2PS-04-02

CHARACTERIZATION OF THE SERUM AUTOANTIGENS RECOGNIZED BY INTRAVENOUS IMMUNOGLOBULINS (IVIg) USING 2D-PAGE AND LC-MS

R. Lemieux, R. Bazin, A. Laroche, C. Philippeau, T. Tremblay
Hema-Quebec, Sainte-Foy, Quebec, Canada

Background: The mechanisms of action of IVIg in autoimmune and inflammatory diseases are unclear. We have reported the isolation from IVIg of a minor (<1%) autoreactive IgG population (auto-IgG) which can form autoimmune complexes (auto-IC) in normal human serum. The auto-IC formed in the plasma of treated patients could play a role in the observed therapeutic immunomodulatory effetcs of IVIg.
Aims and **Methods:** To identify the serum proteins present in auto-IC, the native serum autoantigens (auto-Ags) purified by affinity chromatography on immobilized auto-IgG, were separated by 1- and 2-dimensional PAGE followed by digestion of the eluted proteins, peptide separation by liquid chromatography (LC) and analysis by mass spectroscopy (MS) to identify the proteins.
Results: The PAGE results showed the presence in native auto-Ags of a distinctive set of proteins with MW ranging from 30 to 200 kD. Analysis of the 1D bands and 2D spots by LC-MS first showed the presence of Ig H (50-60 kD) and L chains (25-30 kD) corresponding to the anti-Id Ig known to inhibit the reactivity of auto-IgG in serum of healthy individuals. The analysis also revealed the presence of other proteins including several related to complement (C1, C3, C4, C5, properdin, factor H), coagulation (thrombin, AT III, ·1-PI,) and inflammatory responses (·2-macroglobulin, H4, galectin-3, fibronectin, haptoglobin). Ongoing work will determine if these proteins are directly recognized by auto-Ags or are present in protein complexes containing a bona fide auto-Ag.
Conclusions: The finding that IVIg-derived auto-IgG can bind to and possibly neutralize several inflammation-related proteins may help to explain the therapeutic effects of IVIg in a wide variety of autoimmune and inflammatory diseases. It also opens the door to the targeted development of IVIg substitutes which could be tested in vitro and in animal models. real.lemieux@hema-quebec.qc.ca