EXPERIMENTAL MYOCARDITIS INDUCED IN ALBINO MICE BY INFLUENZA VIRUS

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UDC 616.127.002-022.6;576.858.75]-092.9

Mice of different ages were infected intravenously or intranasally with several strains of A2 influenza virus. The frequency of morphologically detectable lesions of the myocardium was shown to depend on the properties of the particular viral strain. After infection with one of the cardiotropic strains of the virus, 16 of the 56 (28.6%) mice showed residual traces of myocarditis. The course of influenzal myocarditis in the mice was one of typical focal interstitial lesions capable of changing into mixed interstitial and parenchymatous forms. Occasionally extensive areas of the myocardium were affected by necrosis. Preliminary treatment of the animals with the immunodepressant imuran (6-azathioprine) completely prevented the development of the myocardial lesions. No signs of reproduction of the virus could be found in the blood and heart tissues. It is concluded that the toxic properties of the virus and the immunological reactions of the host play an important role in the primary alteration of the blood vessels and capillaries of the heart leading to the development of influenzal myocarditis.

The question of the nature of the myocardial lesions in influenzal infection still remains unanswered. Some workers [1, 8] attribute them to virus myocarditis, others [2, 4] deny that there is any such thing as influenzal myocarditis and consider that the changes in the myocardium in influenza are based on disturbances of the blood supply to that tissue. In experiments on mice infected simultaneously with A1 influenza virus and staphylococci Maksimov et al. [3] found anticardiac antibodies in the blood serum of some of the animals and postulated a possible role of autoimmune processes in the development of influenzal lesions in the myocardium.

In experiments on albino mice to study the properties of various strains of A2 influenza virus the writers found that the frequency of morphologically detectable heart lesions depends on the properties of the virus; some strains produce lesions of the heart tissues consistently more often, others less often. The results of these experiments are described in this paper.

EXPERIMENTAL METHOD

The strains of A2 influenza virus were isolated in 1967 in Smolensk (strain No. 22) and Moscow (strain No. 8) and also in Moscow in the influenza outbreak during the winter of 1968-1969. These strains underwent 10-15 successive passages through the allantois of chick embryos. The titers of their infectivity for chick embryos as a rule were not below 10^7-10^9/ml. Strain A2 Moscow 8/67 was obtained from the Department of Virology, Central Postgraduate Medical Institute, as an inhibitor-resistant variant [7]. The virus was injected into albino mice either as a dose of 1 ml into the caudal vein or as a dose of 0.02 ml intranasally.

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Department of Virology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 11, pp. 105-109, November, 1973. Original article submitted January 9, 1973.

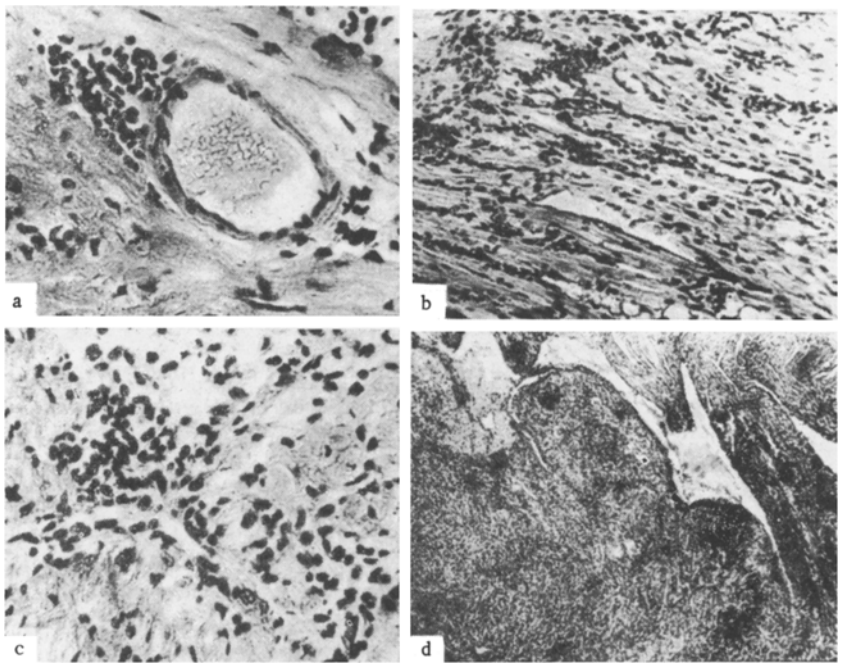

Fig. 1. Myocardial lesions in mice 7 days after intravenous injection of A2 influenza virus (see Table 2 for the preparations of animals used in the control tests accompanying the experiments to study the effect of imuran on the development of myocarditis; stained with hematoxylin and eosin): a) collection of histiocytes and lymphocytes by the wall of a vein in the ventricular septum (500 ×); b) small focus of interstitial myocarditis in wall of right ventricle (500 ×); c) focus of mixed interstitial and parenchymatous myocarditis in wall of left ventricle (220 ×); d) extensive necrotic focus in wall of right ventricle with damage to endocardium and subendocardial zone of ventricular septum (60 ×).

Altogether 780 mice of different ages were used in the experiments: 3 weeks (weight 7-8 g), 6 months (15-20 g), and 1-1.5 years (25-30 g). The animals were killed on the 7th day after injection of the virus. In one experiment 56 mice were kept under observation for 30 days (survival control).

The first step in the experiments with the immunodepressant imuran (6-azathioprine) was to determine the subtoxic dose, which is 20 mg for mice weighing 25-30 g. Imuran solution (30 mg/ml) was injected from a syringe (needle with an olive) into the esophagus in a dose of 0.1 ml daily, under general anesthesia; on the 2nd day the animals received an intravenous injection of 1 ml virus-containing allantoic fluid (strain 2/68).

Virus was isolated from the blood and heart in chick embryos for 7 days after the beginning of the experiment. The heart was fixed in 10% neutral formalin and embedded in paraffin wax; sections 5-7 μ thick were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

All the strains of influenza virus used caused some degree of pathological changes in the heart muscle of the infected animals. In mild cases these changes took the form of circulatory disturbances (severe congestion and stasis), the appearance of "cuffs" of lymphocytes and histiocytes around individual vessels (Fig. 1a), and the development of small foci of degenerative changes in the muscle fibers (unevenness of staining, disappearance of cross striation, and so on). Changes of this type were not regarded as features of myocarditis.

Lesions of the heart muscle of myocarditis type (Table 1) were usually focal in character and were localized in the interstitial tissue. The impression was gained that in mice infected with influenza virus, by contrast with mice infected with Coxsackie virus [6], the primary lesion is in the vascular system of the

TABLE 1. Myocarditis Induced in Mice by A2 Influenza Virus

Strain of virus	Weight of animals (in g)	Mode of injection	Day of taking material	No. of animals infected	Number of animals with myocardial lesions	
					abs.	%
Hong Kong	7—8	Intravenously	7	50	0	
	15—20	"	7	56	9	16,1
Smolensk 22/67	15—20	Intranasally	7	37	2	5,4
	15—20	Intravenously	7	40	6	15,0
Moscow 8/67	15—20	Intranasally	7	20	0	
	15—20	Intravenously	7	90	13	14,4
	25—30	Intranasally	7	17	4	23,5
	25—30	Intravenously	7	40	10	25,0
Moscow 1/68	15—20	"	7	25	3	12,0
	25—30	"	7	25	1	4,0
Moscow 2/68	25—30	"	7	107	30	28,0
	25—30	"	30	56	16	28,6
Moscow 49/68	15—20	"	7	21	0	
	25—30	"	7	19	2	10,5
Moscow 76/68	15—20	"	7	22	2	9,1
	25—30	"	7	25	1	4,0

TABLE 2. Effect of Imuran on Development of Heart Lesions in Experimental Influenza

Material injected into mice	Number of animals investigated	Number of animals with myocardial lesions	
		abs.	%
Imuran	39	0	0
A2 influenza virus . .	48	10	21
Imuran + A2 influenza virus	43	0	0

myocardium; changes in the heart muscle develop secondarily, as a result of disturbance of the blood supply. Consequently, influenzal myocarditis in mice is characterized mostly by focal interstitial lesions (Fig. 1b), which change only in the later stages into mixed interstitial and parenchymatous forms (Fig. 1c). In rare cases the lesions affect wide areas of the heart muscle (Fig. 1d).

After intranasal infection, i.e., the natural mode of entry of the virus, the incidence of myocarditis was rather lower than after intravenous injection. For instance, after infection with A2 influenza virus, strain Smolensk 22/67, intranasally lesions of the myocardium were found in only 2 of 37 mice (5.4%), whereas after intranasal injection of A2 influenza virus, strain Moscow 8/67 into mice weighing 15-20 g no changes were found whatever in the myocardium. However, after injection of the inhibitor-resistant variant of strain Moscow 8/67 into older mice (weighing 25-30 g), the differences in the percentages of incidence of myocarditis depending on whether the virus was given by the intranasal or the intravenous route were not significant (23.5 and 25.0%). In most experiments to test the ability of various strains of A2 influenza virus to produce myocardial lesions the infectious material was therefore injected intravenously.

The data in Table 1 show that when the virus was injected by this route the relative incidence of myocardial lesions depended more on the properties of the viral strains used than on any other factors.

In experiments to study the survival rate of 56 mice, observations continued on the animals for 1 month after infection with the most "cardiotropic" strain (Moscow 2/68). All the animals remained alive, but histological investigations showed that more than half of them had a more intensive pattern of the myocardial vessels, caused by thickening and condensation of the adventitial connective tissue, by microfoci of swollen muscle cells with groups of enlarged nuclei, and by small, dense cuffs of histiocytes near the small blood vessels. Residual traces of previous myocarditis, consisting of small foci of myocardial sclerosis, dense concentrations of histiocytes, and a few macrophages interspersed with them (mainly near the endocardium), remained in 16 of the 56 mice (28.6%). In one case comparatively recent foci of proliferation of connective tissue, rich in cells, were found beneath the endocardium; this created the impression of a more protracted process resembling chronic productive myocarditis in character [1].

The virological investigations showed that whether the virus was injected intranasally or intravenously the various strains could be detected in the blood until the 5th-7th day of the experiment. However, the concentration of the virus in the blood fell sharply after the 1st day. Virus was found in the heart muscle in solitary cases but only in minimal amounts and for 1-2 days in mice which had virus in the blood. Consequently, there is reason to suppose that influenza virus reproduces neither in the blood stream nor in the heart muscle. The lesions discovered in the myocardium (more precisely, primary alteration of the blood

vessels and capillaries of the heart) cannot therefore be connected with reproduction of the virus in the muscle or connective tissue elements of the heart wall or with its direct harmful action.

In view of reports that the myocardial lesions in infectious diseases may be allergic in nature [1, 3, 5], in a series of experiments on 50 mice weighing 25-30 g infection of the animals with influenza virus was preceded by lowering their immunoreactivity with imuran (Table 2). After administration of imuran alone, 11 of the 50 mice died on the 2nd-5th day; after injection of the virus alone 2 mice died on the 3rd day; infection after administration of imuran led to the death of 7 mice on the 3rd-4th day. The results of these experiments were striking: none of the 43 animals showed any sign of a lesion of the heart muscle or the vessels of the heart. It must be particularly emphasized that in control experiments in which virus was given without imuran severe lesions of the heart were found in 10 of the 48 mice (20.8% of the animals). The character of these lesions in the control experiments was the same as that described above. Consequently, there is no doubt that the absence of lesions of the myocardium in the experiments with imuran was in fact due to the action of the immunodepressant.

The absence of reproduction of the virus in the heart tissues thus indicates that influenza virus has no direct harmful action on the heart tissues. The significant differences in the ability of the various strains of A2 influenza virus to cause the development of myocarditis and, in particular, the absence of discernible lesions of the heart tissues in animals receiving the immunodepressant demonstrate the important role of the toxic properties of the virus and the immunological response of the host in the pathogenesis of the cardiac lesions in experimental influenza.

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