Effect of O-linked glycosylation on the antigenicity, cellular uptake and trafficking in dendritic cells of recombinant Ber e 1

ABSTRACT

Ber e 1, a major Brazil nut allergen, has been successfully produced in the yeast Pichia pastoris expression system as homogenous recombinant Ber e 1 (rBer e 1) with similar physicochemical properties and identical immunoreactivity to its native counterpart, nBer e 1. However, O-linked glycans was detected on the P.pastoris-derived rBer e 1, which is not naturally present in nBer e 1, and may contribute to the allergic sensitisation. In this study, we addressed the glycosylation differences between P. pastoris-derived recombinant Ber e 1 and its native counterparts. We also determined whether this fungal glycosylation could affect the antigenicity and immunogenicity of the rBer e 1 by using dendritic cells (DC) as an immune cell model due to their role in modulating the immune response. We identified that the glycosylation occurs at Ser96, Ser101 and Ser110 on the large chain and Ser19 on the small polypeptide chain of rBer e 1 only. The glycosylation on rBer e 1 was shown to elicit varying degree of antigenicity by binding to different combination of human leukocyte antigens (HLA) at different frequencies compared to nBer e 1 when tested using human DC-T cell assay. However, both forms of Ber e 1 are weak immunogens based from their low response indexes (RI). Glycans present on rBer e 1 were shown to increase the efficiency of the protein recognition and internalization by murine bone marrow-derived dendritic cells (bmDC) via C-type lectin receptors, particularly the mannose receptor (MR), compared to the non-glycosylated nBer e 1 and SFA8, a weak allergenic 2S albumin protein from sunflower seed. Binding of glycosylated rBer e 1 to MR alone was found to not induce the production of IL-10 that modulates bmDC to polarise Th2 cell response by suppressing IL-12 production and DC maturation. Our findings suggest that the O-linked glycosylation by P. pastoris has a small but measurable effect on the in vitro antigenicity of the rBer e 1 compared to its non-glycosylated counterpart, nBer e 1, and thus may influence its applications in diagnostics and immunotherapy.