Abstract

Introduction. Interferon beta (IFN-) is a treatment for relapsing remitting multiple sclerosis. However, the therapeutic use of recombinant proteins induces a humoral immunologic response resulting in the induction of binding (BAb) or neutralizing (NAb) antibodies against the biological product. The presence of neutralizing antibodies has been associated with decreased IFN-treatment efficacy. Materials and methods. Two tumor cell lines (K562 and U937) were cultivated with human recombinant IFN-1a at different concentrations and lengths of time in order to measure the expression of intracellular ISG15, an inducible molecule in the IFN-1a signaling cascade. Blood was obtained from non-immunized and IFN-1a immunized (100,000 IU) New Zealand rabbits. The presence of BAb was evaluated by ELISA. For NAb detection, sera 1:20 dilution were added to the IFN-1a stimulated cell lines, and ISG15 expression was evaluated by flow cytometry. Results. K562 cells provided the better cell line for the assay, stimulated with a dose of 1,000 IU of IFN-1a, and a 1:100 dilution for the primary antibody and a 1:200 dilution for the secondary antibody. ISG15 expression was compared between cells alone or cultivated with IFN-1a. Mean fluorescence intensity (MFI) for ISG-15 expression median was 198 arbitrary units (AU) with interquartile ranges of 173-231 AU for non-stimulated cells and 430 AU with interquartile ranges of 316-611.5 AU for IFN-1a stimulated cells (p<0.01). Immunized rabbit sera decreased the expression of ISG-15 in K562 cells stimulated with IFN-1a, whereas non-immunized rabbit sera did not. Conclusions. This rabbit model demonstrates that ISG15 expression evaluated with flow cytometry can be used as a detection assay for NAb.

Keywords
Antibodies, neutralizing, K562 cells, flow cytometry, multiple sclerosis, interferon-beta.