VIROLOGY

Structural Changes in the Liver of Mice Infected with Avian Influenza Virus Subtype H5N1

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Intranasal infection of male outbred rats with isolate of influenza A virus subtype H5N1 (A/Gs/Krasnoozerskoye/627/05) from the Novosibirsk region was followed by high mortality of experimental animals. Morphological study of liver samples revealed subtotal destructive changes in the liver parenchyma (proteinosis and centrolobular necroses), which was related to hemodynamic disorders and cytokine dysregulation. The decrease in reparative activity of hepatocytes was probably followed by hepatocellular failure and contributed to high mortality rate from this infection (up to 85%).

Key Words: *H5N1 avian influenza virus (A/Gs/Krasnoozerskoye/627/05); liver; morphological study*

In 1997-2007 the incidence of human infection with highly pathogenic avian influenza virus (AIV) reached 306, of them 150 patients died [6,8,11]. This disease is associated with direct transmission of AIV subtype H5N1 from infected birds to humans [10,11].

Influenza A virus subtype H5N1 is highly virulent and causes generalized infection in the human population in the absence of previously formed specific immunity. Studies of circulating isolates revealed continuous evolution of this virus and increase in species range of host organisms [5]. Hence, subtype H5N1 of virus A is considered as the most probable cause for influenza pandemics.

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An outbreak of avian influenza erupted in Russia at the end of July 2005 [1]. During this period, highly pathogenic AIV were first revealed in Russia. Hence, the evaluation of viral characteristics and morphological changes in the liver (homeostasis-maintaining organ) is an urgent problem.

This work was designed to study morphological changes in the liver of mice infected with influenza virus subtype H5N1 from Russia (A/Gs/Krasnoozerskoye/627/05).

MATERIALS AND METHODS

Experiments were performed on 125 male outbred mice aging 6 weeks and weighing 20-25 g. This approach allowed us to exclude genotype determination for the response. Influenza A virus subtype H5N1 (strain A/Gs/Krasnoozerskoye/627/05) was isolated from dead birds during influenza pandemic in the Novosibirsk region (July 2005) [9].

The animals were divided into 2 groups. Control group 1 consisted of 30 intact mice. Group 2

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animals were intranasally infected with influenza A virus subtype H5N1 (strain A/Gs/Krasnoozerskoye/627/05) in a dose of MLD_{50} (mouse lethal dose, death of 50% animals). The animals were maintained under standard conditions and had free access to food and water.

The samples were obtained on days 1, 2, 3, 6, and 10 after infection (15 mice per point). The animals were killed by cervical dislocation under ether anesthesia. Liver samples were examined.

For light microscopy, liver samples were fixed in 10% neutral formalin, dehydrated with alcohols in increasing concentrations, and embedded in paraffin. Sections (5 μ) were stained with hematoxylin and eosin (van Gieson technique). The following morphometric parameters were evaluated: volume density of destructive changes, volume density of portal tract infiltration, numerical density (ND) of lymphocytes in sinusoids, and ND of binucleated hepatocytes. The mean values were calculated by methods of variation statistics. The significance of differences between mean values was estimated by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Infection of animals was followed by acute infectious process. The mean life span was 8.19±0.18 days. The mortality rate was 85%.

Light microscopy of the liver parenchyma from infected animals revealed paretic dilation and plethora of large vessels and sinusoids, stasis and hemolysis of erythrocytes, and fibrinoid changes in the vascular wall during the first 6 days after infection.

Starting from day 1 after infection we observed hepatocyte degeneration presented by hydropic changes later transformed into balloon degeneration. Necrotic areas were located centrolobularly. The volume density of degenerative and necrotic changes in the liver parenchyma of AIV subtype H5N1-infected animals was summarized. The volume density of liver destruction reached maximum on day 6 after infection (93.78%). These data illustrate subtotal damage to hepatocytes and development of liver failure. This parameter decreased by 17.6% on day 10, but remained relatively high (77.3% hepatocytes, Table 1).

Reparative activity of the liver parenchyma was evaluated from ND of binucleated hepatocytes. On days 1 and 2 after infection, ND of binucleated hepatocytes was below the control (by 5 and 10 times, respectively), but on days 6-10 this parameter increased by 8 times (Table 1).

The inflammatory cell response was manifested in infiltration of portal tracts with lymphocytes and macrophages and presence of lymphocyte chains in sinusoids. In all periods, the volume density of infiltrates was <1% (Table 1). ND of lymphocytes in the lumen of dilated sinusoids decreased by 4 times on days 1-6 after infection (Table 1). These data suggest that replication of AIV H5N1 is accompanied by inhibition of cell immunity. A significant increase in lymphocyte ND was observed on days 6-10 after infection (by 17.5 times, Table 1).

Histological study showed that morphogenesis of liver changes during viral infection with influenza A virus subtype H5N1 (A/Gs/Krasnoozerskoye/627/05) is mainly characterized by destructive processes and inhibition of the cellular immune response. These changes are probably associated with dysregulation in production of proinflammatory and antiinflammatory cytokines by the mononuclear phagocyte system and natural killer cells. Previous

TABLE 1. Morphometric Parameters of Structural Changes in the Liver of Experimental Animals Infected with AIV H5N1 (A/Gs/Krasnoozerskoye/627/05, *M*±*m*)

Parameter	Control (intact animals)	Infected animals				
		period after infection, days				
		1	2	3	6	10
Volume density of destructive changes, %	1.80±0.06	86.34±0.62*	91.04±0.53*+	90.80±0.48*	93.78±0.43*+	77.30±0.76*+
Volume density of infiltrates, %	—	0.06±0.06	0.28±0.16	0.06±0.04	0.07±0.07	0.53±0.17*+
ND of lymphocytes in sinusoids, 4.2×10^4 μ²	2.45±0.15	3.30±0.24*	1.92±0.14*+	0.84±0.08*+	0.82±0.12*	14.30±0.85*+
ND of binucleated hepatocytes, 4.2×10^4 μ²	9.64±0.26*	1.98±0.17*	1.06±0.14*+	1.34±0.17*	1.03±0.15*	8.02±0.51*+

Note. $p<0.005$: *compared to the control; +compared to previous period.

studies showed that the concentration of proinflammatory cytokines interleukin-6 (IL-6) and TNF-α increases, while the content of IFN-γ decreases in mammalian blood plasma during the early period after infection [2]. Cytokine-inactivated sinusoidal cells synthesize eicosanoids (thromboxanes, prostacyclins, *etc*.), which contributes to paretic dilation of vessels, platelet and erythrocyte sludge in sinusoids, and ischemic hepatocellular necrosis [4]. Activation of macrophages and Kupffer cells is followed by increased production of reactive oxygen and nitrogen species [7], which determines the cytotoxic effect on hepatocytes.

The severity of destructive changes in the liver of survived animals decreased, while ND of lymphocytes in sinusoids increased on day 10 after infection. These changes are probably associated with increased secretion of proinflammatory cytokines IL-10 and IL-2 [2] and alternative activation of macrophages, which results in the decrease in cytotoxic properties, high-intensity endocytosis of cell destruction products [3], and simultaneous elevation of reparative activity of hepatocytes.

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