Immunological Response of Monkeys Infected Intranasally with Human Parainfluenza Virus Type 4

By HIROSHI KOMADA, 1 MASATO TSURUDOME, 1 HISANORI BANDO, 1 MACHIKO NISHIO, 1 MOTOKO UEDA, 1 HIDEKI TSUMURA 2 AND YASUHIKO ITO 1.

1Department of Microbiology and 2Institute of Laboratory Animals, Mie University School of Medicine, 2-174 Edobashi, Tsu-Shi, Mie Prefecture 514, Japan

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SUMMARY

This report describes our attempt to establish an experimental animal model for human parainfluenza virus type 4A (HPIV-4A) and 4B (HPIV-4B) infection, which was used to study the immune response to the viruses. Monkeys were inoculated intranasally with the viruses, and at 10 weeks post-infection they were re-infected with homologous subtype viruses. Virus-specific IgM and IgG serum antibodies were measured by ELISA. A small peak of IgM antibody was detected in the monkeys re-infected with HPIV-4B, whereas this response was not detected after re-infection with HPIV-4A. Virus-specific IgA and IgE antibodies were not detected in sera following infection and re-infection with HPIV-4. However virus-specific IgA and IgE antibodies were found in the saliva and nasal exudates of monkeys infected with either HPIV-4A or -4B. Re-infection of monkeys with HPIV-4B also stimulated an IgA and IgE response. To our knowledge this is the first description of a virus-specific IgE antibody response generated by a paramyxovirus infection of an experimental animal. The kinetics of haemagglutinin-inhibition and neutralization (NT) antibodies were similar to that of virus-specific IgG antibodies. The NT titres of sera from HPIV-4A-infected monkeys were enhanced by the addition of complement, whereas complement did not affect the NT activity of sera obtained from HPIV-4B-infected animals. Antigenic specificities of IgG antibody induced by HPIV-4 infection were analysed with radioimmunoprecipitation followed by SDS-PAGE. Anti-NP, -HN and -F antibodies appeared 2 weeks after infection, and the highest titres were found 2 weeks after re-infection. Anti-F antibody production followed a biphasic pattern previously observed in mumps virus infection.

Human parainfluenza viruses (HPIV), members of the family Paramyxoviridae, cause respiratory tract infections of man in early life. Croup symptoms are characterized by inspiratory stridor, barking cough and hoarseness resulting from laryngeal obstruction caused by the overproduction of virus-specific IgE antibody (Merson et al., 1988). These viruses are divided into four major serotypes: 1, 2, 3 and 4. Type 4 virus is further subdivided into 4A and 4B. Despite their importance as human pathogens, little is known about the structure and replication of HPIV, except for type 3, because of their relatively poor growth in tissue cultures. Ito et al. (1987) identified the polypeptides of HPIV type 2 (HPIV-2) and analysed their synthesis in infected cells. Recently, the HPIV-2 haemagglutinin–neuraminidase glycoprotein (HN) has been characterized with monoclonal antibodies (Rydebeck et al., 1988; Tsurudome et al., 1989).

Type 4 virus was first isolated and characterized by Johnson et al. (1960). Canchola et al. (1964) analysed the antigenic variation of type 4 isolates and divided the viruses into subtypes 4A and 4B according to their patterns of haemadsorption-inhibition. Recently we have
identified some of their polypeptides and have analysed their cross-reactivities with other paramyxoviruses, with polyclonal and monoclonal antibodies directed against HPIV-4A and -4B (Komada et al., 1989a, b). However the nature of HPIV-4 infection and the immune response to these viruses in man are not understood precisely. In this study we have tried to establish an experimental animal model to analyse the immune response against HPIV-4A and -4B.

HPIV-4A (M-25 strain) and HPIV-4B (68-333 strain) were used as pathogens in monkeys. The M-25 strain was obtained from the Denka Institute of Biological Science, Niigata, Japan. The 68-333 strain was donated by Dr F. Nishikawa (National Institute of Health, Japan). Primary monkey kidney (PMK) cells were maintained in Eagle's minimum essential medium (MEM) supplemented with 5% foetal calf serum. Infectivity of HPIV-4 was determined by a haemadsorption test using 0.4% guinea-pig red blood cells 4 days after virus inoculation into PMK cells. The virus titre was expressed as TCID\textsubscript{50}/ml. Eight Japanese red-faced macaques were used: six monkeys were infected with HPIV-4A and two with HPIV-4B. Virus inoculation of the monkeys was carried out by aerosol inhalation via the nasal cavity. Each monkey was exposed for about 10 min to 10\textsuperscript{5.5} TCID\textsubscript{50} of HPIV-4 in 1 ml using a clinical nebulizer. Ten weeks after the first inoculation, the monkeys were re-infected with a homologous subtype virus by the same method. An ELISA was performed as described previously (Tsurudome et al., 1986). Sera obtained from monkeys were diluted 1:400 for IgG and IgM detection, but were used undiluted for IgA and IgE detection. Saliva samples from monkeys were used undiluted for IgA and IgE detection. To obtain nasal washings, the monkey's nasal cavities were washed with 0.5 ml of 150 mM NaCl using applicators, and the nasal washings were used undiluted for IgA and IgE detection. Peroxidase-conjugated goat anti-human IgG (specific for heavy and light chains, Cooper Biomedical) IgM, IgA and IgE (Tago) were diluted 1:6000, 1:3000, 1:1000 and 1:1000, respectively. NT and complement-mediated NT tests were performed by inhibition of haemadsorption. Monkey sera were preincubated at 56 °C for 30 min, and a twofold dilution series was made. Each dilution was mixed with an equal volume of the virus suspension containing 100 TCID\textsubscript{50} virus and held at 37 °C for 1 h. The mixture was then added to PMK cells. For the complement-mediated NT test, the serum sample was mixed with an equal volume of virus plus guinea-pig serum mixture for 2 h. The concentration of the virus was adjusted to give 150 TCID\textsubscript{50} per well and the guinea-pig serum was at a final concentration of 1:500. The haemadsorption assay was carried out 4 days after inoculation. In both assays, the titres were expressed as the reciprocal of the highest serum dilution causing 50% inhibition of haemadsorption. A haemagglutinin inhibition (HI) test was performed using 0.4% guinea-pig red blood cells. Isotopic labelling of the infected cells, immunoprecipitation and SDS–PAGE were carried out according to the method described previously (Ito et al., 1985; Komada et al., 1989a).

Ten weeks after virus inoculation, the monkeys were re-infected with the homologous subtype virus. Although all the monkeys tested in this study showed no clinical signs, HPIV-4 antigen-positive cells were detected in nasal epithelia (data not shown). Serum antibody titres of the infected monkeys were analysed by an ELISA. Fig. 1 shows the kinetic curve of antibody titres in representative cases. Virus-specific IgG antibody was detected 2 weeks post-infection (p.i.) with either HPIV-4A (Fig. 1a) or HPIV-4B (Fig. 1b) and the maximum titre was reached 2 weeks after re-infection. The peak levels of virus-specific IgM antibody were found at week 2 to 3 p.i. in both HPIV-4A- and HPIV-4B-infected monkeys. Whereas IgM antibody production could not be detected in serum from any of the monkeys tested after re-infection with HPIV-4A (Fig. 1a), a small peak of virus-specific IgM production was found in both the monkeys re-infected with HPIV-4B (Fig. 1b). IgA and IgE antibodies were not detected in sera of any of the monkeys infected with either HPIV-4A or -4B, despite the use of undiluted monkey sera and high concentrations of peroxidase-labelled second antibodies in the assay (data not shown).

Virus-specific IgA and IgE antibodies were detected in the saliva and nasal washings of HPIV-4A-infected monkeys, the peak levels of those antibodies being found at 2 weeks p.i. (Fig. 1c, d). Similarly, IgA and IgE antibodies were detected in the nasal washings of HPIV-4B-infected monkeys (Fig. 1e). The antibody titres peaked at weeks 1 to 2 p.i. and then
Fig. 1. Production of virus-specific IgG, IgM, IgA and IgE antibodies detected by ELISA in sera (a, b), nasal washings (c, e) and saliva (d) obtained from monkeys infected intranasally with either HPIV-4A or -4B. Representative cases are shown in the figure. Monkeys A1 (a) and A5 (c, d) were infected with HPIV-4A and monkey B2 (b, e) was infected with HPIV-4B. Antibody titres were analysed by ELISA. Re-infection with homologous subtype virus is indicated (▼).
Fig. 2. Antigenic specificity of IgG antibodies induced by infection with either HPIV-4A or -4B. Representative cases are shown in the figure. Monkey A1 (a) or B2 (b) was infected with HPIV-4A or -4B, respectively and 10 weeks after infection they were re-infected with homologous subtype viruses. Antigenic specificities of antisera were analysed by immunoprecipitation followed by SDS-PAGE. Lane M, M, markers: numbers above lanes represent time (weeks) post-infection; Pre, pre-infection.

sharply declined. After a re-challenge with HPIV-4B at 10 weeks, IgA and IgE antibodies were again detected. The serum HI and NT titres of HPIV-4A- or HPIV-4B-infected monkeys were also studied at different times after injection (data not shown). There was no difference between HPIV-4A- and HPIV-4B-infected monkeys in the onset of the antibody production, although HPIV-4A-infected monkeys produced higher titres of antibody than did HPIV-4B-infected animals. The shape of the curves for the NT titre and HI titres were similar to those from IgG ELISA. The NT titres of sera from HPIV-4A-infected monkeys were enhanced in all the cases tested by the addition of complement, whereas those from HPIV-4B-infected monkeys were not enhanced by complement (data not shown).

Antigenic specificities of IgG antibody induced by HPIV-4 infection were analysed by radioimmunoprecipitation followed by SDS-PAGE. Anti-NP, -HN and -F HPIV-4A antibodies appeared at 2 weeks p.i., and the highest titres were found 2 weeks after re-infection (Fig. 2a). The ratios of HN:NP and F1:NP immunoprecipitated were determined by densitometric analysis and the result is shown in Fig. 3. The ratio of HN:NP was almost constant throughout the period, but the ratio of F1:NP showed a biphasic pattern; that is, peaks
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Fig. 3. The ratio of HN:NP (●) and F1:NP (○) immunoprecipitated with monkey antisera as determined by densitometric analysis. Results obtained using the autoradiogram (Fig. 2a) are depicted in the figure.

were found at 3 weeks p.i. and at weeks 1 to 5 after re-infection. This result showed that anti-F antibody decreased rapidly around 5 weeks either post-infection or after re-infection. As the F polypeptide was poorly labelled in HPIV-4B-infected cells, anti-F antibody from HPIV-4B-infected animals could not be analysed. Concerning anti-HN and -NP antibodies from HPIV-4B-infected animals, the kinetics were similar to those seen in HPIV-4A-infected monkeys (Fig. 2b).

IgG and IgM antibodies directed against the polypeptides of HPIV-4A and -4B were determined by immunoprecipitation using rabbit anti-monkey IgG or IgM serum (a gift from M. Tatsumi, National Institute of Health, Japan). The amount of IgM was greater at 2 weeks p.i. relative to IgG, whereas the amount of IgG was greater at 2 weeks after re-infection, compared with IgM (data not shown). These results were in accordance with those from ELISA.

The biological properties of HPIV-4 have not been fully analysed, largely because of its relatively poor growth in tissue culture systems. The epidemiology, clinical symptoms and the immunological response to HPIV-4 infection have been difficult to study due to the problem of isolating HPIV-4 from patients with respiratory infection and also due to immunological cross-reactions between human paramyxoviruses, namely HPIV types 1, 2, 3, 4A and 4B and mumps virus.

In summary, to study the immunological response against HPIV-4 infection, we established an experimental animal model for HPIV-4 infection. Monkeys, i.e. Japanese red-faced macaques, were infected intranasally with either HPIV-4A or -4B, and at 10 weeks p.i. were re-infected with homologous subtype viruses. Virus-specific antibodies in sera, saliva and nasal washings were measured using ELISA, and HI and NT tests. The viruses were recovered neither from saliva nor from nasal washings from infected monkeys. Furthermore, none of the monkeys infected with the viruses showed clinical signs. However cells bearing HPIV-4-specific antigens were found in nasal epithelia and virus-specific antibodies were detected in sera, saliva and nasal exudates. Interestingly, a small peak of IgM was detected in monkeys re-infected with HPIV-4B, whereas virus-specific IgM antibody was absent from sera of all the monkeys tested after re-infection with HPIV-4A. Although IgA and IgE antibodies were not identified in sera of monkeys infected with either HPIV-4A or -4B, these antibodies were detected in the saliva and nasal exudates of these animals. Furthermore, IgA and IgE antibodies were detected in saliva and nasal exudates of animals re-infected with HPIV-4B (HPIV-4A-re-challenged animals were not tested). Croup symptoms evoked by parainfluenza virus infection were considered to be caused by overproduction of virus-specific IgE antibody (Merson et al., 1988). Similarly, asthmatic attacks have been reported to be induced by infection of the respiratory tract with viruses including parainfluenza virus (Gregg, 1977). The observation that virus-specific IgE
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antibody was produced in saliva and in the nasal cavity of monkeys infected experimentally with HPIV-4 suggests that this might contribute to the pathogenesis of HPIV infections. In addition, repetitive local production of virus-specific IgE in response to re-infection with the virus might be responsible for the persistence of asthma-like symptoms.

The NT titre in HPIV-4A-infected monkeys was enhanced by addition of complement, whereas that in HPIV-4B-infected animals was not enhanced. The complement-enhanced NT activity was also found following mumps vaccination or experimental infection with mumps virus and the activity was reported to be related to the anti-F polypeptide (Hishiyama et al., 1988). The ratio of anti-F:anti-NP antibodies increased transiently and then decreased rapidly after infection or re-infection. The kinetics were similar to that observed in mumps virus infection (Hishiyama et al., 1988). A short half-life of anti-F polypeptide antibody might be a general phenomenon in HPIV infection. The biological significance of the transient anti-F antibody might have implications in immunity to paramyxoviruses.

REFERENCES


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