Lack of cross-protection between European H1N1 and H1N2 swine influenza viruses

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Abstract

Background: Swine influenza viruses (SIVs) of H1N2 subtype have recently become established in several European countries, and they cocirculate with H1N1 and H3N2 viruses. The H1N2 virus haemagglutinin (HA) appears to be of human origin and fails to cross-react with avian-like H1N1 SIVs in vitro. This study examines whether in vivo cross-protection occurs between H1N1 and H1N2 viruses isolated in Belgium. Methods: Influenza virus-seronegative pigs were inoculated first with Sw/Gent/7625/99 (H1N2) or Sw/Belgium/1/98 (H1N1), or left uninoculated. Four weeks later, all pigs were challenged with the H1N2 virus. We examined H1N2 antibody titres prior to challenge, and clinical signs and virus replication after challenge. Results: H1N2 antibodies were found exclusively in the pigs previously infected with H1N2, and these were protected against disease and infection. Fever and respiratory signs typical of H1N2 infection developed in the challenge control pigs and the H1N1-immune pigs. In both groups, all pigs had H1N2 virus in the lungs at 24 h and in nasal swabs during the first week after challenge. In H1N1-immune pigs, however, the mean virus titre in the lungs was 1.9 log10 EID50/g lower than in the challenge controls. Similarly, the total amount of virus excreted was significantly reduced and virus excretion was on the average 1.4 days shorter.

Conclusions: Pigs immune after H1N1 subtype infection are not protected against H1N2 infection and disease. This is in agreement with the antigenic difference in the HA of these viruses. Our data, however, suggest partial heterosubtypic immunity between H1N1 and H1N2, which may be mediated by the cytotoxic T lymphocyte response. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Swine influenza; Virus subtypes; Cross-immunity; Pigs; Virus replication; Disease

1. Introduction

A novel influenza A virus subtype, H1N2, has been associated with swine influenza outbreaks in Great Britain since 1994 [1]. This virus is probably a double genetic
reassortant, with the haemagglutinin (HA) gene of human H1N1 viruses from the early 1980s, the neuraminidase (NA) gene of ‘human-like’ swine H3N2 viruses and the internal protein genes of ‘avian-like’ swine H1N1 viruses [2]. Starting in 1998, similar H1N2 viruses have been diagnosed in Italy, France and Belgium, and they may already be widespread in other European countries [3,4]. Swine H1N2 viruses in Europe coexist with avian-like H1N1 and human-like H3N2 viruses and, importantly, there is growing evidence that all three subtypes are pathogenic [1,3,5]. There is no cross-protection between H1N1 and H3N2 and these subtypes can sequentially hit a swine herd. It is less clear, however, whether cross-protection exists between H1N1 and H1N2. These viruses are of the same HA subtype, but they show little, if any, cross-reaction in haemagglutination-inhibition (HI) tests. Still, the in vivo immune response to influenza infection is complex and the true impact of antigenic variation has to be addressed by in vivo challenge studies. Here, we have studied clinical and virological protection against Sw/Gent/7625/99 (H1N2) challenge in pigs immune against a prototype avian-like H1N1 influenza virus.

2. Materials and methods

Nineteen conventional pigs were used. At the age of 15 weeks, six pigs (H1N2–H1N2 group) were inoculated intranasally with 7.0 log₁₀ EID₅₀ of the Sw/Gent/7625/99 (H1N2) virus, seven pigs (H1N1–H1N2 group) were inoculated similarly with the Sw/Belgium/1/98 (H1N1) virus, and six pigs (H1N2 challenge controls) were left uninoculated. Four weeks later, all pigs were challenged intranasally (7.0 log₁₀ EID₅₀) and intratracheally (7.5 log₁₀ EID₅₀) with the Sw/Gent/7625/99 (H1N2) isolate. All pigs were monitored for HI antibody titres to H1N1 and H1N2 subtypes before challenge, and for body temperatures and respiratory signs at 24 h post challenge (h PC). Three pigs from each group were euthanized 24 h PC for virus titration of lung tissue homogenates. In the remaining three (H1N2 challenge controls and H1N2–H1N2 group) or four (H1N1–H1N2 group) pigs, nasal swabs were collected daily between 0 and 7 days (d) PC and used in virus titrations.

HI tests were performed with 0.5% chicken erythrocytes according to standard procedures. Due to the different pretreatments of sera in H1N1 and H1N2 (treatment with a receptor destroying enzyme) serology, starting dilutions were 1:4 and 1:20, respectively. Virus titrations were performed by inoculation in the allantoic cavity of 10-day-old embryonated hens’ eggs.

3. Results

3.1. Serology at challenge

Pigs were negative for antibodies against Sw/Belgium/1/98 (H1N1) and Sw/Gent/7625/99 (H1N2) at the start of the experiment. At the time of the H1N2 challenge, the control group was still negative and the H1N2–H1N2 (anti-H1N2 titres 80–320) and H1N1–
Fig. 1. H1N2 influenza virus titres in lungs and nasal swabs of challenge control pigs and pigs immune against H1N1. Virus was undetectable in the homologous challenge group (not shown). Data represent means of at least three pigs. Virus titres are expressed per g lung tissue and per 100 mg nasal secret. Detection limits were $10^{1.1}$ EID$_{50}$/g lung tissue and $10^{0.9}$ EID$_{50}$/100 mg nasal secret.
H1N2 groups (anti-H1N1 titres 16–128) only had antibodies against the strain with which they had been inoculated 4 weeks earlier.

3.2. Clinical protection

Twenty-four hours after the H1N2 challenge, fever (body temperature * (40°C) and laboured abdominal breathing were recorded in three and four of the six challenge control pigs, respectively. In contrast, all six pigs of the H1N2–H1N2 group showed evidence of clinical protection. Morbidity rates and disease severities in the H1N1–H1N2 group were similar to those in the challenge controls, with prominent fever and breathing difficulties in six and five of the seven pigs, respectively.

3.3. Virological protection

Virus isolated from lung tissue or nasal swabs was identified as H1N2 influenza by using monospecific swine serum against Sw/Gent/7625/99. The three H1N2 challenge control pigs sacrificed 24 h PC had high virus titres in their lungs (mean 7.6 log$_{10}$ EID$_{50}$/g) (Fig. 1). All three pigs excreted virus during five consecutive days PC, and 1/3 pigs was still virus-positive at 6 d PC. Mean virus titres were between 4.9 and 5.8 log$_{10}$ EID$_{50}$/100 mg nasal secrete from 1 until 4 d PC. In the H1N1–H1N2 group, the lungs of all three pigs euthanized 24 h PC were virus-positive, but the mean virus titre (5.7 log$_{10}$ EID$_{50}$/g) was lower than in the challenge control pigs. All four pigs excreted virus 1, 2 and 3 d PC, but only 3/4 and 1/4 pigs were virus-positive at 4 and 5 d PC, respectively. Mean virus titres in nasal secretions were comparable to those in the challenge controls at 2 and 3 d PC (5.2–5.3 log$_{10}$ EID$_{50}$/100 mg), but lower thereafter.

4. Discussion

This study demonstrates that pigs immune after infection with an avian-like H1N1 influenza virus still excrete large amounts of the H1N2 virus upon challenge. What’s more, H1N1 immune pigs were not protected against H1N2-induced disease. Neutralizing antibodies against the HA are essential to prevent influenza virus infection. The lack of cross-protection between H1N1 and H1N2, therefore, is in agreement with the antigenic difference in their HAs. On the other hand, the H1N2 virus titres in the lungs and nasal swabs of H1N1-immune pigs were generally lower than in H1N2 challenge controls, and virus excretion was shortened. One possibility is that cytotoxic T lymphocytes, which have been primed by the H1N1 infection, facilitate clearance of H1N2-infected cells from the respiratory tract. Interestingly, cell-mediated immunity is primarily directed against the more conserved internal viral proteins and frequently cross-reactive between different subtypes [6].

It remains to be seen whether a previous H3N2 infection can influence the replication of H1N2. The NA antigen is closely related in H1N2 and H3N2 [2] and, though less important than the HA in immunity, anti-neuraminidase antibodies may affect the spread of virus. Overall, however, our data further support the notion that pigs may experience
infection and, theoretically, disease with three different influenza viruses throughout their lifetime. The emergence of a third virus subtype in the European swine population has implications for diagnosis and vaccination.

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References