PREVENTION AND TREATMENT OF LETHAL INFLUENZA A VIRUS BRONCHOPNEUMONIA IN MICE BY MONOCLONAL ANTIBODY AGAINST HAEMAGGLUTININ STEM REGION

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Received July 18, 1997; revised October 15, 1997

Summary. – The protective properties of monoclonal antibody (MoAb) C179 directed to the stem region of haemagglutinin (HA) H2 that possessed fusion-inhibition and unique broad cross-neutralizing activities were examined in a mouse model. The MoAb efficiently protected mice against a lethal challenge with pneumovirulent human (H1) and avian (H2) strains of influenza A virus. Survival rates in mice that received intraperitonealy (i.p.) 1000 μg of the MoAb per mouse a day before the virus challenge were 90% for H1 and 100% for H2 strain. The dose of the MoAb of 100 μg per mouse significantly decreased mortality in mice. Moreover, the MoAb was also efficient in treatment of lethal bronhopneumonia caused by H2 influenza virus. The survival rate in mice that received 1000 μg of the MoAb per mouse 2 days after the virus challenge was 90%, while that in the control group was 30% only. These results indicate that the MoAb was effective in protection of animals against lethal influenza A infection without significant difference between H1 and H2 subtypes. The MoAb exerted significant effect in treatment of mice infected with H2 influenza virus. Thus, these data allow to suggest that the stem region of HA might be a potential target for prevention of influenza virus infection and antiviral therapy.

Key words: influenza A viruses; mice; protection; treatment; monoclonal antibody; haemagglutinin; fusion inhibition

The envelope glycoprotein HA of influenza viruses performs at least two functions which are essential for the initiation of infection (Wiley and Skehel, 1987). The first function is the attachment of the virus to specific cellular receptors. The globular region of HA which contains a receptor-binding site takes part at this stage. The second function is the penetration of the viral genome into cytoplasm through fusion of the viral envelope with cellular membranes (Huang *et al.*, 1981; Klenk and Rott, 1988). The stem region of HA containing a fusion peptide is responsible for this stage of viral reproduction. HA is also the major protein capable of inducing neutralizing antibodies (Murphy and Webster, 1990). Anti-HA antibodies predominantly prevent the attachment of the virus to cellular receptors and possess haemagglutination-inhibition and neutralizing activities mainly within a specific subtype of HA. However, the antibodies may sometimes exert a virus-neutralizing effect in the absence of anti-haemagglutination activity (Yoden *et al.*, 1982; Kida *et al.*, 1982). These antibodies neutralize the infectivity at a post-attachment stage, e.g. the fusion of viral and cellular membranes (Kida *et al.*, 1983).

Okuno *et al.* (1993) produced and characterized a MoAb directed against the HA of H2 subtype. This MoAb designated C179 didn't possess haemagglutination-inhibition activity while it showed unique cross-neutralizing effect among H1, H2 (Okuno *et al.*, 1993, 1994;) and H5 influenza A virus subtypes, and also recognized the immature form of the H6 subtype (unpublished data). It was shown that MoAb C179 recognized a conformational antigenic epitope in the middle of the stem region and inhibited the fusion activity of HA (Okuno *et al.*, 1993).

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Abbreviations: HA = haemagglutinin; i.n. = intranasal(ly); i.p. = intraperitoneal(ly); MoAb = monoclonal antibodies

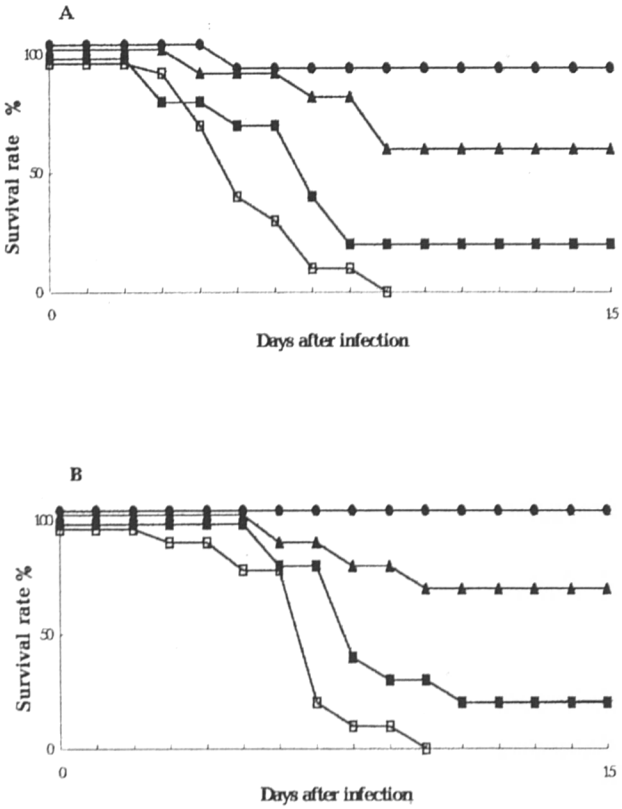

Fig. 1
Protection of mice against lethal challenge with influenza virus USSR/77-MA (H1N1) (A) and DK/NJ/78-MA (H2N3) variants (B) by MoAb C179
MoAb C179 doses: 1000 μg (●), 100 μg (▲) or 10 μg (■). Control (□).

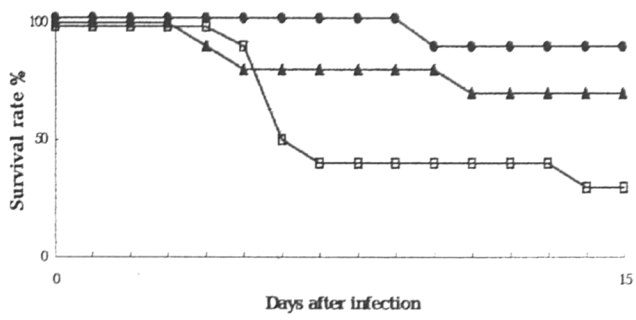

Fig. 2
Treatment of mice infected with influenza virus DK/NJ/78-MA (H2N3) variant by MoAb C179
MoAb C179 doses: 1000 μg (●), 100 μg (▲). Control (□).

In the present study we describe protective properties of MoAb C179 against experimental lethal bronchopneumonia caused by mouse-adapted (MA) variants of human (H1) and avian (H2) influenza A viruses in mice. The MA variant of avian H2 strain was discerned from that of human H1 in reactions *in vitro* with MoAb C179 (Lipatov *et al.*, 1996). To examine whether these strains differ in the neutralization by the same MoAb *in vivo* was one of the aims of this work.

The MA variant of human influenza strain A/USSR/90/77 (H1N1) (USSR/77-MA) was generously provided by Dr. I.A. Rudneva of this Institute (Gitelman *et al.*, 1984; Rudneva *et al.*, 1986; Shilov and Sinitsyn, 1994). The avian influenza strain A/Duck/New Jersey/1580/78 (H2N3) was a kind gift from Dr. R.G. Webster, St. Jude Children's Research Hospital, Memphis, TN, USA. The MA variant of this virus (Dk/NJ/78-MA) was obtained in our Institute by serial lung-to-lung passages and characterized previously (Lipatov *et al.*, 1995). The viruses were propagated in allantoic cavity of 9-day-old embryonated chicken eggs at 37°C for 48 hrs and virus-containing allantoic fluid was used as a virus stock in the experiments. The virulence of USSR/77-MA and Dk/NJ/78-MA variants was measured as $\log_{10}$ of EID_{50}/LD_{50} ratio (Rudneva *et al.*, 1986) and was equal to 5.0 and 3.0, respectively. Four-week-old albino mice were inoculated i.p. with 100 μl of appropriate doses of the MoAb in sterile 0.9% NaCl 24 hrs before or 48 hrs after the virus infection. Each mouse was infected intranasaly (i.n.) under light ether anaesthesia with 50 μl of diluted allantoic fluid containing 10 LD_{50} (for the studies of protection) or 3 LD_{50} (for the studies of treatment) of the challenge virus. These doses resulted in 100% or 70% mortality rate, respectively, in mice as determined in preliminary experiments in which the mice were inoculated with serial ten-fold virus dilutions. The survival rate was calculated after an observation period of 15 days.

The survival rates of mice which received the MoAb a day before the infection with USSR/77-MA variant are shown in Fig. 1A. A total of 40 mice were randomly divided into four groups of 10 animals. Three experimental groups received 1000, 100, and 10 μg of the MoAb per mouse, respectively. The forth (control) group received 0.9% NaCl. The survival rate in the group of mice that received 1000 μg of the MoAb was 90%, while the mortality in the control group was 100%. In the groups that received 100 and 10 μg of the MoAb, the survival rate was 60% and 20%, respectively. These results indicate that 1000 μg of the MoAb was an effective dose to protect animals from lethal infection with USSR/77-MA variant. Fig. 1B shows the protective effect of the MoAb in mice infected with Dk/NJ/78-MA variant under the same conditions as in Fig. 1A. The survival rate of 100% was determined in mice that received 1000 μg of the MoAb. Seventy % and 20% of animals survived in the groups that received 100 μg and 10 μg of the MoAb, respectively. In the control group, the 100% mortality rate was observed. These data show that 1000 μg of the MoAb was enough to protect mice against the lethal effect of Dk/NJ/78-MA variant. The results of these experiments clear indicate that the MoAb efficiently protected mice against infection caused by H1 and H2 influenza viruses. Moreover, there was no significant difference in the capac-

ity of the MoAb to protect mice against the lethal infection with USSR/77-MA or Dk/NJ/78-MA variant. The statistical evaluation of the results presented in Fig. 1 by sign test, an unparametrical statistical test (Korn and Korn, 1978), revealed that the difference in the survival rates between the groups of mice treated with the MoAb (1000 or 100 μg) and the control group was significant at 0.01 level.

The therapeutical effect of the MoAb on the infection of mice caused by Dk/NJ/78-MA variant is shown in Fig. 2. Thirty mice randomly divided into three groups of 10 animals were injected with 1000 or 100 μg of the MoAb or with 0.9% NaCl (control) two days after the virus challenge. A dose of 1000 μg of the MoAb per mouse exerted a significant effect (the survival rate was 90%). The survival rate of 70% was for the dose of 100 μg of the MoAb per mouse, while that for the control was 30%. These results indicate that the MoAb was effective not only in the prevention of influenza virus infection in mice, but in the treatment of lethal pneumonia too. The difference in the survival rates between the group of mice that received 1000 μg of the MoAb and the control group was significant at 0.05 level.

The mechanism underlying the action of MoAb C179 is known in general (Okuno *et al.*, 1993, 1994). This MoAb recognizes a conformational antigenic epitope in the middle of the stem region of HA consisting of two parts: amino acids 318 – 322 of HA1 subunit and 47 – 58 of HA2 subunit. This epitope is conserved among all of H1 and H2 strains of influenza A virus. It has been shown that the process of membrane fusion requires several steps, such as conformational changes in HA at low pH and insertion of the fusion peptide into the target membrane (Skehel *et al.*, 1982; Stegmann *et al.*, 1991). MoAb C179 inhibits the fusion activity of HA most likely by affecting one of these steps (Okuno *et al.*, 1993). *In vivo*, MoAb C179 doesn't prevent the initiation of infection, however, it inhibits the spread of infection in mouse lungs (Okuno *et al.*, 1994). These data were confirmed indirectly in the present study by the finding of haemorrhagic lesions in the lungs. The lesions in the lungs of mice that received 1000 μg of MoAb C179 were localized in small areas only at the sites adjacent to the bronchi. In the control group, those were spread all over the lungs (data not shown).

In our previous study, we have described the differences between the USSR/77-MA and Dk/NJ/78-MA variants in the capacity of MoAb C179 to neutralize them in cell culture and in the precipitability of their HA (Lipatov *et al.*, 1996). MoAb C179 precipitated predominantly the immature form of HA of the avian MA variant and neutralized the infectivity of this variant less efficiently then that of the human variant. We have supposed that differences in the glycosylation of HA1 subunit among the used viruses (Gitelman *et al.*, 1986; Shilov *et al.*, 1991; Lipatov *et al.*, 1995) may influence the recognition of a conformational antigenic epitope by MoAb C179. In the present study, no significant differences in the protective properties of MoAb C179 against the human (H1) and avian (H2) strains were observed. A dissociation between reactions of MoAb C179 with USSR/77-MA and Dk/NJ/78-MA variants *in vitro* and protective properties of the MoAb against the same variants in mice enables to suppose that a component of immune system of the host organism (probably complement- or natural killer cells-dependent mechanisms) is involved in the MoAb-mediated virus clearence.

Recently, Sagawa *et al.* (1996) have observed that a deletion mutant of influenza virus HA, defective in the globular region expression in cell culture, had a potential to induce cross-protection against influenza virus infection in mice. This effect could be caused by antibodies similar to MoAb C179. The stem region of HA is responsible for the induction of these antibodies. The results of present studies together with data of Okuno *et al.* (1994) which describe the protection and treatment of mice by MoAb C179 against lethal challenge with A/FM/1/47 (H1N1) influenza virus indicate that an antibody against the stem region of HA with fusion-inhibition activity is effective in protection and treatment of influenza virus infection caused by H1 or H2 subtypes. It is possible to suggest that the stem region of HA containing a conserved antigenic epitope might be a potential target for prevention and treatment of influenza virus infection.

Acknowledgements. We are grateful to Takara Shuzo Co. Ltd., Biotechnology Research Laboratories, Japan, for the generous supply of MoAb C179. We also thank Drs. E.A. Govorkova and N.V. Kaverin of this Institute for critical comments and helpful discussion.

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