Veterinary Immunology and Immunopathology 152 (2013) 43–49

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

The Fcγ receptor expression profile on porcine dendritic cells depends on the nature of the stimulus

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ARTICLE INFO

Keywords:
Fcγ receptors
Dendritic cells
Immune complex
Pig

ABSTRACT

Nowadays, vaccine research focuses on the development and implementation of subunit vaccines into existing or future vaccination platforms. However, recombinant proteins are often poor immunogens, necessitating adjuvants to activate and direct the immune response. Alternatively, the immunogenicity of antigens can be enhanced by targeting antigens to Fcγ receptors on antigen-presenting cells and as dendritic cells (DCs) are the most potent antigen-presenting cells, orchestrating innate and adaptive immune responses, they are attractive for this selective targeting of vaccine antigens. However, DCs express both inhibitory and activating Fcγ receptors and regulating DC function is pivotal to ensure the induction of effective immune responses and to prevent exaggerated immune responses causing inflammation. Previously, we demonstrated that immature porcine MoDC express FcγRII and FcγRIII on their cell surface, which mediate a functional DC maturation upon activation through immune complexes. In the present study, we clearly demonstrate that immune complexes are primarily internalised via FcγRIII, resulting in DC maturation and that depending on the DC maturation stimulus the FcγR expression profile is differentially regulated. These results could not only expedite the development of FcγR-targeting based vaccines, but also provide insights into FcγR-mediated autoimmune diseases.

1. Introduction

In recent years, vaccine research shifted from live attenuated vaccines to subunit-based vaccines. However, recombinant proteins are often poor immunogens due to the lack of danger signals derived from micro-organisms and damaged cells necessary to properly activate the immune system. This necessitates the use of adjuvants in subunit vaccines to activate and direct the immune response. Alternatively, the immunogenicity of antigens can be enhanced by selectively targeting antigens to endocytotic receptors on antigen-presenting cells (Shortman et al., 2009; Devriendt et al., 2011). As dendritic cells (DCs) represent the most potent antigen-presenting cells uniquely capable of priming naive T cells, they are attractive candidates for the selective targeting of vaccine antigens (Banchereau et al., 2000; Caminschi and Shortman, 2012). In the peripheral tissues immature DCs are proficient in antigen uptake and on activation undergo a complex maturation process involving not only the upregulation of their antigen presentation machinery, resulting in an enhanced T cell stimulatory capacity, but also in the secretion of T cell polarising cytokines, leading to the induction of the desired T cell effector responses (Guermonprez et al., 2002). This DC cytokine secretion profile depends on the nature of the encountered antigen or the triggered receptor (Trinchieri and Sher, 2007).

Several strategies to target vaccine antigens to endocytotic receptors present on DCs are currently being investigated. Both antibody- and ligand-mediated

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http://dx.doi.org/10.1016/j.vetimm.2012.09.021

antigen targeting to DC surface receptors, such as Clec9A, DC-SIGN and Siglec-1, enhanced vaccine efficacy by inducing potent $CD4^+$ T cell effector responses and boosting antibody responses (Revilla et al., 2009; Lahoud et al., 2011; Unger et al., 2012). Besides these examples, numerous reports have demonstrated enhanced cellular and humoural immune responses both *in vitro* and *in vivo* upon antigen targeting to Fcγ receptors (FcγR), even in the absence of adjuvants (Adamova et al., 2005; Boruchov et al., 2005; Rawool et al., 2008; Bitsaktsis et al., 2012). FcγR are a family of membrane glycoproteins belonging to the Ig superfamily, which bind the Fc portion of IgG antibodies. In most species they can be divided into three subclasses: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). In mice however a fourth Fcγ receptor, FcγRIV, has been identified (Nimmerjahn et al., 2005). These FcγR differ in their binding affinity and specificity for the different IgG subclasses. For example, human FcγRI displays a higher binding affinity for IgG_1 than IgG_3, while FcγRIII binds to these IgG subclasses with equal affinity (reviewed in Gosselin et al., 2009). FcγRI is a high affinity receptor, which binds both monomeric IgG and immune complexes, while FcγRII and -RIII are low affinity receptors that only bind antigen-complexed IgG. Both FcγRI and FcγRIII are activating receptors, characterized by the presence of an immunoreceptor tyrosine-based activation motif (ITAM) motif, either intrinsic to the receptor or as part of an associated subunit, the γ chain (Ravetch and Bolland, 2001). In contrast to the activating FcγRIIA, FcγRIIB is an inhibitory receptor, characterized by an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (Nimmerjahn and Ravetch, 2008). Activating and inhibitory FcγR mediate opposing functions, underlining the role of these receptors in regulating adaptive immune responses to efficiently eradicate invasive pathogens as well as to prevent exaggerated responses resulting in overt inflammation and tissue damage (Boruchov et al., 2005; Nimmerjahn and Ravetch, 2008).

Like murine and human DCs, we demonstrated previously that the selective targeting of antigens as immune complex to FcγR on porcine dendritic cells resulted in an enhanced antigen internalisation, accelerated DC maturation and increased the antigen presentation and T cell stimulatory capacity of DCs (Regnault et al., 1999; Boruchov et al., 2005; Dhodapkar et al., 2007; Devriendt et al., 2010). As a more thorough understanding of the role of the different FcγR in porcine DC function is fundamental to accelerate veterinary vaccine development based on antigen targeting to FcγR, we examined the role of these receptors in immune complex uptake and analysed maturation-dependent differences in the FcγRII/III expression profile by porcine DCs.

2. Materials and methods

2.1. Generation of F4/IgG immune complexes

F4 fimbriae were purified from the enterotoxigenic *Escherichia coli* strain IMM01 as previously described by Van den Broeck et al. (1999). Briefly, bacteria were grown in Trypsone Soya Broth (TSB; Oxoid Ltd., Basingstoke, Hampshire, UK) for 18 h at 37 °C and 85 rpm. Subsequently, the F4 fimbriae were isolated from the bacteria by mechanical shearing, precipitated through addition of ammonium sulphate (40%, w/v), dialysed and filtrated (0.2 μm). The protein concentration was determined and the purity was assessed by SDS-PAGE and Western blotting. Flagellin was isolated from strain GIS26ΔFaeG using the same protocol as to purify F4 fimbriae. This strain is derived from the $F4^+$ ETEC reference strain GIS26 by disruption of the gene encoding the major F4 fimbrial subunit FaeG (Verdonck et al., 2008). Porcine F4-specific polyclonal Abs were affinity purified from heat-inactivated porcine hyperimmune serum, obtained from a pig intramuscularly immunized with purified F4 fimbriae. F4 fimbriae (5 μg) and anti-F4 IgG (234 μg) were incubated for 1 h at 37 °C to generate soluble immune complexes (F4-IC) (Devriendt et al., 2010).

2.2. Animals and blood samples

Heparinised blood samples were obtained from the external jugular vein of Belgian Landrace pigs (8–20 weeks old) kept as blood donors under standard conditions at the Faculty of Veterinary Medicine, Merelbeke, Belgium. All animal experiments were in accordance with the local animal welfare regulations of the Faculty of Veterinary Medicine.

2.3. Generation of porcine monocyte-derived dendritic cells

PBMCs were isolated from whole blood by lymphoprep density gradient centrifugation. Monocytes were further enriched to a purity of >95% by positive immunomagnetic bead selection (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) using the anti-CD172a mAb (clone: 74-12-15a; Pescovitz et al., 1984) and goat anti-mouse microbeads together with LS separation columns (MACS, Miltenyi Biotec). Cells were cultured in 24-well plates (Nunc, Thermo Fisher Scientific, Langenselbold, Germany) at a density of 5.0×10^5 cells/ml in phenol red-free Dulbecco's modified Eagle's Medium (DMEM; Gibco, Merelbeke, Belgium) supplemented with 10% (v/v) FCS (Greiner), 100 U/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco), recombinant porcine (rp) GM-CSF (Inumaru et al., 1998) and rpIL-4 (R&D systems) and incubated at 37 °C in a humidified atmosphere at 5% CO_2 to generate monocyte-derived dendritic cells (MoDCs) as previously described (Carrasco et al., 2001). On day 3 of the culture period, MoDCs were fed by addition of fresh medium supplemented with rpGM-CSF and rpIL-4 at the same concentrations. On day 4 of the culture period, immature MoDCs were either immediately used to assess the role of individual FcγR in the uptake of immune complexes or treated with different antigens.

2.4. The role of individual FcγR in immune complex uptake

To investigate the role of individual FcγR in the internalisation of immune complexes (IC), the binding of IC to these FcγR was inhibited. Immature MoDCs were