prepared aqueous solution (20 mg/ml). Mice were injected subcutaneously (s.c.) with 150 mg per kg of body weight 1 day before and 1 day after virus inoculation. In some experiments an additional s.c. Cyc administration was performed on day 4 or 5 after virus infection, as shown in the protocol.

Methotrexate (Mtx; American Cyanamid Co., Pearl River, N.Y.) was prepared as a 5-mg/ml sterile solution and diluted in saline for s.c. injection. Mice received 25 mg per kg of body weight 1 day before and 1 and 3 days after infection.

Procarbazine (Pro) was a gift from W. Bollag, Hoffmann-La Roche AG., Basel, Switzerland. Pro was dissolved in sterile saline (30 mg/ml) and was used at 300 mg/kg 1 day before and at 200 mg/kg 1, 2, and 3 days after infection.

Control animals received virus and saline instead of drug, or drug but no virus. Treated and control mice were kept on drinking water supplied with chloramphenicol (1 mg/ml) to prevent pseudomonas infection. Drug treatment was generally well tolerated. With Mtx some spontaneous deaths among controls occurred 5 to 10 days after inoculation.

Thorotrast treatments. Thorotrast (24 to 26% stabilized colloidal thorium dioxide suspended in 25% aqueous dextrin; Fellows Testagar, Detroit) was kindly supplied by B. Sordat, Institut de Recherches Expérimentales sur le Cancer, Lausanne, Switzerland. Mice were injected i.p. with 0.3 ml 6 h before virus challenge. X rays showed the presence of Thorotrast in the peritoneal cavity at time of challenge and later in the liver of treated animals.

Controls were given 0.3 ml of 25% aqueous dextrin without thorium dioxide.

Silica treatment. Silica (Dörentrup; average particle size < 5 μm), kindly provided by R. Keller, Arbeitsgruppe für Immunobiologie, Zürich, was suspended in isotonic saline at concentrations of 50 mg/ml of i.p. and 10 mg/ml for intravenous (i.v.) inoculations. The suspensions were heat-sterilized and dispersed by ultrasonic vibration immediately before injection. Normal mice received 50 mg i.p. and were used 2 or 72 h thereafter. The i.v.-treated mice were injected with 3 mg of silica through the tail vein 72 h before challenge with TURH virus. Control mice received saline alone.

Partial hepatectomy. Resection of the left lateral and median lobes under ether anesthesia (36) resulted in the removal of 60 to 70% of total liver mass. Sham hepatectomy consisted of simple laparatomy. Treated mice were supplied for 24 h with a 0.5% sucrose solution in place of drinking water and were used 2 days after operation.

RESULTS

Resistance of normal Mx-bearing mice to lethal infection with a hepatotropic influenza virus. To test whether the inborn resistance of mice carrying the dominant gene *Mx* (16),

known to protect lungs and brains from lethal infection with pneumotropic and neurotropic influenza viruses (8, 17, 19), was also expressed in the liver, *Mx*-bearing mice were exposed to the mouse hepatotropic influenza variant TURH (11). Serial titrations of egg-grown and liver-grown TURH virus were performed i.p. in A2G mice homozygous for *Mx*, in heterozygous (A2G × A/J)F_1 mice, and in susceptible ICR controls, and the i.p. LD_{50} values were determined (Table 1). *Mx*-bearing mice proved far less susceptible to TURH virus than the matched controls. Reduction of i.p. LD_{50} titers in A2G mice corresponded to previous findings, with comparative intracerebral and intranasal titrations of the original TUR_0 strain in resistant and susceptible animals (17). The disease produced by TURH virus differed considerably in susceptible and resistant mice. Even with dilute inocula, ICR mice became rapidly ill and succumbed within 2 to 3 days to an acute degenerative hepatitis characteristic of TURH infection (11). In contrast, A2G and F_1 mice infected with moderate doses of virus capable of killing 100% of ICR mice survived indefinitely without any symptoms. Even with very large doses, A2G mice survived for 5 days or longer. Thereafter a proportion of mice developed signs of central nervous system disturbance, particularly paralysis of the hind legs, with scattered deaths occurring 6 to 12 days after infection.

Virus growth in the livers of susceptible and resistant mice. Myxovirus resistance has been shown to be associated with restricted growth of neurotropic influenza virus in brains of infected mice (8, 19). Growth of TURH virus in the liver of normal adult A2G mice was found to be markedly reduced as compared with growth in ICR and A/J livers (Table 2 and Fig. 1). Since A2G mouse liver did support replication of fully infectious virus to a limited degree, attempts to isolate a virus variant more prone to replicate in the livers of resistant mice were made by serial in vivo passages. During seven passages, TURH virus showed no tendency to increased replication in A2G liver; lethal hepatotropic properties for ICR mice were maintained. Serial passages of TURH virus in the livers of Cyc-immunosuppressed adult A2G mice (see below) likewise did not result in better growth.

Influence of Cyc immunosuppression on resistance to TURH infection. Cyc given 1 day before and 1 and 5 days after inoculation of TURH did not affect neither the severity and characteristics of hepatotropic influenza infection in either resistant A2G or susceptible ICR mice (Table 3). In both Cyc-suppressed and normal A2G mice, delayed deaths with signs of central nervous system involvement after high virus inocula occurred. ICR mice succumbed to TURH infection in due time whether Cyc treated or not. Figure 1 shows the growth of TURH in the livers of control and Cyc-treated A2G and ICR mice inoculated i.p. with 10^3

TABLE 1. *Resistance of Mx-bearing mice towards hepatotrophic influenza A virus TURH*

Virus inoculum[a]	Mouse		i.p. LD_{50}[b]	EID_{50}/ i.p. LD_{50}[c]
	Strain	Genotype		
Egg-grown TURH	ICR	+/+	6.7	1.0
	A2G	*Mx/Mx*	2.5	5.2
	F_1 (A × A2G)	*Mx*/+	2.4	5.3
Liver-grown TURH	ICR	+/+	9.2	1.3
	A2G	*Mx/Mx*	5.0	5.5

[a] Intraperitoneal titrations of infected allantoic fluid (= egg-grown TURH, $10^{7.7}$ EID_{50}/ml) or infected liver homogenate (= liver-grown TURH, $10^{10.5}$ EID_{50}/ml).
[b] Log_{10} of titration end point.
[c] Ratio of EID_{50} over i.p. LD_{50} of inoculum, expressed as log_{10}.

TABLE 2. *Comparative virus contents of mouse livers taken from susceptible and resistant mice at indicated times after i.p. infection with $10^{3.0}$ EID_{50} of hepatotropic TURH*

Mouse strain	Virus content (EID_{50}) at:[a]		
	48 h	96 h	168 h
ICR	9.2	–[b]	–
A/J	9.1	–	–
A2G	5.3	5.2	2.0

[a] Egg infectivity of liver homogenates from pools of four livers, expressed as log_{10} per milliliter.
[b] –, All animals were dead by this time.

TABLE 3. *Effect of cyclophosphamide (Cyc) immunosuppression on susceptibility of ICR and A2G mice to TURH virus as measured by lethal end point titrations (i.p. LD_{50})*

Mouse strain	Treatment[a]	i.p. LD_{50}[b]	EID_{50}/ i.p. LD_{50}[c]	Avg time to death (days)[d]
ICR	Saline	8.4	1.3	2.2
ICR	Cyc	8.6	1.1	2.3
A2G	Saline	5.0	4.7	8.8
A2G	Cyc	5.5	4.2	8.2

[a] Cyc was given 1 day before and 1 and (in A2G mice) 5 days after infection with liver-grown TURH (10^3 EID_{50}).
[b] Log_{10} per milliliter calculated from i.p. titrations.
[c] Ratio expressed as log_{10}.
[d] Final readings 2 weeks after infection.

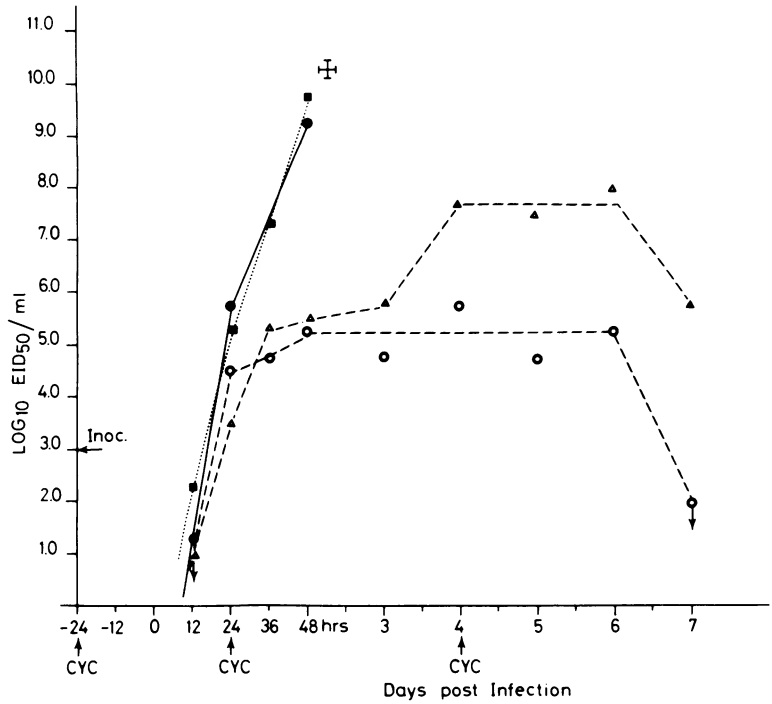

FIG. 1. *Comparative growth curves of hepatotropic influenza virus in livers of normal and Cyc-immunosuppressed adult ICR and A2G mice after i.p. inoculation of 1,000 EID_{50} of egg-grown TURH. Points represent egg infectivity of pools of three livers. Symbols: ●, Normal ICR mice; ■, ICR mice treated with Cyc; ○, normal A2G mice; △, A2G mice treated with Cyc. Cyc (150 mg/kg) was given 1 day before and 1 and 4 days after infection.*

EID_{50}. Immunosuppression did not interfere with TURH replication in susceptible ICR mice. Virus was first detected in their livers by 12 h. Titers rose rapidly to 10^9 EID_{50}/ml by 48 h, when animals were nearing death. In A2G mice the initial rise in infectivity was equally sharp. However, titers reached by days 2 and 3 were about 10,000 times lower than in ICR and were essentially the same in Cyc-suppressed and nonsuppressed animals. Beyond day 3 immunosuppressed A2G mice had more virus in their livers than controls. Attempts to isolate infectious virus from A2G brains late in the disease revealed comparable neurotropism of TURH in immunosuppressed and nonsuppressed A2G mice.

Lesions in livers of normal and Cyc-treated A2G mice after TURH infection. TURH infection of susceptible mice induced gross abnormalities of the livers, which became swollen, friable, and pale yellow by 48 h after infection. Histologically, acute degenerative hepatitis consisted of widespread liver cell necrosis with almost no inflammatory reaction (11). In sharp contrast, infected A2G livers remained macroscopically normal. Liver cell necrosis was confined to minute foci never larger in diameter than about 15 to 20 hepatic cells and showing no preferential location within the various fine structures of the organ. Tiny lesions became first detectable 36 h after infection. They increased slightly in number and size during the next 48 to 72 h and healed spontaneously thereafter (Arnheiter and Haller, manuscript in preparation). A constant and striking feature in normal A2G was the marked inflammatory infiltration by phagocytes invading the lesions (Fig. 2). In Cyc-treated A2G mice such inflammatory infiltrates were absent (Fig. 3). Nevertheless, hepatic cell necrosis remained strictly confined and showed no tendency to spread. Hepatic lesions were not seen in liver sections from control A2G mice receiving the drug alone.

Distribution of viral antigens in the livers of Cyc-treated A/J and A2G mice. Immunofluorescent evaluations of the amount and distribution of virus-specific antigens in the livers of Cyc-suppressed resistant and susceptible mice correlated well with the differences in virus growth and liver pathology described above. At 48 h after infection, liver sections of Cyc-treated

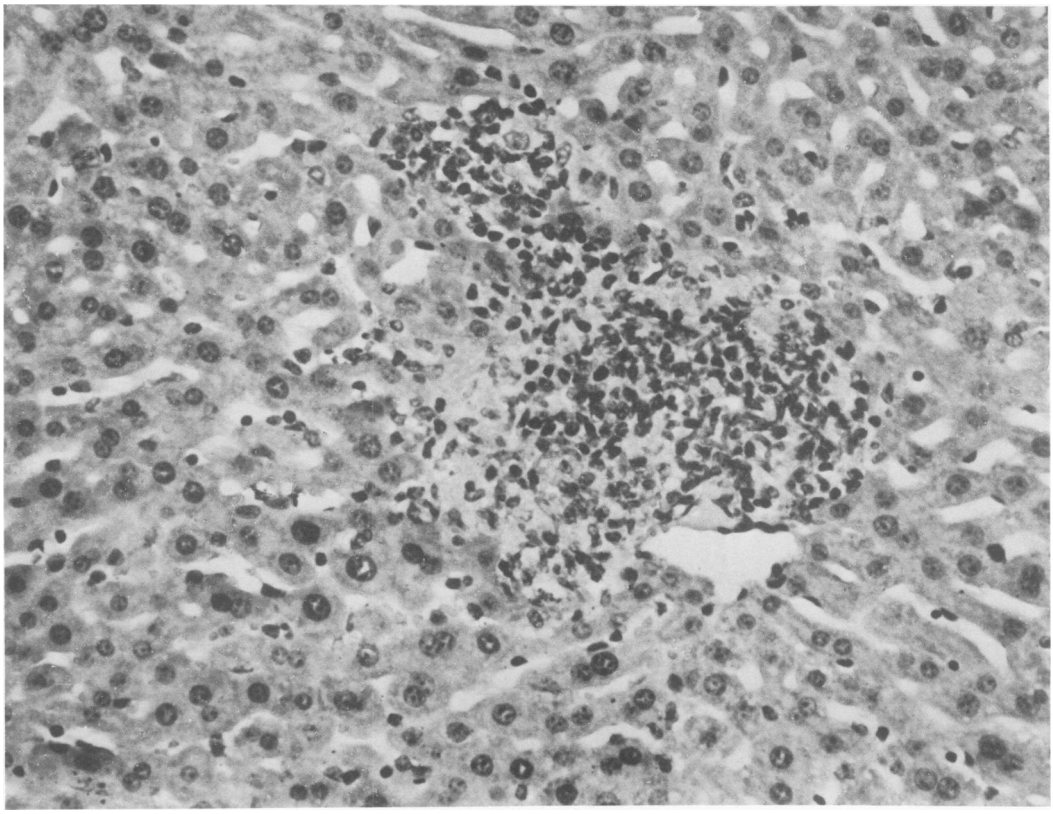

FIG. 2. *TURH-infected liver section from a normal adult A2G mouse 96 h after i.p. inoculation of 1,000 EID_{50}. Focal cellular infiltration. Hematoxylin and eosin; ×250.*

A/J mice showed wide-spread fluorescence of the parenchyma (Fig. 4a), whereas in similarly trated A2G mice only isolated fluorescent foci became detectable by 48 to 96 h (Fig. 4b); since cellular infiltrates were absent, the specific nuclear and cytoplasmic fluorescence of hepatocytes could be better appreciated (Fig. 4c). Fluorescence persisted to days 8 to 10 and was reduced thereafter. Fig. 4d shows the fluorescent pattern found in livers of immunosuppressed A2G mice 10 days after infection with 10^3 EID_{50}.

Effect of immunosuppression with Mtx and Pro. Attempts to break virus resistance of A2G mice by immunosuppressants other than Cyc were not more successful. Both Mtx and Pro are known to suppress humoral and cellular immune responses in mice (20, 21, 33). Furthermore, Mtx is capable of inducing long-term tolerance to viruses (10) and is considered superior to Cyc in inhibiting graft-versus-host disease (31). Pro seems to act predominantly on thymus-dependent cell populations (27) and is even able to prolong second-set homograft survival (9). Nevertheless, treatment of resistant mice with these drugs did not enhance mortality caused by hepatotropic influenza infection. Immunosuppressed A2G mice survived for more than 5 days a 100-fold larger virus dose than that shown to kill all susceptible controls. Later on, intercurrent deaths from general toxicity of Mtx occurred among both infected and noninfected animals.

Effect of reticuloendothelial blockage on resistance to TURH virus. Since recovery of A2G mice from TURH infection pointed to effector mechanisms other than primary immune response, the possible role of the reticuloendothelial system in mediating resistance to lethal hepatitis was investigated. Thorium dioxide is toxic for peritoneal macrophages when injected i.p. and aggravates virus infections in mice (24, 25). Similar effects are exerted by silica (3), which has been found most efficient in potentiating infection of mouse liver by herpesviruses (30, 37).

Virus titers in livers of Thorotrast-treated (see Materials and Methods) A2G mice 48 h after infection with 500 i.p. LD_{50} of TURH virus

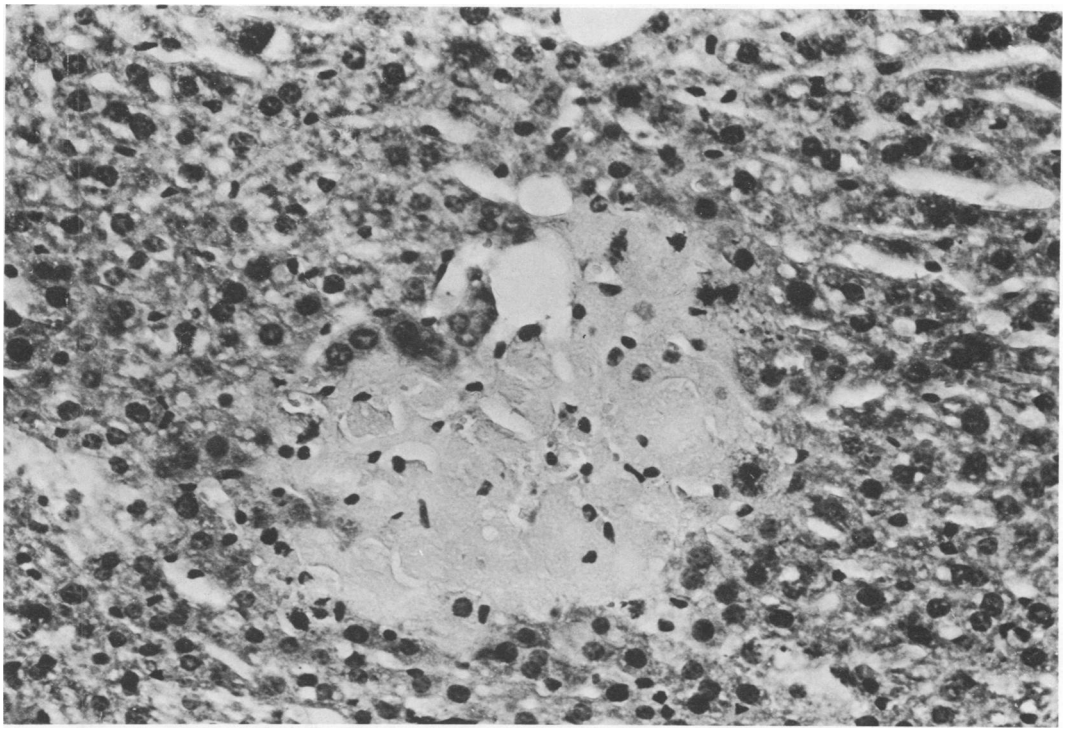

FIG. 3. *TURH-infected liver section from a Cyc-immunosuppressed A2G mouse 96 h after i.p. inoculation of 1,000 EID_{50}. Focal liver cell necrosis without inflammatory infiltrate. Hematoxylin and eosin; ×250.*

were 10,000 times lower than in similarly treated ICR mice and equaled those in non-treated resistant animals.

Pretreatment with silica (50 mg i.p.) was performed 3 days or 2 h before virus challenge. Silica-treated mice were able to resist 100 i.p. LD_{50} of TURH virus. After a 50-fold larger virus dose, part of the treated and untreated A2G mice died between days 7 and 12 after infection. No deaths were observed upon i.p. challenge with 100 i.p. ID_{50} in resistant mice previously injected i.v. with 3 mg of silica. Thus, despite reticuloendothelial blockade, A2G mice remained resistant to TURH infection.

Resistance of regenerating A2G liver after partial hepatectomy. In A2G mice, myxovirus resistance increases with age (16, 19). Maturation to resistance might be related to increasing maturity of the target organ itself rather than to developing systemic mechanisms. We therefore tried to influence the susceptibility of adult A2G livers by partial hepatectomy (36). It was hoped that, in analogy to cultured A2G kidney cells (18), growing liver cells or newly formed hepatocytes might support virus multiplication at higher rates than the resting cells of the intact adult organ. This was not the case. A2G mice challenged with TURH virus 2 days after partial hepatectomy resisted 300 i.p. ID_{50} as did sham-operated A2G controls, whereas control and partially hepatectomized A/J mice died of viral hepatitis in the usual fashion. Furthermore, two independent experiments showed only minor differences in virus titers 42 h after infection between normal and regenerating A2G livers examined 4 days after operation. Histologically, parenchymal cells in regenerating livers appeared larger than normal. Binucleate hepatocytes were numerous and mitotic figures could be detected (34). However, fluorescent staining in resistant hepatectomized mice was confined to the focal lesions already described (Fig. 5a). As in normal A2G mice, cellular infiltrates were seen at 96 h and specific fluorescence was reduced by day 6 (Fig. 5b).

DISCUSSION

Mice bearing the dominant gene *Mx*, known to resist the lethal action of neurotropic and pneumotropic influenza A virus (8, 16, 17, 19), have now been shown also to be highly resistant when infected with a hepatotropic influenza A virus of avian origin (11). Whereas in suscep-

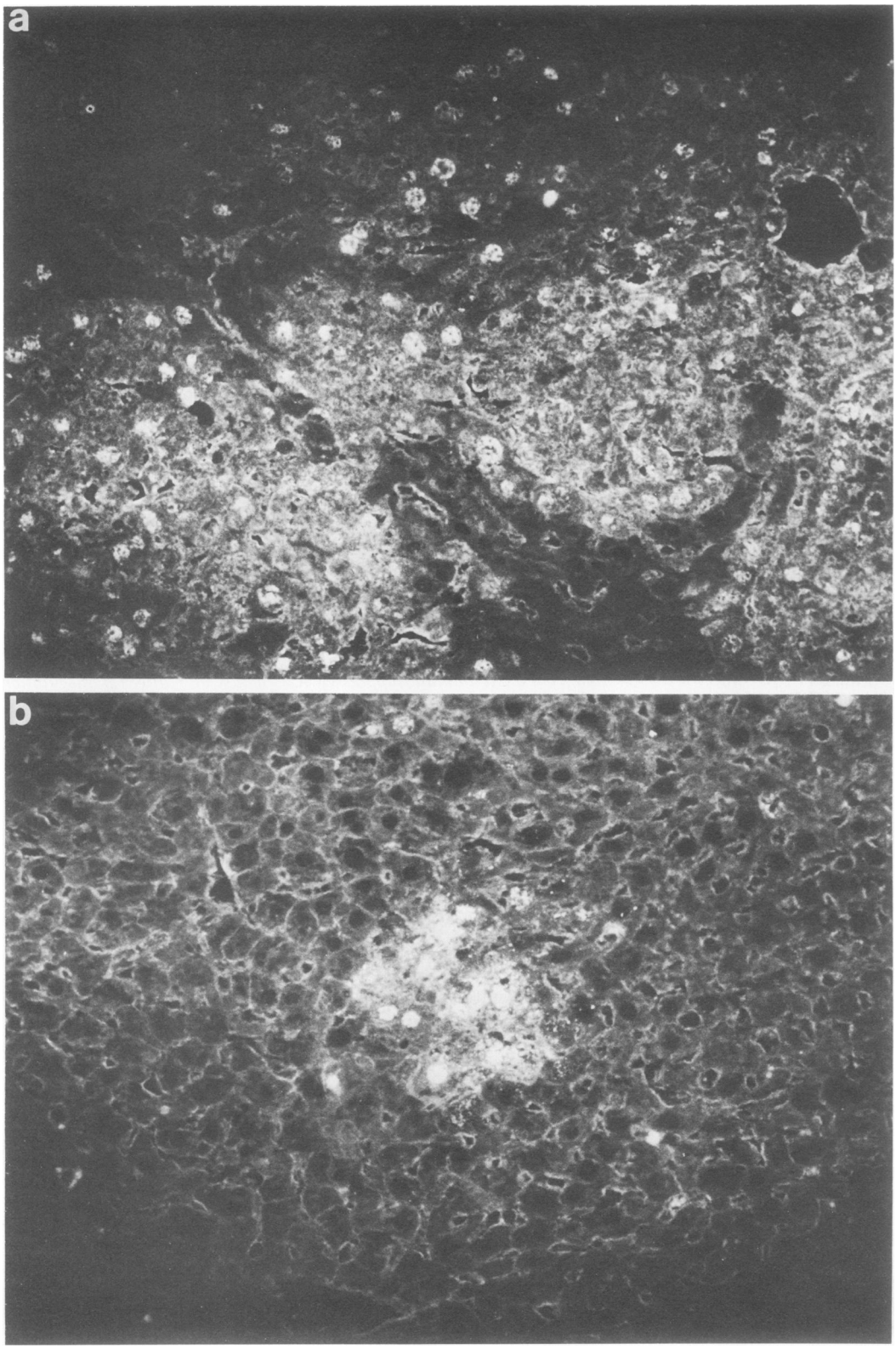

FIG. 4, *a*, *b*.

tible animals the virus spread rapidly throughout the whole liver, resistant animals developed small necrotic foci that were self-limiting (Fig. 2) and eventually healed. That these foci represented sites of virus replication was indicated by immunofluorescence (Fig. 4). Viral titers increased in the livers of resistant animals, but beyond 24 h after infection always were orders of magnitude lower than titers from similarly infected susceptible mice (Fig. 1).

Resistance simultaneously exhibited in different organs would most easily be explained by some systemic factor. Intrinsic resistance of all somatic cells appears unlikely, since previous experiments gave no evidence that cells put in tissue culture were less able to support virus replication when derived from *Mx*-bearing animals (18, 29); also, virus inhibitors were not detected in body fluids or tissue extracts (19). There is a possibility that macrophages might show resistance in vitro (A. C. Allison, personal communication), although this would be at variance with previous work (29). Interferon as a mediator of resistance has been discussed in conjunction with flaviviruses (13), but it is difficult to see how interferon would discriminate between different viruses, for instance, between influenza, to which *Mx*-bearing mice are resistant, and vesicular stomatitis, to which they are fully susceptible (19). Since newborn mice are susceptible (16, 18), we thought that regenerating liver might support high levels of virus replication; this was not the case.

The immune system as an obvious candidate comes next to mind (2, 23). Indeed, Mayer et al. (22) have claimed that immunosuppression abolished resistance in *Mx*-bearing mice. Several observations cast doubt on the validity of this interpretation. Animals immunologically crippled by neonatal thymectomy (P. A. Klein, personal communication) or by genetic manipu-

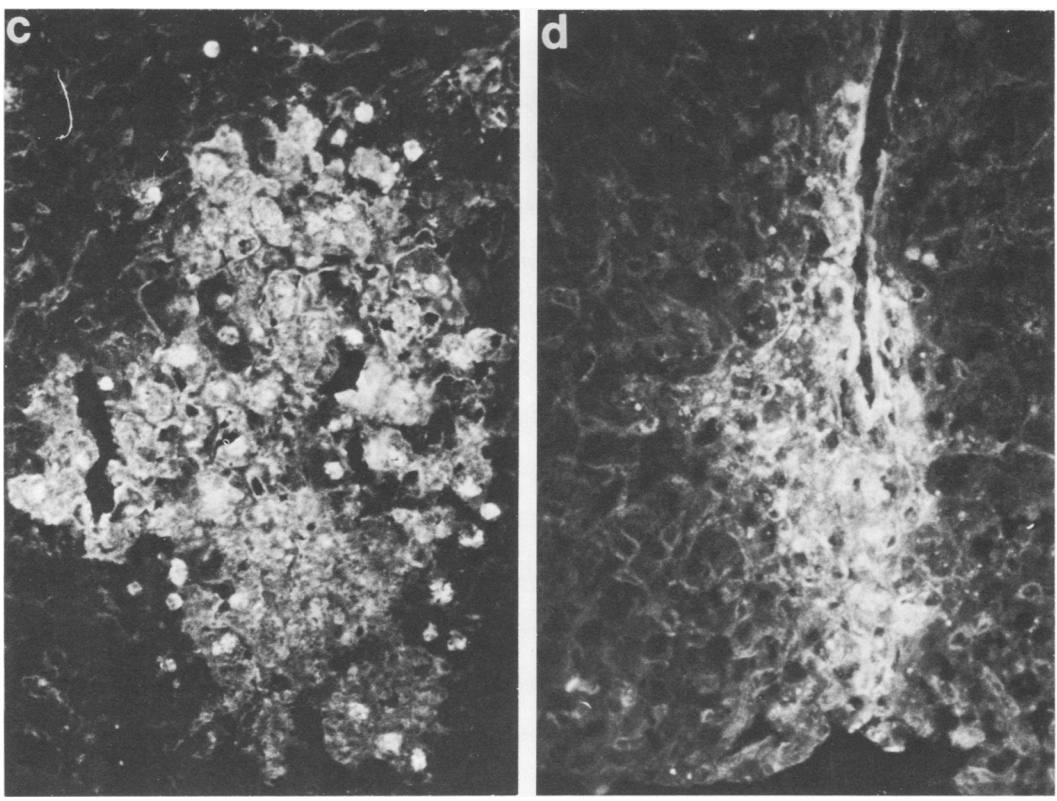

FIG. 4. *Immunofluorescent reaction of liver sections from Cyc-treated mice infected with 100 i.p. LD_{50} of TURH virus. Cyc (150 mg/kg) was given 1 day before and 1 and 5 days after infection. ×320. (a) A/J mouse, 48 h after infection. Widespread fluorescence of parenchymal cells, many of which are distintegrating. (b) A2G mouse, 96 h after infection. Staining is restricted to a single focal lesion. (c) A2G, 4 days after infection. Intense nuclear and cytoplasmic staining of hepatocytes within large focal lesion. Note absence of cellular infiltrates. (d) A2G, 10 days after infection. Parenchymal fluorescence still present. Specific staining is prolonged as compared with findings in normal (not shown) and partially hepatectomized animals (Fig. 5b).*

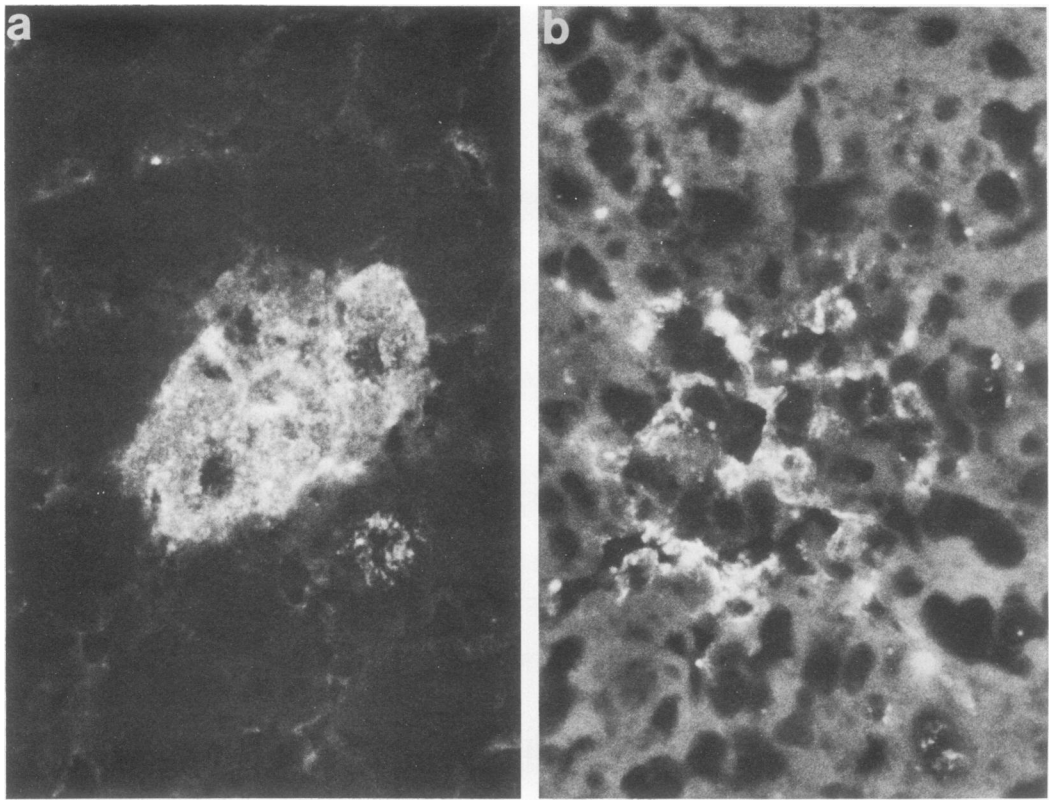

FIG. 5. *Immunofluorescent reaction of liver sections from A2G mice infected with 300 i.p. LD_{50} of TURH virus 48 h after partial hepatectomy. ×800. (a) Specific fluorescence 2 days after infection, involving a few liver cells. Parenchymal cells on the 4th day after partial hepatectomy appear to be larger than normal. (b) Fluorescent remnants and inflammatory cells 6 days after infection. Staining is less marked than in liver sections from Cyc-immunosuppressed animals (for comparison see Fig. 4d).*

lations (12) express resistance just as readily as do immunologically competent animals. Immunosuppression by Cyc or X irradiation did not influence the course of neurotropic infection (8). In the present paper we report failure of various immunosuppressive measures to affect the lethality of hepatotropic infection. The general defense mechanisms of immunosuppressed animals are severely compromised, and intercurrent infections may obscure the results. In our study this could be avoided by keeping experimental animals in a separate room, where they were attended by only one technician specially devoted to this task, and by supplying their acidified drinking water with chloramphenicol.

It might be argued that our immunosuppressive treatment was less efficient than that used by others (1, 4, 6, 14, 22, 26, 28, 32, 35). This is unlikely for several reasons. (i) From unpublished pilot studies, it appears that A2G mice are as susceptible to immunosuppression as are other strains. (ii) In preliminary experiments we had failed to appreciate the necessity of supplying the animals with chloramphenicol in their drinking water; immunosuppression frequently resulted in overwhelming bacterial infection in both virus-resistant and -susceptible mice, indicating that similar degrees of immune impairment had been achieved. (iii) When resistant mice were immunized with large doses of inactivated viral vaccine, those under immunosuppression made significantly lower humoral antiviral response (unpublished observation). (iv) The formation of cellular infiltration around necrotic liver foci, so prominent a feature in resistant normal mice (Fig. 2), was completely inhibited in resistant immunosuppressed mice (Fig. 3). Unless one is prepared to admit that the cellular infiltration had nothing to do with immune reactivity, this finding provides clear evidence that immunosuppression was effective at the very spot where an interaction between virus and host took place. Nevertheless, the foci showed no tendency to

spread, suggesting that the cellular infiltrates were not instrumental in limiting infection (8). (v) Another measurable effect of immunosuppression was delayed clearance of the virus (Fig. 1). A similar observation was made in nude mice carrying the gene *Mx* (unpublished observation). Delayed clearance thus appears to be a consequence of depressed immune reactivity.

The results of macrophage inhibition are more difficult to interpret. Although the dosage schedules were those commonly recommended (3, 25, 30, 37), there is no guarantee that a substantial impairment of macrophage function over some critical time interval was really achieved. Moreover, the effect of treatment on macrophages (or macrophage homologues) residing in the liver and forming possibly the first virus target could not be estimated. A more detailed description of liver pathology after intraperitoneal and intraportal injection of virus will be published elsewhere (Arnheiter and Haller, in preparation).

Whatever explanation for the effects of the gene *Mx* is eventually contemplated, it will have to take into account that resistance operates in three very different organs, brain, lung, and liver, and that it does so in the face of severe immunological malfunction.

ACKNOWLEDGMENTS

We thank M. Acklin for taking care of the mouse colony and for expert assistance, and V. Füllemann, R. Leemann, and H. Schaich for help.

LITERATURE CITED

1. **Aisenberg, A. C.** 1973. Immunosuppression by alkylating agents—tolerance induction. Transplant. Proc. V:1221-1226.
2. **Allison, A. C.** 1974. Interactions of antibodies, complement components and various cell types in immunity against viruses and pyogenic bacteria. Transplant. Rev. 19:3-55.
3. **Allison, A. C., J. S. Harington, and M. Birbeck.** 1966. An examination of the cytotoxic effects of silica on macrophages. J. Exp. Med. 124:141-153.
4. **Camenga, D. L., N. Nathanson, and G. A. Cole.** 1974. Cyclophosphamide-potentiated West Nile viral encephalitis: relative influence of cellular and humoral factors. J. Infect. Dis. 130:634-641.
5. **Carter, W. A., and E. De Clercq.** 1974. Viral infection and host defense. Science 186:1172-1178.
6. **Cole, G. A., and N. Nathanson.** 1968. Potentiation of experimental Arbovirus encephalitis by immunosuppressive doses of cyclophosphamide. Nature (London) 220:399-401.
7. **Darnell, M. B., and H. Koprowski.** 1974. Genetically determined resistance to infection with group B arboviruses. II. Increased production of interfering particles in cell cultures from resistant mice. J. Infect. Dis. 129:248-256.
8. **Fiske, R. A., and P. A. Klein.** 1975. Effect of immunosuppression on the genetic resistance of A2G mice to neurovirulent influenza virus. Infect. Immun. 11:576-587.
9. **Floersheim, G. L.** 1966. Effect of methylhydrazine derivatives on the survival of second-set skin homografts. Nature (London) 211:638-639.
10. **Haas, V. H., and S. E. Stewart.** 1956. Sparing effect of A-Methopterin and Guanazolo in mice infected with virus of lymphocytic choriomeningitis. Virology 2:511-516.
11. **Haller, O.** 1975. A mouse hepatotropic variant of influenza virus. Arch. Virol. 48:77-88.
12. **Haller, O., and J. Lindenmann.** 1974. Athymic (nude) mice express gene for myxovirus resistance. Nature (London) 250:679-680.
13. **Hanson, B., H. Koprowski, S. Baron, and C. E. Buckler.** 1969. Interferon-mediated natural resistance of mice to arbo-B virus infection. Microbios 1B:51-68.
14. **Hashimoto, K., Y. Okada, T. Tajiri, H. Amano, N. Aoki, and S. Saski.** 1973. Intestinal resistance in the experimental enteric infection of mice with a mouse adenovirus. III. Suppressive effect of cyclophosphamide on the establishment and duration of the intestinal resistance. Jpn. J. Microbiol. 17:503-511.
15. **Levaditi, C., and P. Haber.** 1937. Recherches sur le virus de la peste aviaire pathogène pour la souris. Ses affinités pour les néoplasmes. Rev. Immunol. 3:5-24.
16. **Lindenmann, J.** 1964. Inheritance of resistance to influenza virus in mice. Proc. Soc. Exp. Biol. Med. 116:506-509.
17. **Lindenmann, J., and P. A. Klein.** 1966. Further studies on the resistance of mice to myxoviruses. Arch. Gesamte Virusforsch. XIX:1-12.
18. **Lindenmann, J., and P. A. Klein.** 1967. Immunological aspects of viral oncolysis. Recent results in cancer research, vol. 9. Springer, Berlin, Heidelberg, New York.
19. **Lindenmann, J., C. A. Lane, and D. Hobson.** 1963. The resistance of A2G mice to myxoviruses. J. Immunol. 90:942-951.
20. **Liske, R.** 1973. A comparative study of the action of cyclophosphamide and procarbazine on the antibody production in mice. Clin. Exp. Immunol. 15:271-280.
21. **Makinodan, T., G. W. Santos, and R. P. Quinn.** 1970. Immunosuppressive drugs. Pharmacol. R. 22:189-247.
22. **Mayer, V., J. L. Schulman, and E. D. Kilbourne.** 1973. Nonlinkage of neurovirulence exclusively to viral hemagglutinin or neuraminidase in genetic recombinants OF A/NWS (HON1) influenza virus. J. Virol. 11:272-278.
23. **Merigan, T. C.** 1974. Host defenses against viral disease. N. Engl. J. Med. 290:323-329.
24. **Mims, C. A.** 1964. Aspects of the pathogenesis of virus diseases. Bacteriol. Rev. 28:30-71.
25. **Monath, T. P. C., and E. C. Borden.** 1971. Effects of thorotrast on humoral antibody, viral multiplication, and interferon during infection with St. Louis encephalitis virus in mice. J. Infect. Dis. 123:297-300.
26. Multiple authors. 1971. Symposium: effect of immunosuppression on viral infections. Fed. Proc. 30:1822-1964.
27. **Quagliata, F., J. M. Phillips-Quagliata, and G. L. Floersheim.** 1972. Immunosuppression by procarbazine. I. Sites of action of the drug and effect on adjuvant arthritis and circulation antibody responses. Cell. Immunol. 3:198-212.
28. **Rager-Zisman, B., and A. C. Allison.** 1973. Effects of immunosuppression on Coxsackie B-3 virus infection in mice, and passive protection by circulating antibody. J. Gen. Virol. 19:339-351.
29. **Rusanova, N. A., and V. D. Soloview.** 1966. On problem of natural antiviral immunity. The hereditary nature of the resistance mechanism of mice of A2G line to influenza virus. Vopr. Virusol. 11:398-402.
30. **Selgrade, M. K., and J. E. Osborn.** 1974. Role of macro-

phages in resistance to murine cytomegalovirus. Infect. Immun. 10:1383–1390.
31. **Storb, R., T. C. Graham, R. Shiurba, and E. D. Thomas.** 1970. Treatment of canine graft-versus-host disease with methotrexate and cyclophosphamide following bone marrow transplantation from histoincompatible donors. Transplantation 10:165–172.
32. **Thind, I. S., and W. H. Price.** 1969. The effect of cyclophosphamide treatment on experimental arbovirus infections. Am. J. Epidemiol. 90:62–68.
33. **Thomas, E. D., and R. Storb.** 1971. The effect of amethopterin on the immune response. Ann. N. Y. Acad. Sci. 186:467–474.
34. **Wilson, M. E., R. E. Stowell, H. O. Yokoyama, and K. K. Tsuboi.** 1953. Cytological changes in regenerating mouse liver. Cancer Res. 13:86–92.
35. **Worthinton, M., A. S. Rabson, and S. Baron.** 1972. Mechanism of recovery from systemic vaccinia virus infection. J. Exp. Med. 136:277–290.
36. **Yokoyama, H. O., M. E. Wilson, K. K. Tsuboi, and R. E. Stowell.** 1953. Regeneration of mouse liver after partial hepatectomy. Cancer Res. 13:80–85.
37. **Zisman, B., M. S. Hirsch, and A. C. Allison.** 1970. Selective effects of anti-macrophage serum, silica and antilymphocyte serum on pathogenesis of herpes virus infection of young adult mice. J. Immunol. 104:1155–1159.