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Allergen-driven suppression of thiol production by human dendritic cells and the effect of thiols on T cell function

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Abstract

Dendritic cells are a major source of extracellular thiols needed for T cell activation, a process in which CD40-mediated stimulation plays a pivotal role. The *Dermatophagoides pteronyssinus* group 1 mite allergen (Der p 1) has previously been shown to cleave CD40 from the surface of human dendritic cells, thereby suggesting that Der p 1 might compromise the ability of these cells to sustain thiol production during T cell activation. This has therefore prompted us to examine the effect of the mite protease allergen Der p 1 on thiol production by human dendritic cells. Monocyte-derived dendritic cells were treated with either proteolytically active or inactive Der p 1 and then stimulated through CD40 for extracellular thiol detection. The effect of thiol (*N*-acetyl-L-cysteine) and thiol inhibitors on naïve T cell responses, including CD25 and FOXP3 expressions, cell proliferation and cytokine production, was determined. Here, we show that Der p 1-mediated cleavage of CD40 from the surface of dendritic cells suppresses the ability of these cells to produce extracellular thiols, and that reducing thiols are needed for the generation of the T helper type 1 (Th1), T cytotoxic type 1 (Tc1) and T regulatory (Treg) cell phenotypes. We conclude that Der p 1-driven suppression of thiol production by dendritic cells may disrupt Th1/Tc1 and Treg cell development, and in doing so could lead to Th2/Tc2 cell responses and allergy.

Keywords: Allergen; Cysteine; Dendritic cell; Der p 1; Thiols; T cell; Treg

Introduction

The intracellular reduction/oxidation (redox) balance is mainly maintained by glutathione, of which reduced (glutathione, GSH) and oxidised (glutathione disulphide, GSSG) forms exist. Cysteine, a thiol containing amino acid, is the rate-limiting precursor for GSH biosynthesis (Mansoor et al. 1992; Lu 1999), and is therefore the most critical factor in determining the redox status of the cell. In the extracellular space, cysteine is present at very low concentrations, whereas its oxidised form cystine is present in substantial amounts (Mansoor et al. 1992). Thus, cystine is taken up by cells equipped with a cystine transporter and reduced intracellularly to cysteine (Ishii et al. 1987; Gmunder et al. 1990).

The intracellular redox status of antigen-presenting cells (APCs), including dendritic cells (DCs) and

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macrophages, is thought to play an important role in determining the outcome of an immune response (Peterson et al. 1998; Murata et al. 2002a, b). Previous studies have indicated that the thiol *N*-acetyl-L-cysteine (NAC), a less readily oxidised derivative of L-cysteine, may favour a T helper type 1 (Th1) cytokine response (Jeannin et al. 1995; Eylar et al. 1995; Bengtsson et al. 2001). This suggests that the presence of cysteine in the microenvironment constitutes a further costimulatory factor that may play an essential role in determining Th1 versus Th2 cell polarisation. However, T cells lack an efficient system of cystine import, and thus rely on alternative sources for cysteine (Ishii et al. 1987; Gmunder et al. 1990), namely those generated by APCs, such as DCs (Angelini et al. 2002).

During naïve T cell activation, DCs generate extracellular cysteine via two mechanisms. Firstly, they take up extracellular cystine, reduce it intracellularly to cysteine and then release it into the microenvironment. Secondly, DCs secrete a cytosolic enzyme, namely thioredoxin (TRX), which in turn reduces extracellular cystine to cysteine (Angelini et al. 2002), thereby maintaining physiological levels of cysteine in the microenvironment during an immune response.

Cysteine production by DCs has been shown to increase upon maturation with lipopolysaccharide (LPS) or tumor necrosis factor-α (TNF-α), and upon interaction with T cells, in which the interaction of CD40 with CD40 ligand (CD40L) is thought to play a pivotal regulatory role (Angelini et al. 2002). The cysteine protease activity of Der p 1, a group 1 allergen of the mite species *Dermatophagoides pteronyssinus*, has previously been shown to cleave CD40 from the surface of human DCs (Ghaemmaghami et al. 2002), thereby suggesting that Der p 1 might also compromise the ability of DCs to sustain CD40-mediated production of reducing thiols during naïve T cell activation. Thus, the aim of this work was to determine the effect of the proteolytic activity of Der p 1 on thiol production by DCs and to investigate how might thiols affect T cell subset polarisation.

Materials and methods

Materials

Mouse anti-human CD1a (PE, clone BL6, IgG1), CD3 (ECD, clone UCHT1, IgG1), CD4 (PC5, clone 13B8.2, IgG1), CD11c (PE, clone BU15, IgG1), CD14 (ECD, clone RMO52, IgG2a), CD25 (PE, clone B1.49.9, IgG2a), CD40 (PE, clone MAB89, IgG1), CD45RA (PE, clone ALB11, IgG1), CD80 (FITC, clone MAB104, IgG1), CD83 (FITC, clone HB15a, IgG2b), CD86 (PE, clone HA5.2B7, IgG2b), HLA-DR (ECD, clone IMMU-357, IgG1), interferon-γ (IFN-γ, FITC, clone 45.15, IgG1), interleukin 4 (IL-4, PE, clone 4D9, IgG1) and isotype control (PE and FITC, clone 15H6, IgG1; PE, clone 7T4-1F5, IgG2a) antibodies were purchased from Beckman Coulter (Highwycombe, UK). Mouse anti-mouse/rat/human FOXP3 (Alexa Fluor® 488, clone 150D, IgG1) and isotype control (Alexa Fluor® 488, clone MOPC-21, IgG1) antibodies were purchased from Cambridge BioScience (Cambridge, UK). Mouse anti-human CD40 antibody (purified, clone 5C3, IgG1) was purchased from BD PharMingen (Oxford, UK). Mouse anti-human CD28 antibody (purified, clone 37407, IgG1) was purchased from R&D Systems (Oxford, UK). Mouse anti-human CD3 antibody was purified on a Protein G column from the supernatant of a hybridoma cell line (OKT3) (ECACC, Porton Down, UK).

The house dust mite allergen Der p 1 was purified and tested for enzymatic function using our previously published procedure (Gough et al. 1999).

Generation of immature DCs from peripheral blood monocytes

Peripheral blood samples were obtained from healthy non-atopic donors (with prior consent and ethical committee approval) and peripheral blood mononuclear cells (PBMCs) were separated on a histopaque density gradient. $CD14^+$ monocytes were purified from PBMCs by positive selection with anti-CD14 microbeads (Miltenyi Biotec, Bisley, UK) to 95–98% purity. Immature $CD1a^+$ $CD83^-$ DCs were generated from monocytes as detailed elsewhere (Ghaemmaghami et al. 2002; Sallusto and Lanzavecchia 1994). Briefly, monocytes were cultured with recombinant human (rh) granulocyte macrophage-colony stimulating factor (GM-CSF) (50 ng/ml; R&D Systems, Oxford, UK) and rhIL-4 (10 ng/ml; R&D Systems, Oxford, UK) in serum-free AIM V medium (1×10^6 cells/ml in a 24-well culture plate) (Invitrogen, Paisley, UK), in which Der p 1 remains proteolytically active (Schulz et al. 1998), at 37 °C in a humidified atmosphere of 5% CO_2. On day 7, most of the cells were immature $HLA\text{-}DR^+$ $CD1a^+$ $CD80^+$ $CD83^-$ $CD86^+$ DCs.

Maturation and restimulation of monocyte-derived DCs

Immature DCs were cultured in AIM V medium (1×10^6 cells/ml in a 24-well culture plate), in the presence or absence of LPS (500 ng/ml; Sigma, Poole, UK) and TNF-α (25 ng/ml; R&D Systems, Oxford, UK), at 37 °C in a humidified atmosphere of 5% CO_2 for 30 h. Cells were then either harvested and stained for surface molecules (to confirm the induction of DC