Serum IgE isolation and purification from betalactam allergic patients

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Objective: Total Immunoglobulin E isolation and purification have been scarcely described in literature. Purification process is complicated due to its low concentration comparing with the rest of the immunoglobulins (0.002%) and the homology between them. However, having purified IgEs from allergic patients can be very useful to develop in vitro diagnostic tools and to study the role of the protein in allergic disease. The aim of this study is to compare different total IgE isolation and purification methods in sera from antibiotic allergic patients and tolerant controls.

Material and methods: 40-100 ml of whole blood from 8 patients and 11 controls were collected at the Allergy department of La Fe Universitary and Polytechnic Hospital. Total IgE purification was performed using 2 methods: 1) Ion exchange chromatography and 2) Dynabeads magnetic particles (Invitrogen™) conjugated to 2 different human anti-IgE antibodies (monoclonal Omalizumab, Xolair®, and policlonal from Dr. Fooke Laboratorien GmbH). Quantitative and specific total IgE evaluation against betalactams was determined by ImmunoCAP™ (Thermo Fisher Scientific).

Results: Efficiency obtained using ion exchange chromatography was 40-75% with more diluted samples and with the presence of other proteins. Efficiency with magnetic particles was 3.8-47.2% with a higher IgE concentration and less presence of proteins. Regarding the antibodies used, efficiency was higher with the monoclonal antibody (22.7%) than with the polyclonal one (4.1%).

Conclusion: Ion exchange chromatography shows better efficiency in the IgE purification process. However, purification is optimized when using magnetic particles conjugated to a specific anti-IgE antibody compared to ion exchange chromatography: we obtain more purified IgE and is more time efficient.

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