

PROTEOMIC ANALYSIS OF ALLERGIC MAST CELL ACTIVATION

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Asthma patients produce high levels of IgE against environmental allergens leading to airways inflammation. A crucial early event in allergy is the IgE-mediated mast cell activation. The aim of this study was to assess the 2D-electrophoresis profiles of the murine mast cell lineage subjected to sensitization with IgE anti-dinitrophenylated bovine serum albumin (DNP-BSA) (0.5 µg/ml), followed by DNP-BSA (10 ng/ml) challenge. These profiles will be compared to those of cells sensitized but not challenged and naïve cells. Protein extracts were obtained after incubating cells in 7 M urea, 2 M thiourea, 4% CHAPS, 60 mM DTT, 40 mM tris and 1% IPG buffer, in the presence of Roche protease inhibitor cocktail. The isoelectric focusing step was performed on 18 cm 3-10 IPG strips, followed by SDS-PAGE (12%T) separation in the second dimension and staining with colloidal Coomassie. Although spots could be detected all over the gels, most of them were concentrated between pH 4-8, with molecular masses ranging from 20.1 to 97 kDa. Gel images from cell extracts were compared, and 25 protein spots that showed consistent differences in expression levels were detected. Mass spectrometry analysis of these spots are now underway and may contribute to a better understanding of the molecular mechanisms involved in the pathogenesis of allergic diseases.

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