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Maternally-derived antibodies do not inhibit swine influenza virus replication in piglets but decrease excreted virus infectivity and impair post-infectious immune responses

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ABSTRACT

Maternally-derived antibodies (MDA) reduce piglet susceptibility to swine influenza A virus, but interfere with post-infectious immune responses, raising questions about protection after waning of passive immunity. We therefore analysed the impact of different levels of residual MDA on virus excretion and immune responses in piglets born to vaccinated sows (MDA+) and infected with H1N1 at 5, 7 or 11 weeks of age, in comparison to piglets born to unvaccinated sows (MDA-). Subsequent protection against a second homologous infection occurring 4 weeks after the primo-infection was also investigated. MDA- pigs showed clinical signs, shed the virus, and developed specific immune responses despite some age-dependent differences: 7-week-old pigs were less affected clinically, showed a 2-day delayed excretion peak and excreted less virus than younger pigs. In MDA+ animals, clinical signs increased together with the decrease of MDA levels related to the age at infection-time. Virus shedding was not prevented and genome quantification profiles were similar to those obtained in MDA- piglets. However, viral particles excreted by 5-week-old MDA+ piglets appeared to be less infectious than those shed by MDA- piglets at the same age. Humoral response was affected by MDA as illustrated by the absence of HI and neutralizing response regardless the infection age, but anti-NP/M responses were less affected. Proliferative T cell responses were slightly delayed by high MDA levels. Nevertheless, MDA+ animals were all protected from a second infection, like MDA- piglets. In conclusion, responses of pigs to H1N1 were affected by both the physiological development of animals at infection and the MDA level.

1. Introduction

Swine influenza is a highly contagious respiratory infection in pigs. The disease is characterized by acute respiratory problems, fever, loss of appetite and lethargy. A flu outbreak has a substantial economic impact on the infected farm because of medication costs and the growth retardation, especially in the case of porcine respiratory disease complex (Opriessnig et al., 2011) or persistent flu (Rose et al., 2013). The disease is caused by type A influenza viruses. Swine influenza A viruses (swIAV) are enzootic in areas densely populated with pigs and may occasionally be transmitted to humans (Freidl et al., 2014). Three subtypes of swIAV, i.e. H1N1, H3N2 and H1N2, are simultaneously circulating within the swine production worldwide and many genetic lineages have been found within each subtype, depending on the geographic location

Abbreviations: ANSES, French Agency for Food, Environmental and Occupational Health and Safety; AUC, area under the curve; CFSE, carboxyfluorescein succinimidyl ester; EMEM, Eagle's minimum essential medium; FBS, fetal bovine serum; HA, hemagglutinin; HAU, hemagglutinating units; HI, hemagglutination inhibition; IRPC%, Relative Index Percent; M, matrix protein; MDA, maternally-derived antibodies; MWG, mean weight gain; NA, neuraminidase; NP, nucleoprotein; PBMC, peripheral blood mononuclear cells; RDE, receptor destroying enzyme; RPMI, Roswell Park Memorial Institute; SPF, specific pathogen-free; swIAV, swine influenza A virus; $TCID_{50}$, 50% tissue culture infectious dose; TPCK, tosyl phenylalanyl chloromethyl ketone; wks, weeks

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(Simon et al., 2014; Vincent et al., 2014).

Vaccination is the most common strategy to prevent swine influenza and/or limit the impact of the disease. All current commercial swIAV vaccines in the EU are whole inactivated virus vaccines (Rajao et al., 2014). In keeping with the diversity of swIAV strains, vaccines for each geographic area are produced locally and may differ in strains, antigen dose and adjuvant formulation (Van Reeth and Ma, 2012). Vaccination with a killed swIAV vaccine reduces clinical signs and viral load in the lungs in case of infection, but does not fully prevent viral replication and excretion (Kyriakis et al., 2010; Van Reeth et al., 2012).

Vaccination of growing pigs is globally rarely practiced due to economic considerations balancing costs and benefits. Therefore, many farms practice vaccination on breeding sows, most often at each reproduction cycle (Hervé et al., 2014; Vincent et al., 2008), to reduce the infection pressure in the breeding part of the herd, to prevent the consequences of swIAV infections on reproductive performance, and to transfer maternally-derived antibodies (MDA) to offspring through colostrum (Cador et al., 2016a). In the case of a homologous viral infection, with antigens from the same genetic lineage for infection of piglets and vaccination of sows, MDA may induce partial or total clinical protection of piglets during early infections but these antibodies also appear to have some disadvantages. In a previous study we have shown that swIAV spread within a swine population is modified by the maternally-derived immune status of piglets at the time of infection, but remains still effective (Cador et al., 2016a). Animals with MDA (MDA+) had reduced susceptibility to homologous infection compared to piglets without MDA (MDA-), but the reproduction number, although 3 times lower than in MDA- piglets, was still higher than 1, reflecting an efficient dissemination process. Based on mathematical modeling, the presence of MDA appears to extend the duration of the epidemics within a batch, favoring transmission between successive batches of pigs in intensive batch-segregated swine production systems, and thus favoring a longer swIAV persistence at the herd level (Cador et al., 2016b). Besides this impact on viral transmission, MDA also appears to interfere with the establishment of post-infectious immune responses in piglets (Kitikoon et al., 2006; Loeffen et al., 2003; Rose et al., 2013). A longitudinal study of swine farms affected by persistent flu in animals at 7–8 weeks or at 11 weeks of age has shown that piglets infected in the presence of MDA did not develop a serological response (Rose et al., 2013). Moreover, experimental studies have also evidenced an impairment of immune response in MDA+ piglets following an influenza infection at 7 weeks of age (Kitikoon et al., 2006; Loeffen et al., 2003), but underlying mechanisms are still poorly understood. We therefore performed an experimental study with MDA- and MDA+ animals infected at different ages: 5, 7 and 11 weeks to investigate the impact of the physiological development of animals, as well as their different levels of residual MDA, on virus excretion and immunological responses developed after an H1N1 infection. The quality of immune responses was studied through different approaches, i.e. measurements of anti-NP, anti-M, anti-HA and neutralizing antibodies in sera, quantification of swIAV-specific T cells in blood as well as assessment of the protection acquired against a second homologous challenge 4 weeks after the primo-infection.

2. Materials & methods

2.1. Animals and inoculum

Specific pathogen-free (SPF) animals were obtained from the biosecurity level 3 and air-filtrated pig herd of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) in Ploufragan (France). Sixty-six SPF Large White piglets were used: 33 with maternal antibodies directed against swIAV (MDA+) and 33 without maternal antibodies (MDA-). The MDA+ piglets were born to 3 SPF sows that had been vaccinated. Primo-vaccination was performed 6 and 3 weeks before insemination with a 2 mL intramuscular injection of an inactivated trivalent vaccine (Respiporc Flu®3, formerly GRIPOVAC®3, IDT Biologika GmbH, Dessau-Rosslau, Germany) containing antigens representative of three out of the four most widespread enzootic lineages circulating in Europe, i.e. “avian-like swine H1N1” ($H1_{av}N1$), “human-like reassortant swine H3N2” (H3N2) and “human-like reassortant swine H1N2” ($H1_{hu}N2$) (Simon et al., 2014; Van Reeth and Ma, 2012). Vaccination of sows was followed by three boosters 6, 3 and 2 weeks before farrowing, in order to induce high antibody levels in the colostrum (Cador et al., 2016a).

Sera were collected from vaccinated sows at farrowing and from their piglets at 3 days of life in order to evaluate postvaccinal and maternally-derived antibody levels, respectively. Hemagglutination inhibition (HI) tests using virus strains representative of the three $H1_{av}N1$, $H1_{hu}N2$ and H3N2 lineages (Rose et al., 2013) were performed as described below. Sows exhibited HI antibodies towards the three antigens, as previously reported (Cador et al., 2016a). Although all SPF piglets were fed as far as possible by their own dam, some individual variations in HI titers were observed among batches, most probably linked to colostrum intake. In order to ensure high and homogeneous MDA levels in piglets included in infected MDA+ groups, animals with the lowest HI titers were voluntarily placed in control groups. The other group compositions were balanced according to the weight, sex, dam’s origin of the piglets and their MDA level based on HI titers.

Inoculations were performed with the virus strain A/Sw/Cotes d’Armor/0388/09, of the $H1_{av}N1$ subtype, like the vaccine H1N1 antigen, both bearing an H1 gene from the 1C.2 genogroup (Anderson et al., 2016). This strain was isolated in a herd in France from pigs with acute respiratory disease. The amplification and the titration of the inoculum were performed as previously described (Deblanc et al., 2012). Inoculations of piglets in the infected groups were performed intratracheally with 5 mL of $10^5 TCID_{50}$ (50% tissue culture infectious dose) of the $H1_{av}N1$ strain, whereas mock-inoculated groups similarly received 5 mL of Eagle’s Minimum Essential Medium (EMEM, Lonza, Belgium).

2.2. Experimental designs

Two experiments were performed in the ANSES level 3 biosecurity facilities which have an agreement for animal experimentation delivered by the *Directions Départementales de la Protection des Populations des Côtes d’Armor* [Departmental Directorate for Protection of the Population] (ANSES registration number C-22-745-1). These experiments were approved by the French national committee for ethics in animal experimentation ANSES/ENVA/UPEC and authorized by the French Ministry for Research (approval No. 11/03/14-17 for experiment 1 and No. 08/12/15-6 for experiment 2).

2.2.1. Experiment 1

In the first experiment (E1), 9 MDA- and 9 MDA+ piglets were first inoculated with $H1_{av}N1$ at 5 weeks of age (D0) and received a second challenge at 9 weeks of age (D28) (5/9wMDA- and 5/9wMDA+ groups, Table 1 and Fig. 1A). Three mock-inoculated (at D0 and D28) MDA+ and 3 mock-inoculated MDA- piglets were placed in an independent room (MDA- and MDA+ control groups). Animals were euthanized, bled and necropsied at 11 weeks of age (at D42).

2.2.2. Experiment 2

A second experiment (E2) was performed with older animals, thus presenting lower MDA levels at D0 than animals in E1. Fourteen MDA- and 14 MDA+ 7-week-old pigs were inoculated with $H1_{av}N1$, while 10 other MDA- and 10 MDA+ pigs were mock-inoculated with EMEM (D0). Four weeks later (at D28), 7 MDA- and 7 MDA+ $H1_{av}N1$-infected pigs, as well as 7 MDA- and 7 MDA+ mock-inoculated pigs, were inoculated with $H1_{av}N1$ (7/11wMDA-, 7/11wMDA+ , 11wMDA- and 11wMDA+ groups, respectively), while the remaining 20 animals received EMEM (7wMDA-, 7wMDA+ , MDA- control and MDA+ control