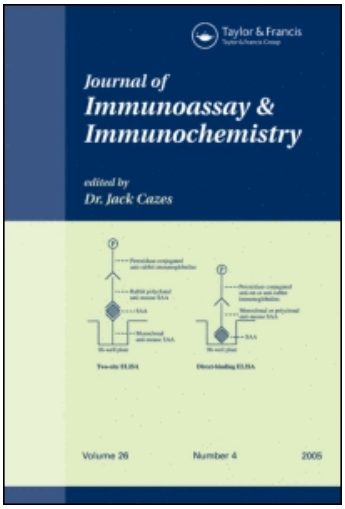

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IgG Interference in Second Generation Enzymeimmunoassays for Anti-Hepatitis C Virus Antibodies

Luis Borque[a]; Carlos Maside[a]; Antonio Rus[a]; Angel Elena[b]; Jorge del Cura[a]; Maria J. Gastañares[a]
[a] Laboratorio Central, Hospital "San Millàn", Logroño, Espanña [b] Sección de Reumatología, Hospital "San Millàn", Logroño, Espanña

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IgG INTERFERENCE IN SECOND GENERATION ENZYMEIMMUNOASSAYS FOR ANTI-HEPATITIS C VIRUS ANTIBODIES.

Luis Borque, M.D., Carlos Maside, Ph.D., Antonio Rus, Ph.D., Angel Elena, M.D.*, Jorge del Cura, Ph.D., Maria J. Gastañares M.D.

Laboratorio Central; * Sección de Reumatología.
Hospital "San Millàn", Logroño, España.

ABSTRACT

The interference of endogenous IgG in the identification of anti-HCV antibodies was studied in three second-generation enzymeimmunoassays. The addition of increasing concentrations of this immunoglobulin led to the appearance of false positives. The results obtained confirm the hypothesis that a non-specific binding of the IgG with the support material used was responsible for this interference.

Key words: hepatitis C virus antibodies, IgG interference, enzymeimmunoassay.

Correspondence and reprint request to:

Dr. Luis Borque de Larrea.
Laboratorio Bioquímica
Hospital "San Millàn"
c/ Autonomía de La Rioja, 3.
26004 Logroño, La Rioja.
Spain.

INTRODUCTION

Since Choo et al. (1) identified and described the genome of the infecting agent of post-transfusion non-A, non-B hepatitis (NANBH), HCV, various enzymeimmunoassays (EIAs) to identify the anti-HCV antibodies have been developed. The antigen used in the first-generation EIAs for HCV antibody was non-structural virus protein encoded by the initial clone. Not all post-transfusion NANBH sera reacted in these tests, in part because of delayed immune response to that antigen, and false positive reactions were also a problem (2). Recently, we described a type of interference attributable to high levels of serum IgG in a first-generation EIA (3).

The development of second-generation EIA's, which use structural virus proteins, has increased the sensitivity though not the specificity of these assays. In this paper we study the interference of IgG in three commercial second-generation EIAs.

MATERIALS AND METHODS

The following enzymeimmunoassays were used: Abbott HCV EIA 2nd Generation (Abbott Diagnostics Division, Chicago, USA); Ortho HCV™ ELISA Test System 2nd Generation (Ortho Diagnostic Systems Inc. Raritan, NJ 08869 USA); and Monolisa® anti HCV (Diagnostics Pasteur. Marnes-La-Coquette, France). The three assays use recombinant antigens: c22-3, c33c and c100-3 antigens (Abbott HCV EIA 2nd Generation); c100-3, 5-1-1, c33c and c22-3 antigens (Ortho HCV™ ELISA Test System 2nd Generation); and NC450 and 409-1-1 antigens (Monolisa® anti HCV). All tests were performed according to the manufacturers' instructions. Similar washing protocols between exposure to serum and application of conjugate

are carried out for all EIAs. Polystyrene beads for Abbott HCV EIA test and polystyrene microwells plates for Ortho HCV™ ELISA and Monoelisa® assays were use as the solid support phase.

Four anti-HCV negative sera were selected from healthy volunteers with IgG concentrations ranging from 9.5 to 12 g/L. Their absorbance values were similar to the negative controls provided with the three EIAs. In all four sera, negative values were also obtained with INNO-LIA HCV Ab test (Innogenetics N.V. Antwerp, Belgium), which uses synthetic peptides in discrete lines on a nylon strip. The IgG was isolated from these four sera using 40% ammonium sulphate precipitation, followed by dialysis against a phosphate buffer (70 mmol/L, pH 6.3) and subsequent purification by DEAE-cellulose chromatography. To exclude contamination of immunoglobulins by IgG fragments and aggregated IgGs that might have been generated by the isolation procedure, the isolated IgG was subjected to gel chromatography on Sephacryl S-200. A peak corresponding to monomeric IgG was concentrated by ultrafiltration on an Amicon 8050 cell, using a PM 10 membrane.

RESULTS AND DISCUSSION

Increased amounts of each sample's own IgG were added to each serum, and anti-HCV absorbance values were determined by the three EIAs. We observed increased absorbances as the concentrations of added IgG increased (Figure 1). Absorbances became greater than the respective cut-off values at concentrations of added IgG of 10 g/L for Monolisa, 12 g/L for Ortho, and 20 g/L for the Abbott assay. The total IgG present would be in the range of 20, 22, and 30 g/L respectively, before a signal exceeding the cut-off was observed, levels seen in

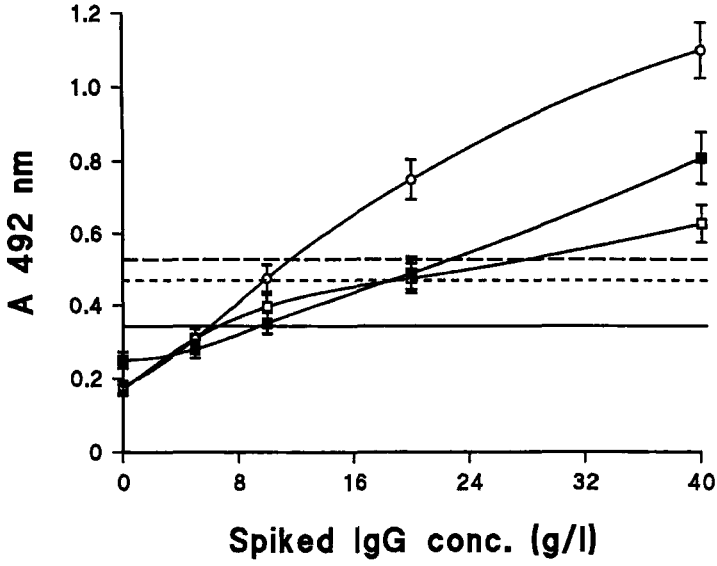

Fig. 1. Effect of added endogeneous IgG on four serum samples in different EIAs (□-□ Abbott HCV EIA 2nd Generation; ○-○ Ortho HCV™ ELISA Test; ■-■ Monolisa® anti HCV. The horizontal lines represent the cut-off values of the assays (----- Abbott HCV EIA 2nd Generation, cut-off value = 0,473; — — — Ortho HCV™ ELISA Test, cut-off value = 0,536; ——— Monolisa® anti HCV, cut-off value = 0,356). Values are expresed as mean ± SD.

polyclonal hypergammaglobulinemic conditions. Using Abbott Diagnostics' c100-3 antigen, a neutralization assay was carried out in the false positive samples with the Abbott HCV 2nd Generation EIA, and no significant inhibition was observed.

All samples with added human IgG which were positive with the 2nd generation assays, were negative with the INNO-LIA HCV Ab test. To rule out cross-reaction between human IgG added and recombinant antigens, the experiment was repeated on one of the

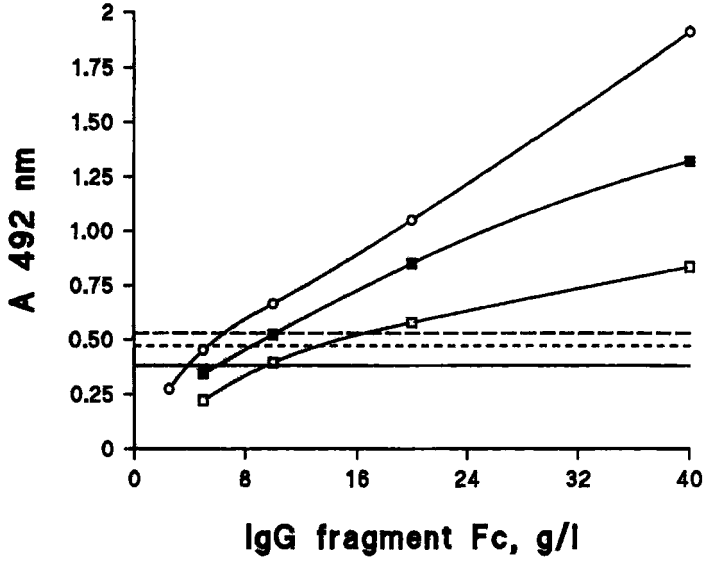

Fig. 2. Effect of added human Immunoglobulin G Fc fragment on a serum sample from healthy volunteer in different EIAs (□–□ Abbott HCV EIA 2nd Generation; ○–○ Ortho HCV^{TM} ELISA Test; ■–■ Monolisa^{R} anti HCV. The horizontal lines represent the cut-off values of the assays (------ Abbott HCV EIA 2nd Generation, cut-off value = 0,473; — — — Ortho HCV^{TM} ELISA Test, cut-off value = 0,536; ——— Monolisa^{R} anti HCV, cut-off value = 0,356).

HCV negative sera using added human purified Immunoglobulin G Fragment c (Fc)(Serva, Heilderberg, Germany) instead of whole IgG. Fc purity was checked by double immunodiffusion using a sheep anti-human IgG Fab (Serotec, Oxford, England) and by electrophoresis in 15% SDS-PAGE. The results are shown in Figure 2. Concentrations of Fc greater than 6,25 g/L for Ortho, 13,5 g/L for Abbott and 5,8 g/L for the Monolisa assay produce absorbances greater than the corresponding cut-off values.

Fc fragments cannot recognize antigenic epitopes and they are binding to plastic support through non-specific hydrophobic interactions (4). The interference due to Fc and IgG have approximate molar equivalence.

These findings suggest a non-specific binding of the Fc fragment of IgG to the solid support material of the EIAs, rather than specific binding to the recombinant antigens used in these assays.

On the other hand, in 36 patients with rheumatoid arthritis, we found a significant correlation ($r = 0.587$, $p<0.01$) between IgG serum values and optical density obtained by Abbott HCV EIA 2nd Generation test.

In conclusion, we have demonstrated one explanation of an interference found in many anti-HCV assays. The interference attributable to endogenous IgG is significant and must be taken into consideration in the evaluation of EIA results in patients with polyclonal hypergamma-globulinaemia and could explain the existence of false positives in rheumatoid arthritis (5), Sjögren's disease (6), auto-immune hepatitis (7) and primary biliary cirrhosis (8), and possibly in mixed cryoglobulinaemia (9).

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