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# IL-13 replaces IL-4 in development of monocyte derived dendritic cells (MoDC) of swine

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**Abstract**

Dendritic cells (DCs) are a critical aspect of innate immune responses in addition to initiating adaptive immunity. In vitro generation of monocyte derived dendritic cells (MoDC) by culturing cells in IL-4 and granulocyte/macrophage colony stimulating factor (GM-CSF) has been reported for multiple species including swine. However, IL-4 is not a prominent cytokine detected in the periphery of common breeds of swine such as Yorkshire pigs. In this study, we report the generation and characterization of porcine MoDC in vitro using porcine IL-13 and porcine GM-CSF. These cells have the predicted expression of Class II MHC and T cell costimulatory molecules, phagocytic capacity and the ability to process and present antigen. Critically, porcine IL-13/GM-CSF MoDC have the unique ability to stimulate a primary mixed lymphocyte response in vitro. The type I interferon response of these MoDC to poly I:C (TLR3 ligand), LPS (TLR4 ligand) and CpG (TLR9 ligand) was tested. Of these TLR agonists, LPS or CpG did not stimulate induction of type I interferons, but a strong response was observed to poly I:C. This analysis shows that the generation of MoDCs in IL-13 yields cells of equivalent phenotype and function as IL-4 generated DC. However, for swine, in vitro generation of MoDC in IL-13 is likely to induce a more physiological cell population to study given expression of IL-4 is lacking in the periphery of these animals.
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*Keywords:* Dendritic cell; IL-13; TLR agonists; Porcine

## 1. Introduction

The interface between the mammalian host and pathogens is a critical consideration in the design of strategies to combat an infectious disease. Dendritic cells are a heterogeneous population with important functional and phenotypic immune properties (Steinman and Nussenzweig, 1980; Steinman et al., 1980) critical in the defense against pathogens, in part by linking innate and adaptive immunity (Le Bon and Tough, 2002). Two of the major populations of DC in peripheral blood are the plasmacytoid dendritic cells and monocytic dendritic cells, combined accounting for less than 1% of circulating leukocytes. Being such a small fraction of the peripheral blood cells, their study requires complex sorting and concentration methods to isolate enough cells for analysis. In vitro derived DCs have been obtained from cultured monocyte precursors by the use of various cytokine cocktails. Incubation of monocytes of various mammalian species with GM-CSF in combination with IL-4 yields in vitro derived functional dendritic cells (Chapuis et al., 1997; Gieseler et al., 1998; Kiertscher and Roth, 1996; O'Doherty

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et al., 1997; Peters et al., 1996; Pickl et al., 1996). In swine, various dendritic cell populations, including in vitro derived dendritic cells with IL-4 and GM-CSF have been described (Bautista et al., 2002; Carrasco et al., 2001; Paillot et al., 2001; Summerfield et al., 2003). However, it is not clear whether IL-4 in swine is the physiological cytokine involved in such DC differentiation.

The evolution and biological function of cytokine genes and their receptors is an example of common mechanisms within the mammalian species that have been studied. The Th1 versus Th2 paradigm was initially described in vitro (Mosmann et al., 1986) and the importance of this dichotomy relative to pathogen/host interaction was elicited in part from the study of murine immune responses to *Leishmania major* (Boom et al., 1990; Rogers et al., 2002). There are now many examples of immune responses of humans, nonhuman primates, rodents and livestock animals to pathogens, allergens and in autoimmune mediated disease that are marked by a particular pattern of T helper cell cytokines (Guo et al., 2004). While IL-4 is a major Th2 cytokine in man and mouse, this cytokine is rarely detected in the peripheral lymphoid organs of swine (Diaz and Mateu, 2005; Raymond and Wilkie, 2004). Contrarily, IL-13 mRNA is readily expressed by porcine PBMC (Bailey et al., 1998). The successful use of IL-4 to generate MoDC from pigs in vitro (Carrasco et al., 2001) is likely due to the existence of common receptor for IL-13 and IL-4.

In species where these receptors have been cloned and analyzed for biological function, the pattern of IL-4R and IL-13R expression and relative affinity of these receptors for these two cytokines have revealed the complex nature of the biology of these Th2 cytokines. Chains of the IL-4R are involved in receptor complexes that bind IL-13 and chains of the IL-13R are known to contribute to IL-4 binding (Hilton et al., 1996; Murata et al., 1998; Obiri et al., 1997; Zurawski et al., 1993). The differential expression of IL-4R and IL-13R chains within these receptors by different cell types responsive to IL-4 and/or IL-13 often regulates the role of these cytokines in differentiation and activation of lymphoid cells.

We have developed a method of expressing porcine cytokines with replication-defective adenovirus vectors, initially for expressing the type I interferons, α and β (Chinsangaram et al., 2003; Moraes et al., 2001). Using this system, we have cloned the genes for porcine granulocyte/macrophage colony stimulating factor (GM-CSF) (Caron et al., 2005), porcine IL-4 (Moraes and Grubman, unpublished data) and IL-13 (this report) into this vector for expression in vitro and in vivo. We now show that culturing PBMC from naïve, healthy swine in the presence of GM-CSF and IL-13 generates monocyte derived dendritic cells. These cells were analyzed for expression of cell surface markers, important coreceptors for T cell activation, phagocytic activity, the ability to process and present antigen and induction of primary mixed lymphocyte responses. Having established that these in vitro derived cells are monocyte derived dendritic cells (MoDC) by such criteria, analysis of the innate response to PAMPs was undertaken.

## 2. Materials and methods

### 2.1. Construction of Ad5-pIL-13 virus

Porcine IL-13 was cloned from con-A-stimulated PBMC by PCR cloning using primers from the 3′ and 5′ end of the gene sequences derived from GenBank accession no. NM_213803/AF385626. The generation of the recombinant replication-defective adenovirus (Ad5) expressing the porcine IL-13 gene (Ad5-pIL-13) was performed according to methods previously established in our laboratory (Moraes et al., 2001). Briefly, the cloned p-IL-13 gene sequences were subcloned into the Ad5-Blue expression vector. Positive clones were confirmed by restriction enzyme analysis and sequencing. Purified DNA was digested with *Pac*I and tranfected into 293 cells (Fig. 1). Plaques from the 293 transfected cells were collected and virus was further propagated in 293 cells and purified by CsCl density centrifugation.

### 2.2. Rabbit antisera to IL-13 and antigen capture ELISA

Polyclonal rabbit antisera to IL-13 were generated and affinity purified by custom order at Zymed Laboratories Inc., South San Francisco, CA, USA. The carboxi-terminus immunizing peptide (CRDTKIEVAQFVKDLLKHLRMIFRHG-COOH) and to the amino-terminus peptide (GPVPPHSTALKELIEELVNITQNQKC-COOH) of porcine IL-13 were inoculated into two rabbits each (C1b, C2b and N1a, N2a). Specificity of the reactivity of purified antibodies to the immunizing peptides was confirmed by solid phase ELISA. To develop a capture ELISA for the detection of native protein, 1 mg of antibodies were labeled with biotin (Pierce, Rockford, IL, USA) according to manufacturer directions. Plates were coated with 0.2 μg/well of either N-terminus or C-terminus specific

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