Serum IgG isolation and purification from betalactam allergic patients

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Objective: Immunoglobulin G (IgG) is the most prevalent immunoglobulin in serum (IgG represents the 80%). Specific betalactam IgG can be found in serum from allergic patients, although we do not know the role of this protein. The aim of this study is to purify IgG from serum from antibiotic allergic patients and tolerant controls, in order to evaluate the role of these antibodies in the etiopathogenesis of the allergic reactions and its interference with methods of diagnosis.

Material and methods: 40-100 ml of whole blood from 10 patients and 12 controls were collected at the Allergy department of La Fe Universitary and Polytechnic Hospital. Total IgG purification from serum was performed using 2 methods: 1) Ion exchange chromatography and 2) chromatography columns containing protein G, which specifically bind IgG. The IgG recovery was quantified by ELISA for IgG IgG (Abcam™) and by measuring the absorbance at 280 nm. Specific IgG evaluation against betalactams was determined by ImmunoCAP™ (Thermo Fisher Scientific). The purity degree of the isolated IgGs was analysed by gradient gel electrophoresis (Bis-Tris 8%) developed with silver staining and Coomassie.

Results: With both methodologies, in patients and controls, we have obtained high quantity of total IgG (17,85 mg-123,25 mg) and specific betalactam IgG (<2 µg/ml a 39,9µg/ml). The column with protein G is easier, we obtain more purified IgG and is less time consuming (2h) than the ion exchange chromatography (24h).

Conclusion: We obtain high quantity of IgG with the ion exchange chromatography and with the column with protein G. However, using the second methodology, we optimize the purification protocol.

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