VIROLOGY

Antiviral Activity of Technologically Processed Antibodies to CD4 Receptor against Influenza Infection

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The antiviral activity of technologically processed antibodies to CD4 receptor was evaluated a model of sublethal A/California/04/2009 (H1N1)pdm09-induced influenza infection in female BALB/c mice. The technologically processed antibodies increased animal survival rate by 50% in comparison with the placebo group ($p<0.05$), which correlated with significant inhibition of virus replication in the lungs ($p<0.05$). The reference drug Tamiflu increased mouse survival rate (by 47%), decreased the virus titer in the lungs, and prevented body weight loss ($p<0.05$ in comparison with the placebo group by all parameters). The intrinsic protective activity of technologically processed antibodies to CD4 receptor was demonstrated, which manifested in a decrease in viral load in the lower respiratory tract and an increase in the survival rate.

Key Words: *influenza A infection; technologically processed antibodies; CD4 receptor; Ergoferon; Raphamin*

The incidence of influenza virus in the era of a novel coronavirus infection caused by SARS-Cov-2 has sharply declined in many countries [1]. The main reasons for this were: travel restrictions, wearing masks, social distancing, as well as a policy on the issue of vaccination. Nevertheless, vaccination rate has not reached a desired level [1], and certain population of people remain vulnerable to influenza and the other respiratory infections, which are not associated with COVID-19. Indeed, children between the age of 5 and 17 years are more susceptible to influenza infection than to COVID-19 [2].

The world community is expecting that a positive trend in reducing the number of new cases of influenza infection will continue to strengthen. However, the scale of mortality and morbidity will directly depend on the measures taken for the prevention and treatment of this disease.

Drugs based on the technologically processed antibodies (TPA; LLC "MATERIA MEDIC HOLDING") are the products of multiple serial dilutions combined with hydrodynamic influence at each stage. The dilutions manufactured according to this technology acquire special long-lasting physical, chemical, and biological properties that differ from the properties of the original substance [3]. In practical terms, the most important property of technologically processed substances is their ability to cause conformational changes in the target molecule [4,5], thus affecting the biological pathways in which this target is involved [4]. Drugs of this class of substances proved to be effective and safe in the prevention and treatment of viral infections [6-8]. The protective mechanism of action of the drug, apparently, consists in the enhancement of the specific T-cell response, as was shown earlier [9].

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However, antiviral activity of TPA to CD4 receptor as an individual component has not been studied yet.

The aim of this study was to evaluate the intrinsic protective effect of TPA to CD4 receptor in a well-known *in vivo* model of influenza infection.

MATERIALS AND METHODS

Compounds. Affinity-purified rabbit polyclonal antibodies to CD4 receptor in a stock concentration of 2.5 mg/ml were manufactured (Ab Biotechnology) in accordance with the GMP requirements for active pharmaceutical substances. Technological processing of antibodies to CD4 consisted in successive multiple dilutions followed by controlled intensive hydrodynamic treatment at each dilution step [10]. For the preparation of all dilutions (1:100 at each stage), a water-alcohol solution was used, except for the final ones, which were obtained with purified water. The theoretical level of reduction in the concentration of the original antibodies is at least 10^{24} times. Despite the high level of dilution, it was shown that molecules could be retained even in the solutions of TPA, which can be due to flotation effect [11]. However, according to experts in the field of physics of aqueous solutions, special physical-chemical and biological properties of the solutions subjected to the external influence can be attributed to spontaneously formed nanoassociates [12-14]. Purified water subjected to the identical treatment procedure was used as the placebo. All samples were supplied and tested blinded.

Oseltamivir (the active component of commercially available drug Tamiflu, Hoffmann-La Roche Ltd.) was used as a reference drug and in order to confirm the validity of the results obtained in the experiment. The dose was calculated according to the animal's body weight in mg/kg based on the content of oseltamivir in the dosage form.

Animals. Influenza infection was reproduced in female BALB/c mice ($n=45$) weighing 12-14 g (SMK STE-SAR). The animals were housed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and SP 2.2.1.3218-14 "Sanitary and epidemiological requirements for the organization, equipment, and maintenance of experimental biological clinics (vivaria)". The following conditions were maintained in the room with the animals: 18-26°C; 12/12 h light/dark cycle at a relative humidity of 30-70%. Water and food were provided *ad libitum*.

All procedures were approved by the Ethical Committee of the I. I. Mechnikov Research Institute of Vaccines and Sera.

Evaluation of antiviral efficacy of the samples against influenza infection. The animals were anesthetized with ether and intranasally infected with mouse-adapted Influenza A/California/04/2009 (H1N1pdm2009) (CDC) as it was described in paper [15]. Sublethal infection dose was used (LD_{70}): 10^4 $TCID_{50}/0.1$ ml.

Animals were randomized into body weight-matched groups (no more than 10% deviation): group 1 comprised mice orally treated with Tamiflu 4 h before the infection (10 mg/kg/day, divided into 2 doses) and then for 4 days; group 2 mice were orally treated with TPA to CD4 (20 ml/kg/day) 3 days before the infection and for 6 days after the infection; group 3 (placebo) included animals that received purified water according to the dosing regimen used for TPA to CD4. Each group consisted of 15 mice. On day 4 after infection, 5 animals from each group were sacrificed by cervical dislocation, the lungs were isolated and used for evaluation of virus titer. The remaining animals ($n=10$ per group) were observed over the next 11 days; their condition was visually assessed (consumption of water, food, *etc.*).

Survival rate and the mean lifespan (MLS) were the primary criteria in assessment of the efficacy of the test drug. The secondary criteria were virus titer in the lungs and dynamics of body weight.

Statistical analysis. The data were analyzed using the RStudio 1.4.1717 software and the R package 4.1.0. The normality of data distribution was estimated by the Shapiro−Wilk test and variances homogeneity was assessed by the Bartlett test. The Kaplan−Meier method was used to assess the survival rate of mice (in %), the differences in the MLS ($M \pm SE$) and viral load (Me (Q1; Q3)) were estimated by the permutation test, the Holm adjustment was used to correct for multiple comparisons. Comparison of the animal's body weight changes (estimated marginal means$\pm SE$) was performed by polynomial regression model, the animal number was taken as a random effect of the model, Tukey's correction was used to correct for multiple comparisons. All differences were considered significant at $p \leqslant 0.05$.

RESULTS

In the placebo group, the infection dose used induced death of 80% of mice, MLS was low (8 days), and body weight loss was intensive (22.9% by postinfection day 9 (Table 1, Fig. 1). The virus titer in the lungs in this group on postinfection day 4 was 7 lg $TCID_{50}/0.1$ ml. Thus, the chosen infection dose allowed successfully reproducing the influenza infection (Table 1).

The administration of TPA to CD4 receptor led to an increase in the animal survival rate by 50% in comparison with the placebo group ($p<0.05$) and prolonged MLS ($p<0.05$). TPA to CD4 also reduced the viral load by 2 lg $TCID_{50}/0.1$ ml ($p<0.05$ in comparison

TABLE 1. Survival Rate, MLS, and Viral Load in the Lungs of BALB/c Mice after Intranasal Infection with Influenza A/California/04/2009 (H1N1pdm2009)

Group	Survival rate, %	MLS, days ($M \pm SE$)	Virus titer, lg $TCID_{50}$/0.1 ml (Me (Q1, Q3))
Tamiflu	67*	12.90±1.07*	5.0 (0.0; 5.0)
TPA to CD4 receptor	70*	12.90±1.07*	5.0 (5.0; 5.0)*
Placebo	20	8.09±1.22	7.0 (6.0; 7.0)

Note. *$p<0.05$ in comparison with the placebo group.
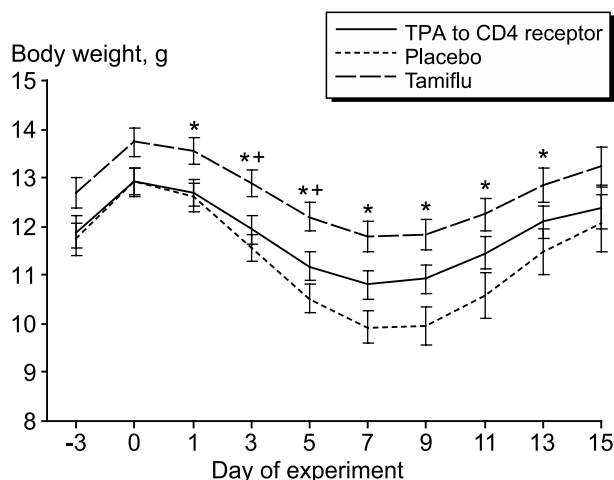
Fig. 1. Changes in body weight of BALB/c mice infected with mouse-adapted influenza A/California/04/2009 (H1N1). $p<0.05$ in comparison with *placebo, +TPA to CD4 receptor.

with placebo); however, complete inhibition of virus replication in the lungs was not achieved. The body weight loss in this group was less intensive (8.15% *vs* 15.6% in the placebo group by postinfection day 9); however, no significant differences from the placebo and Tamiflu groups were found (Fig. 1).

TPA to CD4 receptor are a component of the complex drugs Ergoferon and Raphamin. The antiviral efficacy of these drugs was studied previously [6-8]. The data presented in this study show that activity of TPA to CD4 against influenza infection is comparable to that of TPA to IFNγ (also a component of Ergoferon and Raphamin drugs) [4].

The antiviral effect demonstrated for TPA to CD4 receptor, apparently, is the result of enhanced activation of CD4+ T lymphocytes. This assumption is consistent with the data previously obtained *in vitro*. It has been shown that TPA to CD4 affect activity of Lck-kinase [16] associated with the cytoplasmic part of the CD4+ and CD8+ co-receptors on T helpers and T killers, respectively, and is involved in the signal transduction from the T-cell receptor. In addition, administration of TPA to CD4 receptor to Jurkat cells increased secretion of IL-2, the main participant of T-cells differentiation and proliferation [9].

Thus, therapeutic and prophylactic application of TPA to CD4 receptor is effective against influenza A virus and positively affects clinical manifestations of the infection.

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