Mast cell degranulation and histamine release during A/H5N1 influenza infection in influenza-sensitized mice

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ABSTRACT

Here we evaluate the role of mast cells in infection with influenza A/H5N1 virus in immunized mice. CBA mice were immunized intramuscularly with formalin-inactivated A/Vietnam/1194/2004 (H5N1)NIBRG-14 (H5N1). Serum samples were obtained on days 7, 12, 14, 21 after immunization. At day 14, the mice were infected intranasally with the A/Indonesia/5/2005 (H5N1)IDCDC-RG2 (H5N1) influenza virus with half of the animals receiving a mixture of the antihistamines. 67% of the vaccinated mice were protected from the lethality compared to 43% in the PBS-immunized group. Administration of antihistamines increased survival up to 85%–95%. Immunohistochemical examination using CD117 staining of the lungs demonstrated a larger quantity of activated mast cells after infection of immunized mice compared to mock-immunized mice. This was correlated to increased histamine level in the lungs and blood. Our experimental results suggest the involvement of mast cells and the histamine they produce in the pathogenesis of influenza infection in case of incomplete formation of the immune response to vaccination and mismatch of the vaccine and infection influenza viruses.

1. Introduction

Avian H5 influenza viruses (the genus *Influenzavirus*, the family *Orthomyxoviridae*) present great concern to health authorities around the world due to sporadic human infections causing serious illness and high mortality [1]. The A/H5N1 influenza virus was considered to have potential to cause a possible pandemic after it had been isolated from a child who died of viral pneumonia during a massive chicken outbreak in Hong Kong in 1997 [2]. According to the WHO data for the period from 2003 to 2019, the mortality rate from laboratory confirmed A/H5N1 influenza was 30%–66% [https://www.who.int/influenza/human_animal_interface/2020_MAY_tableH5N1.pdf?ua=1]. From 2014 to 2019, the incidence and mortality rate of H5 influenza decreased, and no human cases of this disease have been registered anywhere in the world in 2020 [3]. From 1997 to 2001, A/H5N1 viruses remained antigenically conserved, but from 2003 an unusually high level of A/H5N1 evolution has been observed. Highly pathogenic A/H5N1 viruses isolated from poultry and humans were divided into 3 phylogenetic branches (clades), which differ antigenically and genetically [1]. Since 2003, 10 distinct clades of avian influenza A/H5N1 have been established. In 2014, HA gene segments of H5N1, H5N2, H5N5, H5N6 and H5N8 subtypes were designated as clade 2.3.4.4, which was found in birds in 40 countries in Africa, Asia and Europe [4].

In 2019, clade 2.3.4.4 cocirculated with other clades of H5 viruses such as clade 2.3.2.1 [https://www.who.int/influenza/vaccines/virus/201909_zoonotic_vaccinevirusupdate.pdf?ua=1].

The clinical features of a human infection caused by highly pathogenic A/H5N1 viruses are characterized not only by primary viral pneumonia, but also by complications with acute distress syndrome and multiple organ lesions [5]. The development of vaccines against A/H5N1 viruses is included in the overall pre-pandemic preparation plan. Candidate vaccine strains for the preparation of inactivated A/H5N1 vaccines recommended by the WHO include A/Viet Nam/1194/2004(H5N1) NIBRG-14 viruses (clade 1) and A/Indonesia/5/2005(H5N1) CDC-RG2 (clade 2.1.3.2) produced using reverse genetics based on the laboratory strain A/Puerto Rico/8/34 (H1N1) [https://www.who.int/influenza/vaccines/virus/201902_zoonotic_vaccinevirusupdate.pdf?ua=1]. It is known that inactivated influenza vaccines administered parenterally, are aimed primarily at the formation of serum antibodies with strain-specific properties. In case of mismatch between vaccine and infecting viruses, protection may be reduced [6].

The main goal of vaccination is to induce an antibody immune response. However, for some viral infections in individuals who have previously been infected or vaccinated, an antibody-dependent enhancement (ADE) phenomenon is described, with patients developing a

severe course of infection. ADE is characteristic of dengue fever, in which it received attention and was first described [7], but is also observed in other viral infections [8–11] including Zika, Ebola, HIV, respiratory syncytial virus and SARS-CoV infections [12,13].

Instead of a complete neutralizing antiviral immunity, so-called "sensitization" may occur as a result of primary infections with heterotypic viruses of the same genera, as well as divergent antigenic variants of the infectious virus during chronic infections, or after immunizations resulting in incomplete protective immunity. This can lead to an antibody-dependent increased secondary infection [14].

There is an assumption that if there is a lack of virus-neutralizing antibodies, the non-neutralizing influenza-specific antibodies may participate in cellular reactions [15]. During the 2009 influenza pandemic, the use of seasonal vaccines and the presence of non-neutralizing antibodies against A/H1N1pdm09 correlated with an increased risk of a more severe flu-like illness in infected people [16].

The low avidity of non-neutralizing antibodies can lead to the formation of immune complexes and the activation of the complement cascade [17], which caused severe disease in young people during the 2009 pandemic [18].

Scientific literature discusses the ADE phenomenon with the participation of immune complexes of IgG antibodies, which mediate exacerbation of diseases associated with a wide range of pathogens. The biological activity of immune complexes and ADE complicate the development of novel vaccines. The aim of the study was to evaluate the role of antibody-dependent mast cell reactions in influenza-sensitized mice.

2. Materials and methods

2.1. Ethics statement

All procedures involving animals were performed according to the "Rules Laboratory Practice" Ministry of Health of the Russian Federation № 708 n. The study was approved by the Local Ethics Committee for Animal Care and Use at the Institute of Experimental Medicine, Saint-Petersburg, Russia (protocol № 3/17 of 30.11.2017). Non-terminal procedures were performed under ether anesthesia. To control viral load in the lungs, animals were euthanized under ether anesthesia and cervical dislocation. The health status of the challenged mice was monitored and recorded once a day for 10–14 days post infection.

2.2. Viruses

The study used influenza viruses A/Vietnam/1194/2004(H5N1) NIBRG-14 (National Institute for Biological Standards and Control, UK) [A/Vietnam] and A/Indonesia/5/2005(H5N1) IDCDC-RG2 (Centers for Disease Control and Prevention, USA) [A/Indonesia]. These strains were produced by reverse genetics by the National Institute of Biological Standards and Control (NIBSC, United Kingdom) and the Center for Disease Control and Prevention (USA) using Vero-certified vaccine-producing cells and laboratory protocols that take into account that the end use of the vaccine is administration to humans. The WHO Influenza Center recommends these strains for the preparation of inactivated influenza vaccines against avian influenza. Viruses contain modified H5 subtype hemagglutinin and are safe for humans.

The viruses used in the study were cultivated in the allantoic cavity of 10-day-old developing chicken embryos (Skvoritsy poultry farm, Leningrad region, Russian Federation).

2.3. Preparation of inactivated viral antigen for immunization of mice

Inactivated whole virus vaccine was prepared as previously described [19] from A/Vietnam/1194/2004(H5N1) NIBRG-14 after purification and concentration on a stepwise 30/60% sucrose gradient.

Stock formaldehyde solution (37% w/w) was diluted in PBS (0.1. ml to 4 ml PBS). Purified virus at a concentration of 40,000 hemagglutination units (HAU) per ml was mixed with a 40-fold diluted 37% formaldehyde solution, in a ratio of 1:100 (0.04 ml to 4 ml virus), followed by an exposure of at least 3 days at $+4$ °C. To determine the completeness of inactivation, 100-fold and 10–fold virus dilutions and undiluted virus were inoculated into the allantoic cavity of 10-day-old chicken embryos and incubated for 2 days at 33 °C. The virus content was determined in a hemagglutination reaction.

2.4. Mouse model of lethal influenza infection after immunization

A formaldehyde-inactivated concentrated vaccine strain of influenza A/Vietnam/1194/2004(H5N1) NIBRG-14 virus was used to immunize mice intramuscularly (IM) at a dose of 20,000 HAU/1 ml in a volume of 0.1 ml. The control group received IM placebo — phosphate buffered saline (PBS) in the same volume.

Blood sera were taken from the mice on days 7, 12, 14 and 21 after immunization and the sera were stored at -20 °C until serological tests were performed. For the hemagglutination-inhibition assay (HI) sera were treated with receptor-destroying enzyme (RDE, Denka Seiken, Tokyo, Japan) and tested for HI antibodies against A/Vietnam/1194/2004(H5N1) NIBRG-14 or A/Indonesia/5/2005(H5N1) IDCDC-RG2 virus as previously described [19]. The enzyme-linked immunosorbent assay (ELISA) was conducted to determine serum IgG, IgG1, IgG2a and IgG3 antibodies against A/Indonesia/5/2005(H5N1) IDCDC-RG2 in 96-well microplates (Sarstedt AG & Co, Nümbrecht, Germany) as previously described [20]. For absorption we used 20 HAU/0.1 ml of the whole purified A/H5N1 viruses. The end-point ELISA titers were expressed as the highest dilution that yielded an optical density at 450 nm (OD_{450}) greater than the mean OD_{450} plus 3 standard deviations of negative controls at an equivalent dilution of sera.

On day 14 after immunization, the mice were infected with the A/Indonesia/5/2005(H5N1) IDCDC-RG2 virus at a concentration of one 50% murine lethal dose (LD_{50}) which was approximately $10^{4.5}$ EID_{50}. LD_{50} was determined after infection with serial dilutions of virus A/Indonesia from 10^2 to 10^7 EID_{50}.

Simultaneously with the infection, half of the immune mice were given intraperitoneal injections with a mixture of histamine receptor blockers H1 and H2, chloropyramine (Egis, Guyancourt, France) and quamatel (Gedeon Richter, Budapest, Hungary), each 6.7 mg/kg body weight, in a volume of 0.1 ml, or PBS in the same volume. The experimental design is shown in Fig. 1.

To determine the viral titer in the lungs, the samples were collected from mice at 72 h after viral infection, homogenized in PBS containing 100 U/ml penicillin, 100 μg/ml streptomycin and centrifuged for 10 min at 6000*g*. The viral titers were calculated as a $\log_{10}$ of the 50% embryonic infectious doses (EID_{50}) using hemagglutination as the endpoint, as described previously [19].

To determine the histamine levels in the lungs and blood of mice by ELISA, samples were collected on the 5th day after infection with the A/Indonesia/5/2005(H5N1) IDCDC-RG2 virus. Histamine production was evaluated using the EIA Histamine kit (Beckman Coulter, Brea, United States) according to the manufacturer's instructions.

For immunohistochemical examination, lung sections were obtained at day 8 after viral infection, fixed in 10% neutral buffered formalin and embedded in paraffin. Immunohistochemical staining for CD117 (mast cell marker) was done using rabbit polyclonal antibodies (mAb) to CD117 (Agilent, Santa Clara, United States), additional staining was performed using toluidine blue. Additionally, sections were stained with hematoxylin and eosin (H&E).

2.5. Statistics

Data was processed using Statistica software, version 6.0 (StatSoft, Inc. Tulsa, Oklahoma, USA) and graphics data were generated using
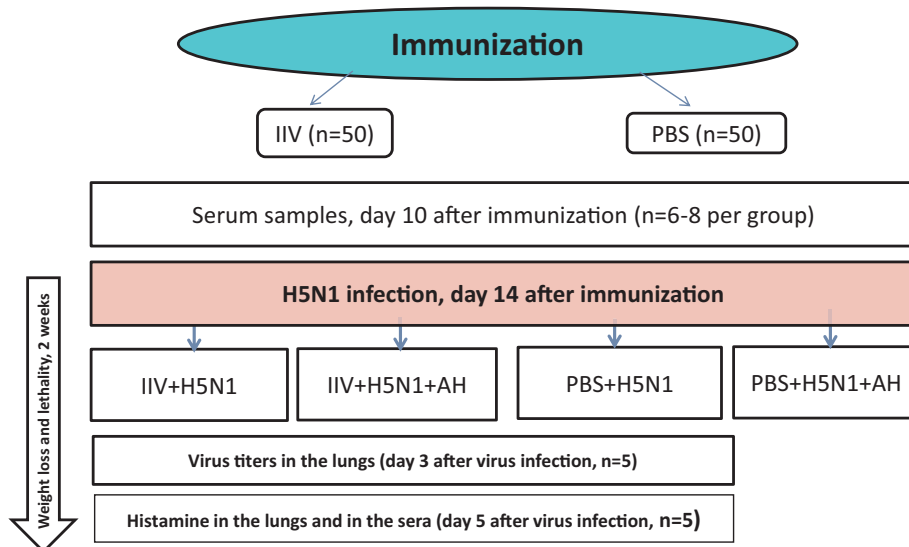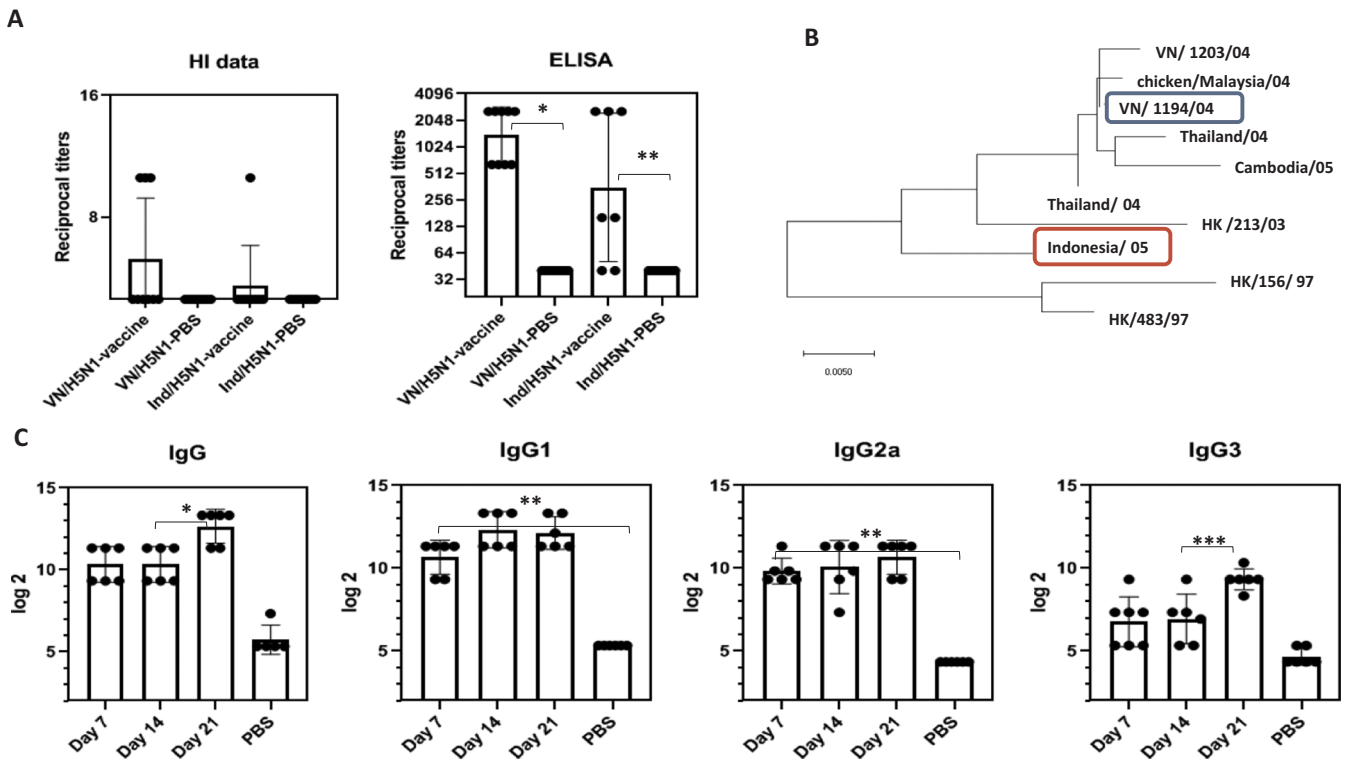
Fig. 1. Experimental study design.

Fig. 2. Immunogenicity after parenteral immunization of mice with A/Vietnam/2004/PR8/RG-23(H5N1) influenza virus. A. The immune response against vaccine virus and drift variant A/Indonesia/5/2005(H5N1) IDCDC-RG2 on day 12 after immunization according to hemagglutination-inhibition assay (HI) and ELISA test (* - P = 0.0004; ** - P = 0.02). B. Phylogenetic differences between vaccine and infectious A/H5N1 viruses. C. The dynamics of the serum IgG and IgG subclasses against A/Indonesia/5/2005(H5N1) IDCDC-RG2 influenza virus on day 7, 14 and 21 after immunization (* - P = 0.045; ** - P = 0.002; *** - P = 0.012).

Prism 8 (GraphPad software, San Diego, USA). Means and standard errors of means (SEM) were calculated to represent virus titers. Reciprocal antibody titers were expressed as log2 and presented as mean ± SEM. To compare two independent groups, we used a Mann-Whitney *U* test. To compare multiple independent groups, we used a Kruskal-Wallis ANOVA test. The P-value < 0.05 was considered to be statistically significant.

3. Results

3.1. Immunogenicity

On day 12 after immunization the levels of HI antibodies to vaccine and infecting viruses were lower than 1:40 (Fig. 2, A), i.e. did not reach a protective level which is associated with at least a 55% reduction in the risk of influenza [20]. At the same time, the levels of serum IgG specific to homologous A/Vietnam/1194/2004(H5N1) NIBRG-14 and drift A/Indonesia/5/2005(H5N1) IDCDC-RG2 influenza viruses among
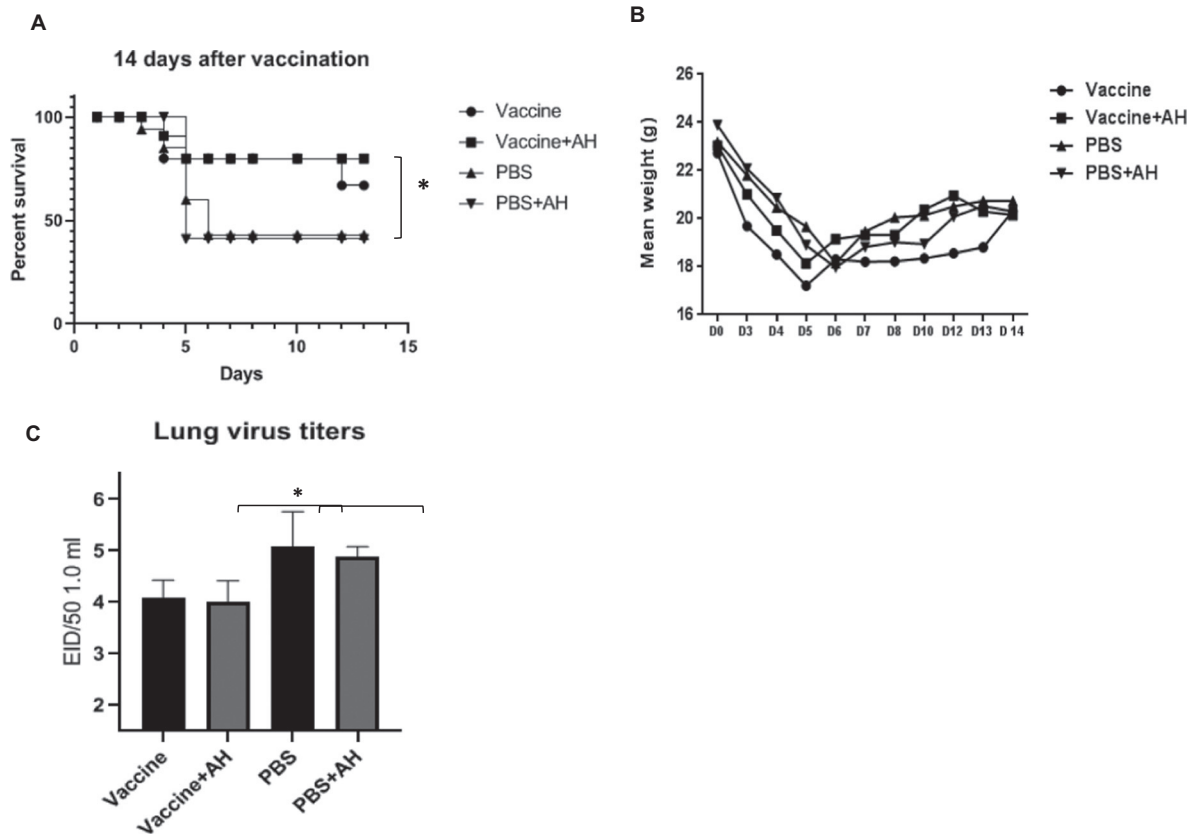
Fig. 3. Challenge with A/Indonesia/5/2005(H5N1) IDCDC-RG2 influenza virus on day 14 after immunization with A/Vietnam/1194/2004(H5N1) NIBRG-14. Three independent experiments were carried out with similar results; the figure shows the data of one of the experiments. P-values provided compared to PBS-immunized group. A – survivals after infection; half of the immunized or mock-vaccinated mice were administered antihistamines (AH) simultaneously with infection (n = 10–12). B – weight loss after infection; C - infectious virus isolation from the lungs (n = 5).

immunized mice significantly exceeded the levels in the control group (Fig. 2, B). Thus, the antibodies formed as a result of intramuscular immunization with A/Vietnam/1194/2004(H5N1) NIBRG-14 inactivated virus could represent mainly non-neutralizing antibodies by day 12 after immunization. To follow the dynamics of the serum IgG antibodies after parenteral immunization with an inactivated vaccine, we determined IgG of different subclasses at day 7, 14, and 21 after immunization. As shown in Fig. 2, C, serum IgG levels and IgG3 levels to A/Indonesia/5/2005(H5N1) IDCDC-RG2 influenza viruses on day 21 were significantly higher than on day 14 after vaccination. The average levels of virus-specific IgG1 increased by the 14th day after immunization, and the IgG2a increased by day 7 (Fig. 2, C). Thus, although all subclasses of IgG significantly increased by the third week of vaccination compared with non-vaccinated animals, the dynamics of this increase was different.

3.2. Influenza virus challenge on day 14 after immunization

Fig. 3, A shows that 70% of mice immunized with A/Vietnam/1194/2004(H5N1) NIBRG-14 influenza virus survived when A/Indonesia/5/2005(H5N1) IDCDC-RG2 infection was started on day 14 after immunization (P = 0.2 compared to PBS-immunized group where 33% animals survived). Thus, vaccination increased survival by 37%. The introduction of antihistamines (chloropyramine + quamatel) increased the survival of immunized animals by another 13% up to 83% (P = 0.046 compared to PBS-immunized group). Administration of antihistamines did not affect survival in the control PBS-immunized group (Fig. 3, A). It is noteworthy that in the group of immune mice without the use of antihistamines, mortality and a decrease in the average weight of animals were observed up to 13 days after infection,

while in the remaining groups the maximum mortality and weight loss occurred 5–6 days after infection (Fig. 3, A, B), although differences in weight were not statistically significant. The titers of the infectious virus in the lungs of immune mice after infection accompanied by antihistamine administration were significantly lower than in PBS-immunized mice without administration of antihistamines (P = 0.045, Fig. 3, C).

The decreased mortality due to antihistamine administration in infected mice previously immunized with A/H5N1 suggests the role of mast cells (or the histamine secreted by mast cells) perhaps under the influence of immune complexes containing IgG antibodies.

3.3. Immunohistochemical examination of the lungs

The assumption that mast cells participate during the infection process in immune mice was confirmed during immunohistochemical examination of the lungs, which is presented in Figs. 4, 5, 6 where present the specificity of the CD117 expression is shown. CD117 is a membrane tyrosine kinase receptor for stem cell factor (SCF) widely used as a mast cell marker [21]. After infection of non-immune mice with A/Indonesia/5/2005(H5N1) IDCDC-RG2, the CD117 + mast cells were not detected in peri-bronchial tissues; only moderate or small amounts of intact mast cells were found in the thymus and mediastinal tissue (Fig. 4, B, C, mast cells indicated by arrows). Few mast cells were located mainly in the perivascular space in an amount of two to four in visual field; in areas of edema there were up to 16 in the visual field. After infection of immune mice, from 2 to 10 mast cells were identified the in visual field mainly in the perivascular space; in areas of edema and red blood cell transudation from vessels up to 37 degranulated mast cells was found in visual field (Fig. 5, A, B, C, mast cells are indicated by
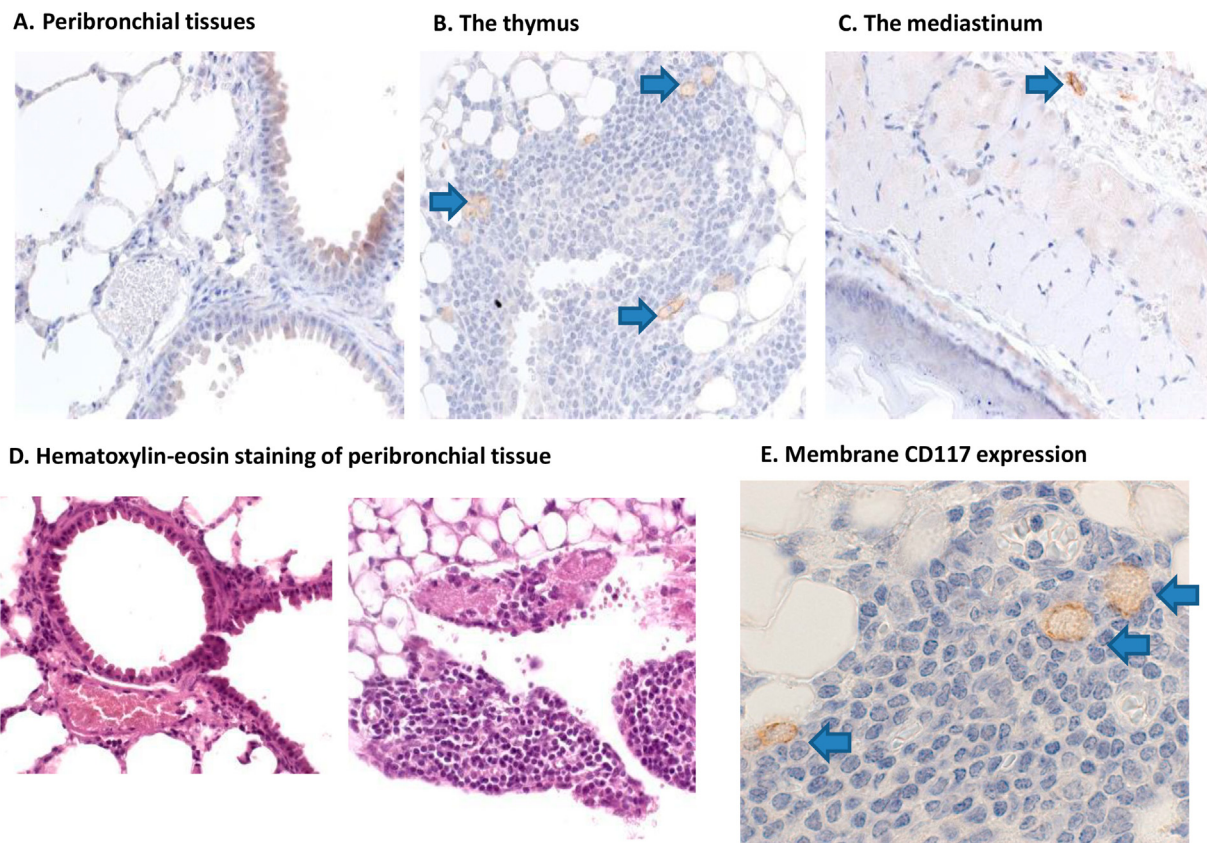
A. Peribronchial tissues

B. The thymus

C. The mediastinum

D. Hematoxylin-eosin staining of peribronchial tissue

E. Membrane CD117 expression

Fig. 4. Histological examination of the lungs on the PBS-vaccinated mice 8 days after infection with A/Indonesia/5/2005(H5N1) IDCDC-RG2. Mast cells were located mainly in the perivascular space in an amount of 2–4 in visual field; in areas of edema there were up to 16 in the visual field. The arrows indicate mast cells. A, B, C, D - immunohistochemical staining using mAb to CD117. The mast cells indicated by arrows. A- peri-bronchial tissues (no CD117); B – the thymus, C - mediastinal tissue; D - intact mast cells. E – H&E staining. A,B,C, E – ×200; D – ×1000.

arrows). After administration of antihistamines to immune mice during infection, mast cells were also detected in the peribronchial region, from 2 to 10 in the visual field (Fig. 6, A). In this case, both intact and degranulated mast cells were detected (Fig. 6, C, D). The data obtained do not allow us to conclude that the administration of antihistamines reliably affects the number of mast cells in the lungs and their localization. We assume that antihistamines, by blocking H receptors on histamine-sensitive structures, may abolish the reaction of vessels to histamine and thereby reduce blood cell transudation from vessels. Hematoxylin-eosin staining revealed the presence of peribronchial infiltrates in case of infection of immunized mice, both without antihistamines (Fig. 5, E) and when antihistamines were used (Fig. 6, B), in contrast to infection of non-immunized mice (Fig. 4, E) when peribronchial infiltrates were not detected.

3.4. The histamine content in the lungs and blood serum

The content of histamine secreted by activated lung tissue mast cells and blood basophiles was evaluated in the lungs and blood serum of mice on the 5th day after infection with A/Indonesia/5/2005(H5N1) IDCDC-RG2. As the data in Fig. 7 show, the histamine content in the lungs was higher than in the blood. In mice from all vaccine groups infected with the A/Indonesia/5/2005(H5N1) IDCDC-RG2 virus on the 14th day after immunization, the histamine level in the lungs was increased compared to control non-vaccinated mice that did not receive the virus. Moreover, the histamine level in the lungs and in the sera significantly differed from the control mice only in immune mice (Fig. 7, B). This may indicate that the release of histamine was induced not only by the development of a viral infection, but also by the antibody + virus immune complex as IgG-containing immune complexes

are able to activate mast cells to release histamine [22,23]. The administration of antihistamines reduced the histamine content in the lungs of immune animals infected with the drifted A/H5N1 virus very slightly. The introduction of antihistamines to infected immune animals slightly decreased the histamine content in the blood, although the differences were not statistically significant.

3.5. Influenza virus challenge on day 21 after immunization

Since it was found that on the 21st day the antibody response exceeded that on the 14th day, we conducted an experiment with the infection of mice at a later day after immunization. Three weeks after vaccination, 75% of vaccinated animals were protected from mortality due to A/Indonesia/5/2005(H5N1) IDCDC-RG2 virus infection (P = 0.023 compared to mock-vaccinated animals, Fig. 8, A) while in the group of non-immunized animals only 12.5% survived. The titers of the infectious virus in the lungs and weight dynamics were significantly different from those in non-immunized animals (Fig. 8, B, C). Thus, vaccination of mice using inactivated A/H5N1 vaccine provided more pronounced protection on the 21st day compared to the 14th day. In the case of a more complete immune response of antibodies, which are known to be the main protective factor for immunization with an inactivated vaccine, the protective effect of vaccination should prevail over possible antibody-dependent adverse reactions.

4. Discussion

The scientific literature widely discusses the involvement of IgG-containing immune complexes in ADE development, which may mediate exacerbation of diseases associated with a wide range of pathogens.
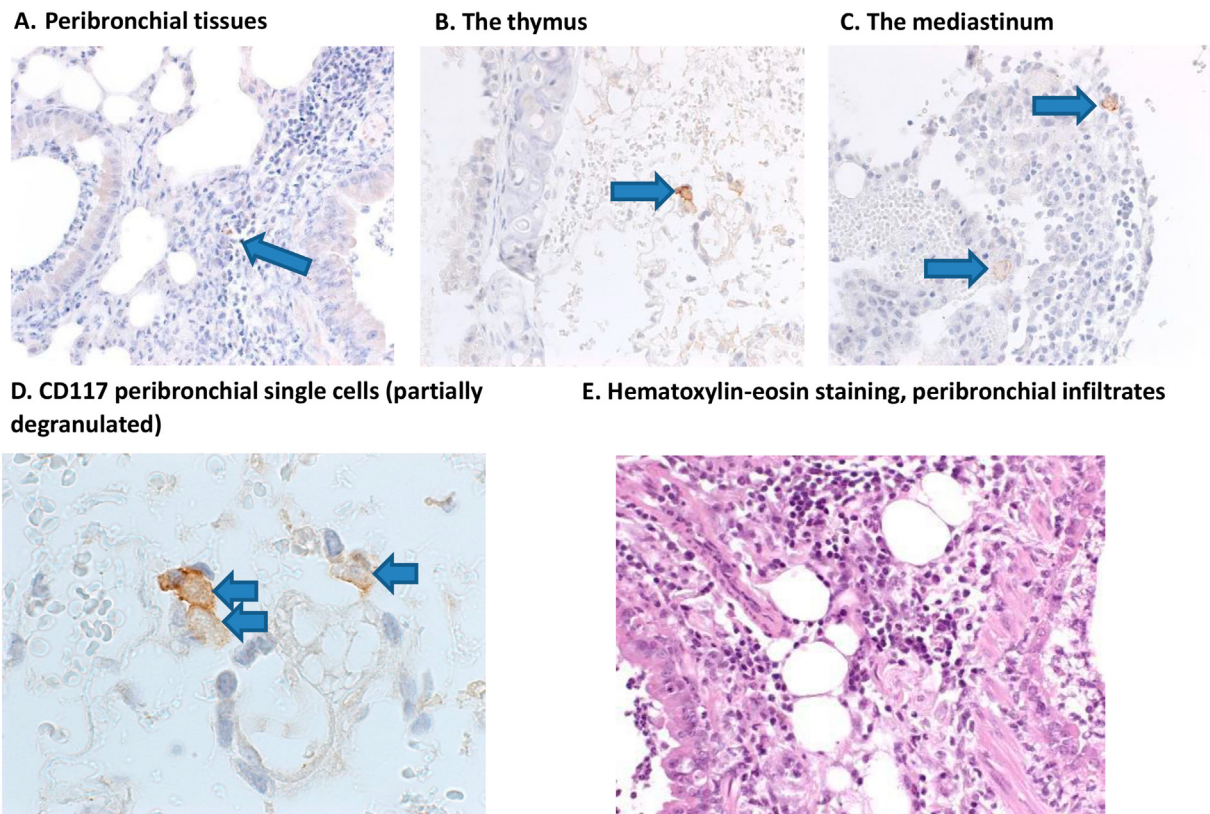
A. Peribronchial tissues

B. The thymus

C. The mediastinum

D. CD117 peribronchial single cells (partially degranulated)

E. Hematoxylin-eosin staining, peribronchial infiltrates

Fig. 5. Histological examination of the lungs on the A/H5N1-vaccinated mice 8 days after A/Indonesia/5/2005(H5N1) IDCDC-RG2 infection. From 2 to 10 mast cells were identified the in visual field mainly in the perivascular space; in areas of edema and red blood cell transudation from vessels up to 37 degranulated mast cells was found in visual field. A, B, C, D - immunohistochemical staining using mAb to CD117. The mast cells indicated by arrows. A- peri-bronchial tissues; B – the thymus, C - mediastinal tissue; D – partially degranulated mast cells. E – H&E staining. A, B, C, E – ×200; D – ×1000.

Non-neutralizing antibodies with low avidity to influenza virus that does not match the vaccine can activate the complement cascade and, in combination with dysregulation of cytokines, cause a damaging effect in the absence of neutralizing immunity [24].

Previously, it was shown that whole inactivated A/H1N2 influenza vaccines may provide only partial protection against drift variants of HA subtype in pigs, but also may induce vaccine-associated enhanced respiratory disease [25]. This study investigated the immune response against HA, neuraminidase (NA) and nucleoprotein (NP) which may play a role in obtained infection enhancement. It was shown that mismatched HA between vaccine and challenge virus was necessary to induce this, although vaccines containing a matched NA facilitated severity of infection due to HA mismatch and this was correlated with NA-inhibiting (NI) antibodies [25].

Wang et al. [26] studied whether vaccination with killed A/H5N1 virus (Re-6) while administering the antihistamine nizatidine protect mice against lethal influenza infection. Animals were vaccinated with a chemically inactivated virus with or without nizatidine (or cimetidine, another histamine receptor blocker) and then the mice were infected with a live virus 2 weeks after vaccination [26]. It was shown that vaccination with the killed virus alone, as expected, reduced the viral load in the lungs on day 6 after infection. However, a more significant decrease in viral load was observed in mice immunized with an inactivated virus with nizatidine or cimetidine, and in these mice, survival was significantly greater than that of mice immunized without antihistamines. Thus, nizatidine and cimetidine exerted an adjuvant effect, since their use demonstrated higher titers of virus-specific antibodies and higher T-killer activity. Unfortunately, the authors did not touch on the role of mast cells in the discussion, despite the fact that nizatidine and cimetidine are primarily H-receptor antagonists that block the tissue effects of histamine. Nevertheless, this publication is

devoted to the effect of antihistamines on the post-vaccination course of a viral infection and demonstrates, albeit indirectly, the possibility that mast cells participate in the pathogenesis of severe cases of the post-vaccination viral infections. Mast cells, sedentary elements of tissues, and circulating blood basophils, are the main source of histamine in the whole body; therefore, the positive effects of antihistamines can be attributed to turning off or reducing the effect of mast cells.

Mast cells belong to the innate immune system [27] and express receptors characteristic of this system: receptors for microbial and viral substances (TLR), complement factors (CR3, CR5) and Fc receptors for IgE and IgG. Mast cells synthesize and store cytokines in granules. Mast cells have been shown to produce cytokines IL-1β, IL-6, and TNF-α, a potent pro-inflammatory cytokine [28]. The role of mast cells and their Fc Ig epsilon receptors in the mechanism of immediate-type hypersensitivity reactions is well-known. Mast cells are also supposed to participate in immune complex type reactions (Arthus phenomenon, etc.), however, the role of mast cells and their Fc receptors for IgG (FcγR) in these processes is much less studied.

Mast cells possess CR3 and FcγR and therefore have the ability to recognize pathogens opsonized by complement, IgG or IgG and complement together. Mast cells also have an innate ability to recognize pathogens in the absence of opsonins via toll-like receptors (TLR) or the mannose receptor (CD48) [29]. It is suggested that mast cells that react with antigen via IgG antibodies may be involved in the development of ADE.

In our murine study, it was shown that when mice immunized with A/Vietnam/1194/2004 (H5N1) NIBRG-14 influenza virus were infected with A/Indonesia/5/2005(H5N1) IDCDC-RG2, which was antigenically different, the mortality rate of sensitized animals during reinfection decreased with the introduction of antihistamines, and more mast cells were found in the lungs than after infection of immune
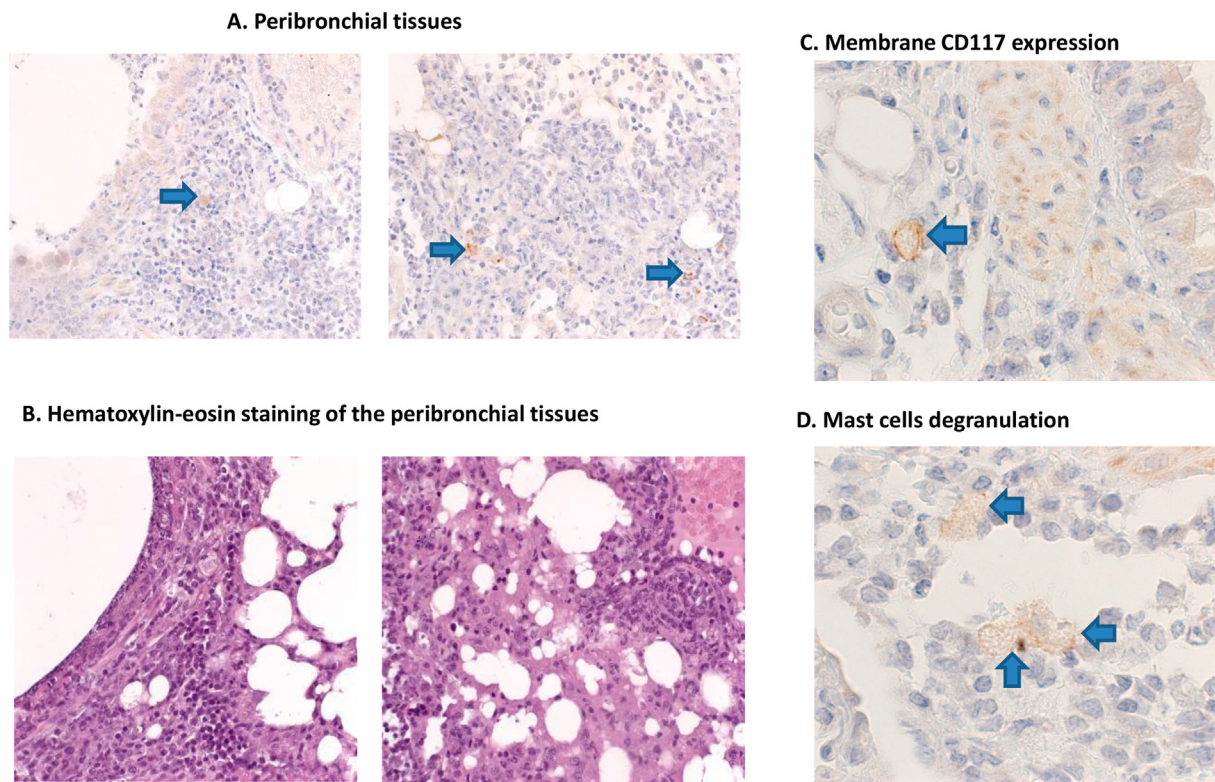
A. Peribronchial tissues

C. Membrane CD117 expression

B. Hematoxylin-eosin staining of the peribronchial tissues

D. Mast cells degranulation

Fig. 6. Histological examination of the lungs on the A/H5N1-vaccinated mice administered with antihistamines 8 days after A/Indonesia/5/2005(H5N1) IDCDC-RG2 infection. The mast cells were detected in the peribronchial region, from 2 to 10 in the visual field. A, C, D - immunohistochemical staining using mAb to CD117. The mast cells indicated by arrows. A - the mast cells in peri-bronchial tissues; B - H&E staining of peri-bronchial tissues; C - intact mast cells; D – degranulated mast cells. A, B – ×200; C, D – ×1000.
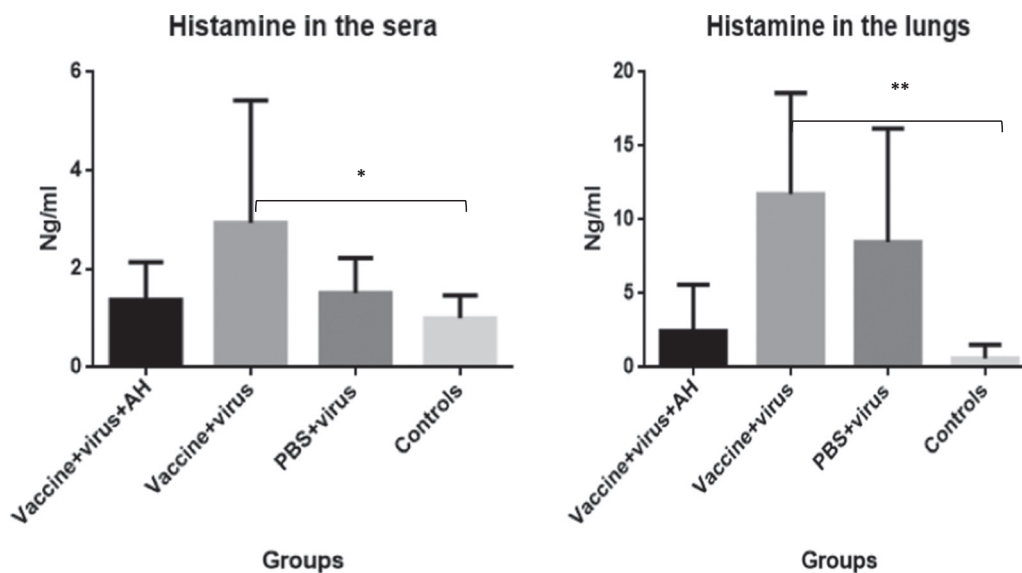

Fig. 7. Histamine levels in the sera and lungs on day 5 after virus infection, n-6. * - P = 0.016; ** - P = 0.033.

animals compared to mock-immunized mice. The histamine levels in the lungs of immune mice after reinfection were higher than in the lungs of mock-immunized animals. The fact that antihistamines did not significantly reduce the histamine content in the lungs and blood serum of immune animals after infection confirms the fact that antihistamines affect the ability of histamine receptors to perceive histamine and the ability of cells to respond to histamine, but not the number of histamine molecules released by mast cells. These data may indicate at the role of histamine secreted by activated mast cells in pathology when mice

sensitized to A/H5N1 virus lacking neutralizing antibodies were infected with a drift influenza virus. It was assumed that the animals lacked virus-neutralizing antibodies by the time of infection based on the fact that HI antibodies did not reach the protective level (1:40), although ELISA IgG of different subclasses was detected. It has been shown previously that H5 HA is poorly immunogenic and may need either booster or adjuvanted immunization for eliciting protective titer. In our previous studies in mice, it was shown that immunization with an inactivated influenza vaccine (IIV) of A/H5N2 subtype elicited
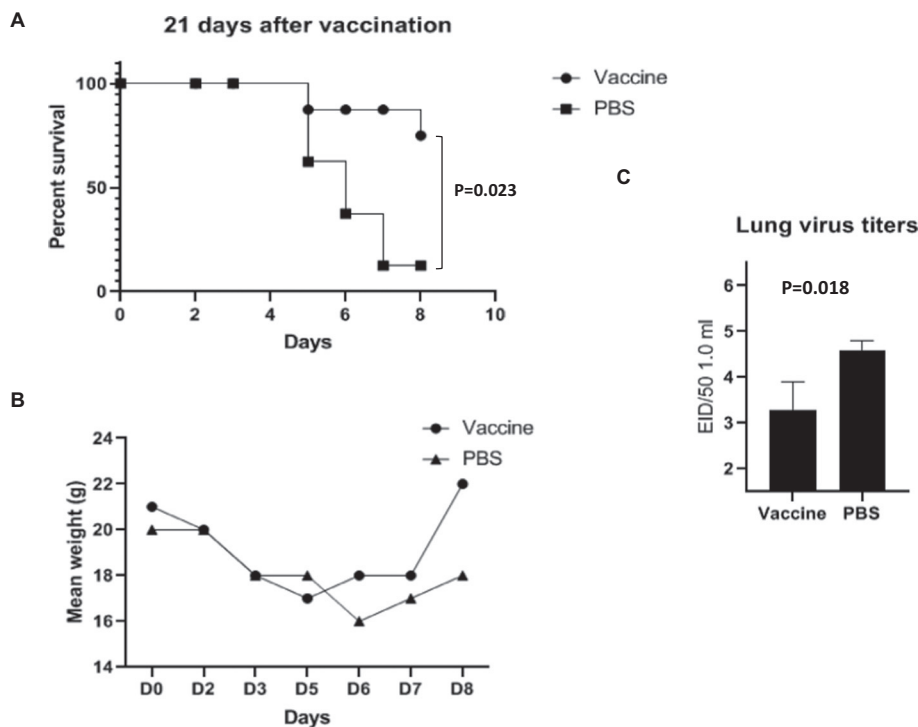
A

B

C

Fig. 8. Infection of immune mice on day 21 after immunization. A. Survival rates (n = 8). B. Body weight dynamics (n = 12). C. Infectious virus isolation from the lungs (n = 5). P-values provided compared to PBS-immunized group. In this experiment, neither vaccinated mice nor those receiving PBS were treated with antihistamines.

neutralizing antibodies to the homologous H5 virus one month after a single vaccination [30]. In the same study, it was shown that after double immunization with A/Hong Kong/213/2003(H5N1) IIV with aluminum, neutralizing antibodies to the antigenic variant A/Vietnam/1203/2004 (H5N1) were detected two months after booster immunization. In those cases, we were dealing with completed immunization, when antibodies were detected a month or more after immunization. The current study had other tasks, because, on the contrary, we simulated the so-called "sensitization" of mice. For this reason, serum antibodies were determined even before the development of a full-fledged immune response, and then a challenge was performed. Along with this we were interested in the further development of the immune response up to three weeks after immunization. It was confirmed that, on the 21st day after immunization, serum levels of serum IgG for the test virus were significantly higher than those on the 14th day (Fig. 2, C).

As shown in Fig. 2, C, the highest antibody titers by day 14 after vaccination were demonstrated with respect to IgG1 and IgG2a subclasses. The IgG2a antibodies serve as a ligand for the high affinity FcγRI receptor and the medium affinity receptor FcγRIV [31]. Thus, IgG2a subclass, on high and moderate affinity mast cell receptors, could play a role in mortality. More abundant antibodies of the IgG1 subclass (along with the viral antigen) could also be involved in mast cell activation through the low affinity FcγRIII receptors [32]. Thus, most of the antibodies of IgG1 and IgG2a subclass were quite suitable for the formation of immune complexes with the infecting virus that could activate mast cells and release vasoactive mediators.

Previously it was shown that in mice influenza infection or vaccination did not increase the level of serum IgE compared to non-immunized and uninfected animals [33]. Repeated sensitization of mice with ovalbumin was needed during bronchial asthma modeling to achieve increased IgE during influenza infection or vaccination, only in this case there was an increase in serum IgE.

In our previous studies, a model of virus-bacterial vaccination against influenza and pneumococcal complications of influenza showed an antibody-dependent increase in pneumococcal infection in mice with high titers of antibodies to the surface streptococcal proteins. This effect was also stopped by the introduction of antihistamines, which

confirmed the role of histamine produced by mast cells in the development of severe systemic infection [34].

In a number of infections, the presence of specific IgG antibodies and their complexes with an antigen is associated with pathological reactions [35]. However, the mechanisms, ways of development and prevention of immunocomplex pathology are not fully understood.

Immunization with vaccines based on avian influenza viruses is often accompanied by reduced immunogenicity in both animals and humans compared to seasonal vaccines [36,37]. The A/Vietnam/1194/2004(H5N1) NIBRG-14-based inactivated influenza vaccine was previously demonstrated to be only moderately immunogenic in humans as 2 doses of 45 μg of HA—the dose used in those study—resulted in HI seroconversion in only 41%–56% of study subjects in phase 1 clinical trials [36]. Therefore, the formation of a certain part of non-neutralizing antibodies that could take part in immune-complex reactions cannot be ruled out. Moreover, if virus-specific antibodies are the main protective component for immunization with inactivated influenza vaccines, the time interval before the formation of a complete antibody immune response, i.e. between the second and third week after immunization, should be considered. If an infection occurs during this interval, it is possible that the use of antihistamines can help reduce the severity of the consequences of such an infection.

5. Conclusions

In the model of infection with a drift variant of the A/H5N1 influenza virus, it was shown that on day 14 after immunization with the virus A/Vietnam/2004/PR8/RG-23(H5N1), the immune mice were only partially protected from infection: mortality decreased by 25.9%, the level of pulmonary infection decreased 10 times. The introduction of antihistamines additionally reduced mortality in the group of immunized mice by 12.5% and did not affect neither lung virus titers in infected immune animals nor mortality among infected non-immunized animals. A greater number of activated mast cells as well as a higher histamine content were detected in the lungs of immunized animals after infection compared to non-immune animals. This data confirm mast cell involvement during reinfection of influenza-sensitized mice with the drift variant of the A/H5N1 virus.

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Declaration of competing interest

The authors declare no commercial or financial conflict of interest.

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