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The detection of ADAM8 protein on cells of the human immune system and the demonstration of its expression on peripheral blood B cells, dendritic cells and monocyte subsets

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Abstract

A disintegrin and metalloprotease (ADAM) proteins have wide ranging functions, including proteolytic cleavage of cell surface molecules, cell fusion, cell adhesion and intracellular signalling. Recent evidence suggests the involvement of ADAM8 in allergic responses. For instance, ADAM8 is amongst a number of genes up-regulated in experimentally induced asthma in animals. In order to further define the involvement of ADAM8 in allergic responses, we sought in the first instance to examine its distribution on human peripheral blood B cells, resting and activated T cells, monocyte subsets and monocyte derived dendritic cells. Here we demonstrate for the first time ADAM8 protein expression on B cells and dendritic cells, and its higher expression on $CD14^{2+}CD16^{-}$ monocytes compared to $CD14^{+}CD16^{+}$ cells. Immature dendritic cells expressed low levels of ADAM8 when treated with a combination of GM-CSF and IL-4, but stimulation with LPS resulted in a higher level of expression, which was TLR-4 independent. Up-regulation of ADAM8 expression on dendritic cells was also observed after stimulation with TNF-α, but not after stimulation with anti-CD40. The demonstration of ADAM8 expression on these cells provides an opportunity for addressing the potential role of inhaled protease allergens, such as Der p 1, in modulating ADAM8 functions, particularly with regards to innate immune responses by dendritic cells and IgE synthesis by B cells.

Keywords: ADAM; Allergen; Asthma; Lymphocyte; Metalloprotease; Monocytes

Introduction

Allergic diseases such as asthma affect more than 30% of the population, and they are the result of a complex interaction between host genetic components and environmental factors. Studies aimed at elucidating asthma-related genes have highlighted chromosomal regions that have significant links to disease susceptibility. One such study identified the a disintegrin and

Abbreviations: ADAM, a disintegrin and metalloprotease; Der p 1, group 1 allergen of *Dermatophagoides pteronyssinus*

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metalloprotease (ADAM) protein family as potential susceptibility genes, by demonstrating an association between single nucleotide polymorphisms in the ADAM33 gene and asthma (Van Eerdewegh et al., 2002).

ADAM proteins are type-1 transmembrane glycoproteins consisting of 700–1200 amino acids and belong to the zinc protease superfamily. To date, 40 ADAM proteins have been identified, and of these 23 are human, seven of which (ADAM 8, 9, 10, 17, 19, 28, 33) are expressed on cells that are potentially involved in the pathogenesis of asthma or allergy. ADAM proteins have wide ranging functions, including proteolytic cleavage of cell surface molecules, cell fusion, cell adhesion and intracellular signalling (Seals and Courtneidge, 2003; Huovila et al., 2005).

Recent evidence suggests the involvement of ADAM8 in allergic responses, including experimental asthma in animals (King et al., 2004; Matsuno et al., 2006). For instance, ADAM8 is amongst a number of genes up-regulated in experimentally induced asthma in mice (King et al., 2004). It has also been demonstrated to cleave the low affinity IgE receptor, also known as CD23 (Fourie et al., 2003). On B lymphocytes, CD23 is an important component of the regulatory mechanisms controlling IgE synthesis. When bound by IgE, membrane CD23 (mCD23) delivers a negative regulatory signal to B cells switching off further IgE synthesis. However, unoccupied CD23 can undergo proteolytic cleavage by membrane metalloproteases (Wheeler et al., 1998), including ADAM8 (Fourie et al., 2003), to produce soluble fragments of varying molecular sizes (sCD23). Depending on their oligomerisation state, soluble fragments of CD23 can either up-regulate or down-regulate IgE synthesis (Gould et al., 2003).

Expression of ADAM8 is mainly restricted to cells of the immune system, having been demonstrated on the monocytic cell lines U937 and THP-1, and on monocytes and granulocytes (Yoshiyama et al., 1997; King et al., 2004). ADAM8 has also been detected in very low quantities on the B cell line Raji, but could not be detected on the T cell line Molt-4 (Yoshiyama et al., 1997). In addition, expression of ADAM8 has been demonstrated throughout the central nervous system (CNS) of mice (Schlomann et al., 2000) and on human osteoclasts (Choi et al., 2001). Levels of mouse ADAM8 mRNA can be increased upon IL-4 and IL-13 stimulation (King et al., 2004), whilst other studies have indicated that ADAM8 expression can be up-regulated on various cell types by TNF-α (Schlomann et al., 2000), IFN-γ and LPS (Kataoka et al., 1997).

In order to further define the involvement of ADAM8 in allergic responses, we sought in the first instance to examine in greater detail its distribution on human peripheral blood B cells, T cells, monocytes and monocyte derived dendritic cells. Here we demonstrate for the first time the expression of ADAM8 protein on B cells, dendritic cells and its differential expression on the two monocyte subsets.

Materials and methods

Antibody reagents

Mouse anti-human CD3 (ECD, clone UCHT1), CD4 (PC5, clone 13B8.2), CD8 (PC5, clone B9.11), CD11c (PE, clone BU15), CD14 (ECD or FITC, clone RM052), CD16 (PE, clone 3G8), CD19 (PE, clone J4.119), CD25 (PE, clone B1.49.9), CD83 (PC5, clone HB15a), HLA-DR (PC5, clone IMMU-357) and IgG1 (FITC, clone 679.1Mc7) were purchased from Coulter Immunotech (Luton, UK). Mouse anti-human TLR-4 (PE, clone HTA125) was purchased from Serotec (Oxford, UK). Mouse anti-human ADAM8 (unlabelled or FITC-labelled, clone 143338), CD28 (clone 37407) and goat anti-mouse IgG (HRP-labelled) were purchased from R&D Systems (Oxford, UK). Mouse anti-human CD3 antibody was purified on a protein G column from the supernatant of the hybridoma cell line OKT3 (ECACC, Porton Down, UK).

Cell culture reagents

RPMI 1640 medium (with L-glutamine and $NaHCO_3$), HEPES buffer and Histopaque were purchased from Sigma (Poole, UK). Penicillin/Streptomycin was purchased from Gibco (Invitrogen, Paisley, UK). Human recombinant (rh) IL-4 and rhGM-CSF were purchased from R&D Systems (Oxford, UK). LPS (*E. coli* 055:B5) was purchased from Sigma (Poole, UK).

Staining for cell surface markers

Cells were washed with PBA (PBS, 1% v/v BSA and 0.1% w/v sodium azide) and stained with the appropriate antibodies for 30 min, at 4 °C in the dark. Cells were then washed twice with PBA and fixed in 0.5% v/v formaldehyde in isotonic azide free solution. Flow cytometry of antibody labelled cells was performed using an EPICS Altra Flow Cytometer (Beckman Coulter, Buckinghamshire, UK). Approximately 50,000 cells were collected for each sample (200,000 for monocytes) and isotype matched antibodies were used to determine binding specificity. The data were analysed using WinMDI version 2.8 (Joseph Trotter, Scripps Institute, La Jolla, CA, USA). Dead cells were excluded from analysis according to their forward and side scatter characteristics.