Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Immunology and Immunopathology 111 (2006) 175–185
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Effects of antigen and recombinant porcine cytokines on pig dendritic cell cytokine expression in vitro

Claudine R. Raymond *, Abubaker M.E. Sidahmed, Bruce N. Wilkie

Department of Pathobiology, University of Guelph, Guelph, Ont., Canada NIG-2W1

Received 14 July 2005; received in revised form 18 October 2005; accepted 22 December 2005

Abstract

To evaluate variables influencing in vitro immune response induction, pig monocyte-derived DCs (moDCs) were treated with putative type-1 and type-2 antigens (Ags, killed *Mycobacterium tuberculosis* (Mtb) and hen egg white lysozyme (HEWL)) and recombinant porcine cytokines (IL-6, IL-10, IL-12, IFN-γ and TNF-α). Responses were measured as moDC cytokine mRNA expression. Treatment of moDCs with HEWL increased IL-13 but not IL-12, IFN-γ or IL-10 mRNA, suggesting a DC2 phenotype. Addition of TNF-α, IFN-γ or IL-12 to HEWL-treated moDCs increased IL-12p35 and reduced IL-13 mRNA; suggesting a DC1 phenotype. Mtb increased moDC IL-12p35, IFN-γ and to a lesser extent IL-13 mRNA. This DC1 bias was enhanced by TNF-α, IFN-γ or IL-12, which increased IL-12p35 and to a lesser extent IL-10 mRNA but reduced IL-13 mRNA. Addition of IL-10 to Mtb-pulsed moDCs reduced IL-12p35, IFN-γ and IL-13, but increased IL-10 mRNA, suggesting diversion from DC1 to DC2. Thus porcine moDCs treated with Ag and/or cytokines alter moDC cytokine expression confirming their likely ability to initiate and steer acquired immune response.

Keywords: Pig; Monocyte-derived dendritic cells; Cytokines; Antigens; Immune response steering; Dendritic cells types 1 and 2

1. Introduction

Dendritic cells (DCs) are professional antigen presenting cells (APCs) and the only APCs capable of inducing primary immune response (Banchereau et al., 2000) hence DC function is a key regulator of immune response. Immature tissue DCs respond to infection or tissue damage by secreting cytokines and activating other innate and acquired immune system cells (Banchereau et al., 2000). In response to danger signals such as tissue damage, pathogens or inflammatory cytokines, the efficiency of antigen (Ag) uptake and presentation by DCs are increased (Guermonprez et al., 2002; Lipscomb and Masten, 2002). Mature DCs have up-regulated expression of costimulatory molecules, adhesion molecules and

Abbreviations: BMC, blood mononuclear cells; HEWL, hen egg white lysozyme; IR, immune response; Mtb, killed *Mycobacterium tuberculosis*; moDC, monocyte-derived dendritic cell; Th, T-helper

* Corresponding author at: Institute for Animal Health, Neuropathogenesis Unit, Ogston Building, West Mains Road, Edinburgh EH9 3JF, UK. Tel.: +44 131 319 8211; fax: +44 131 668 3872.
E-mail address: claudine.raymond@bbsrc.ac.uk (C.R. Raymond).

0165-2427/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2005.12.004

MHC complexed with antigen for recognition by T-cell receptors (TCRs) (Lipscomb and Masten, 2002; Palucka and Banchereau, 1999). Maturation of DCs induces their migration out of tissues, into the afferent lymph to enter T-cell zones of lymph nodes where they secrete cytokines and select and activate Ag-specific T-cells (Guermonprez et al., 2002). The profile of cytokines secreted by DCs depends on the inducing organism and secreted cytokines influence $CD4^+$ T-helper (Th) cell phenotype and their cytokine profile. Activated Th cells secrete cytokines, which can activate macrophages, NK cells and eosinophils (Banchereau et al., 2000). The activated mature DCs and $CD4^+$ T-cells interact with B-cells activating them and causing their migration to the B-cell areas of the secondary lymphoid organs where they differentiate into antibody (Ab) producing plasma cells (Banchereau et al., 2000; Lipscomb and Masten, 2002). Antibodies as well as effector cells, including activated macrophages, NK cells, eosinophils and CTL, leave via the efferent lymph, enter the blood and move to sites of infection to facilitate pathogen clearance (Banchereau et al., 2000). The net effect is induction of cell-(CMI) and antibody-mediated immune response with variable proportions of type-1 and type-2 bias due in part to the cytokines produced by the DC.

Two lineages of DCs have been identified in mice and humans. Human myeloid-derived DCs (DC1) produce pro-inflammatory cytokines as well as IL-12, 15 and 18, and were assumed to stimulate Th-1-type responses, including CMI, which protects against intracellular pathogens (de Saint-Vis et al., 1998). Plasmacytoid DCs (DC2) produced IL-7, IL-10, IL-13 and IFN-γ and were assumed to stimulate Th-2/Ab response, principally mediating resistance to extracellular pathogens (de Saint-Vis et al., 1998). More recent evidence suggests that both myeloid and plasmacytoid DCs can induce $CD4^+$ T-cells to Th-1 or Th-2 phenotypes. Plasmacytoid DCs when stimulated by virus, produced large amounts of IFN-α, IFN-γ and TNF-α, stimulated naive $CD4^+$ T-cells to secrete IFN-γ and IL-10 and triggered antiviral (Th-1) response (Kadowaki et al., 2000; Liu et al., 2000). Maturation of human myeloid DCs into stable Th-1-promoting DC1 or Th-2-promoting DC2 cells depends on the microbial ligands with which they were stimulated. Stimulation of myeloid DCs with *Schistosoma mansoni* protein extract promoted development of DC2, which induced Th-2 cells while poly-IC, an IFN inducer, promoted the development of DC1, which induced Th-1 cells (de Jong et al., 2002).

Earlier evidence suggested that in vivo, mouse lymphoid ($CD8\alpha^+$) DCs produced high amounts of IL-12, IFN-γ and α leading to Th-1 responses while myeloid DCs ($CD8\alpha^-$) directed the development of Th-2 responses, induced IFN-γ and IL-2 along with large amounts of the Th-2 cytokines IL-4 and IL-10 and favoured a predominantly IgG1 response (Maldonado-Lopez et al., 1999; Ohteki et al., 1999; Pulendran et al., 1999). Recent evidence suggests plasticity among murine DC subsets in their ability to polarize Th-1/Th-2 responses depending on pathogen/Ag, Ag dose and site of exposure to Ag. The results from two sets of experiments demonstrated that both myeloid and lymphoid DCs producing IL-12 may promote Th-1 response but the presence of other cytokines, such as IL-10, down-regulates IL-12 and steers the response toward Th-2 (Huang et al., 2001a; Maldonado-Lopez and Moser, 2001). Incubation of both subsets with IFN-γ down-regulated the capacity of $CD8\alpha^-$, myeloid DCs to promote Th-2 and increased their ability to generate Th-1 responses (Maldonado-Lopez and Moser, 2001). In mice as for human DCs, the nature of the microbial stimulus determines whether the DC subsets generate Th-1 or Th-2 responses. Murine bone marrow-derived DCs stimulated with *Escherichia coli* LPS matured into DC1 and promoted a Th-1 response (increased IFN-γ and decreased IL-4 production), while stimulation with glycoprotein ES-62 of *Acanthocheilonema vitea*, a nematode parasite, generated DC2 with the capacity to induce Th-2 (increased IL-4 and decreased IFN-γ production) (Whelan et al., 2000).

Previous investigations of the effects of stimulating pig moDCs with pathogen associated molecular patterns (PAMPs) have demonstrated that like those of human and mice, pig moDCs responded to microbial PAMPs by altering Toll-like receptor expression, up-regulating MHC II and B7 and altering cytokine expression towards type 1 or type 2. The resulting cytokine profile depended on the PAMP to which the moDCs were exposed and may in turn steer immune response (Raymond and Wilkie, 2005). Paillot et al. (2001) have described allogeneic mixed leukocyte reactions induced by pig moDCs which