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Short communication

Polyclonal IgM and IgA block *in vitro* complement deposition mediated by anti-ganglioside antibodies in autoimmune neuropathies

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ABSTRACT

Intravenous immunoglobulin (IVIG), consisting of IgG, is the first-line treatment for Guillain–Barré syndrome and multifocal motor neuropathy. IgG, but neither IgM nor IgA, has been demonstrated *in vitro* to inhibit complement deposition mediated by anti-ganglioside autoantibodies in sera from patients with both conditions. The objective of this study is to investigate the *in vitro* effectiveness of IgM and IgA in inhibiting complement deposition to ganglioside/anti-ganglioside antibody complexes. Serum samples were obtained from patients with multifocal motor neuropathy associated with anti-GM1 IgM antibodies, Guillain–Barré syndrome associated with anti-GM1 IgG antibodies and Miller Fisher syndrome associated with anti-GQ1b IgG antibodies. Inhibition of complement deposition using different immunoglobulin preparations was measured by enzyme-linked immunosorbent assay. IgM/A-enriched IVIG and immunoglobulin isotypes (polyclonal IgM and IgA) showed higher potential in inhibiting complement deposition than standard IVIG. Although the safety concerns about the use of IgM and IgA for an immunotherapy still remain, IgM and IgA may serve as an alternative immunotherapy in those anti-ganglioside antibody-mediated neuropathies.

1. Introduction

Polyclonal IgG concentrates for the attenuation of autoimmune neuropathies are a success story [1]. Treatments of Guillain–Barré syndrome (GBS), chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy (MMN) are labelled indications in some countries [2–4]. GBS is the most common cause of acute flaccid paralysis with demyelinating and axonal forms. Molecular mimicry between micro-organisms and gangliosides can induce the development of anti-GM1 or anti-GD1a IgG antibodies in patients with axonal GBS. The pathogenic autoantibodies bind to GM1 and activate complement at the nodes of Ranvier in the peripheral nerves, leading to formation of the membrane attack complex at nodal axolemma, resulting in muscle weakness. Miller Fisher syndrome (MFS) associated with anti-GQ1b IgG antibodies and MMN associated with anti-GM1 IgM antibodies are also mediated by complement-dependent cytotoxicity. Intravenous immunoglobulin (IVIG) is the first-line treatment of GBS and MMN. The therapeutic effect of IVIG is likely to inhibit complement deposition, probably by anti-idiotypic activity, scavenging nascent C3b and specific inhibition of the alternative pathway of C3 convertase assembly, mediated by anti-GM1 IgM antibodies in MMN and anti-ganglioside IgG antibodies in GBS [5].

The study using secreted IgM-deficient mice has shown that secreted IgM lessened the severity of autoimmune pathology related to IgG autoantibodies [6]. However, clinical proof of their effectiveness in human autoimmune disease remains scarce and is even rarer for IgA-enriched preparations (oral and topical applications, no infusions reported). Hints for an immunomodulatory potential of polyclonal IgM in humans were obtained from the preparation (IgM/A-enriched IVIG, Pentaglobin®, Biotest Pharma, Dreieich, Germany), which contained ~12% IgA, ~12% IgM and ~76% IgG, sharing a similar distribution of immunoglobulin isotypes in plasma. In contrast, IgG concentrates consist of >97% IgG, negligible IgM and traces (<2.5%) of IgA. The studies of IgM/A-enriched IVIG in human neurological disease were conducted in Lennox-Gastaut syndrome [7], critical illness polyneuropathy/myopathy [8] and lung transplantation [9]. However, the effectiveness of IgM/A-enriched IVIG has yet to be established in GBS, MFS and MMN.

In vitro and animal studies have shown considerable immunomodulatory effects of polyclonal IgM mediated by the idiotypic network and complement attenuation [10,11]. Polyclonal IgM contains the majority

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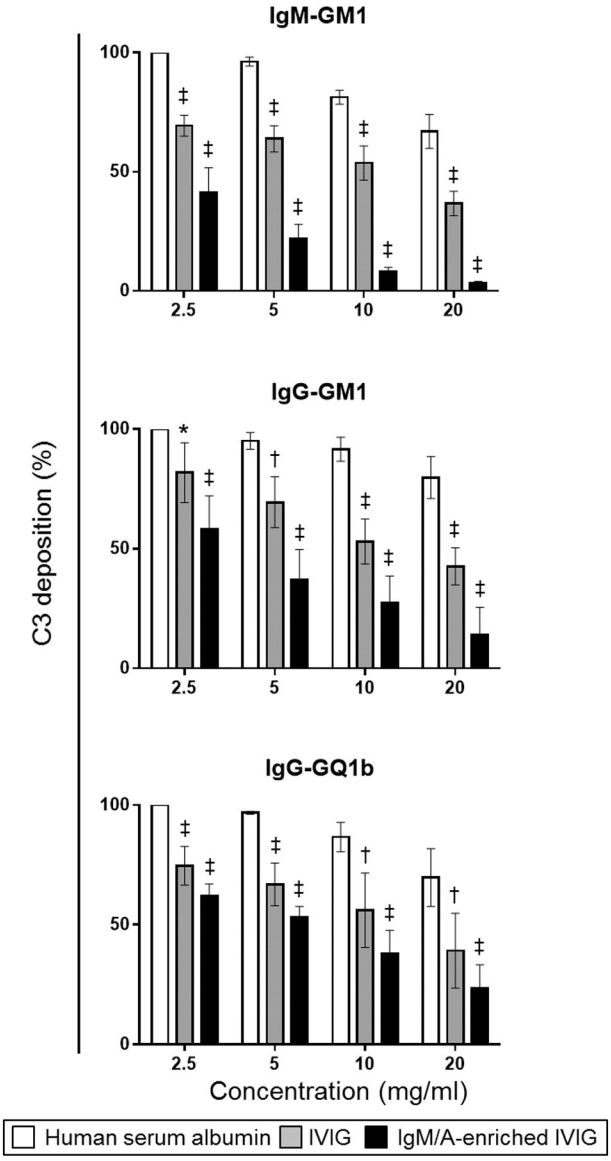

Fig. 1. Dose-dependent inhibitory effects of C3 deposition by IgM/A-enriched intravenous immunoglobulin (IVIG) and standard IVIG at different concentrations (2.5–20 mg/ml). Each experiment was performed at least 3 times for each serum sample with anti-GM1 IgM (n = 5), anti-GM1 IgG (n = 5) and anti-GQ1b IgG (n = 5) antibodies. The values are normalized to each treatment of 2.5 mg/ml of human serum albumin, and data are showed as mean values ± SD. *P < 0.05, †P < 0.005 and ‡P < 0.001 *vs.* treatment with human serum albumin (open bar).

of xeno-reactive antibodies, and these may interfere with heterologous assay systems. In the present study, we compared *in vitro* efficacy in inhibiting complement deposition mediated by immune complexes of ganglioside and anti-ganglioside antibodies using standard IVIG, IgM/A-enriched IVIG, IgM concentrate purified from human plasma and pharmaceutical preparations of IgM and IgA.

2. Materials and methods

2.1. Blood samples

Sera from patients with GBS associated with anti-GM1 IgG antibodies (n = 5), MFS associated with anti-GQ1b IgG antibodies (n = 5) and MMN associated with anti-GM1 IgM antibodies (n = 5) were obtained at Dokkyo Medical University (Tochigi, Japan). The presence of anti-GM1, anti-GQ1b IgG antibodies and anti-GM1 IgM antibodies was determined by enzyme-linked immunosorbent assay as described elsewhere [12]. As a complement source, normal human sera were obtained from five healthy subjects. Written informed consent was obtained from every patient. The study was approved by the Dokkyo Medical University Ethics Committee and National University of Singapore Medical Research Ethics Committee.

2.2. IgM purification

Investigational human IgA was from side fractions of an IgG preparation and was kindly provided by CSL Behring (Bern, Switzerland). Highly purified pentameric IgM was either a gift of CSL Behring (investigational preparation) or was prepared as follows. Pooled human plasma was diluted with phosphate-buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, pH 7.3). After centrifugation, the supernatant was first filtrated with paper filter and then with 0.22 μm-filter. The filtrate was concentrated and dialyzed against PBS. To remove IgG, the solution was applied to Protein G Sepharose (GE Healthcare, Tokyo, Japan) equilibrated with PBS. The flow-through fractions were then applied to KAPTIV-M® (Tecnogen, Piana di Monte Verona, Italy). The column was equilibrated with 50 mM sodium phosphate (pH 7.0) and the column-bound IgM was eluted with 0.1 M acetic acid. The eluted IgM fraction was immediately neutralized and dialyzed against PBS at 4°C. The dialyzed samples were concentrated and purified using Superdex 200 (GE Healthcare, Tokyo, Japan) equilibrated with PBS at the flow rate of 0.5 ml/min. The purity of IgM was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

2.3. Complement deposition assays inhibited by IgM and IgA

We investigated whether standard IVIG (Glovenin-I®, Nihon Pharmaceuticals, Tokyo, Japan), IgM/A-enriched IVIG (Pentaglobin®) and plasma-derived, unmodified IgM and IgA (industrially manufactured preparations) inhibited the complement deposition mediated by the ganglioside and anti-ganglioside antibody immunocomplex. The assays were performed as described elsewhere [13]. The optimal doses of IVIG and dilution rate for each serum sample were determined by the preliminary experiments. Briefly, individual wells of microtitre plates were coated with 5 pmol of each ganglioside. Diluted patients' sera

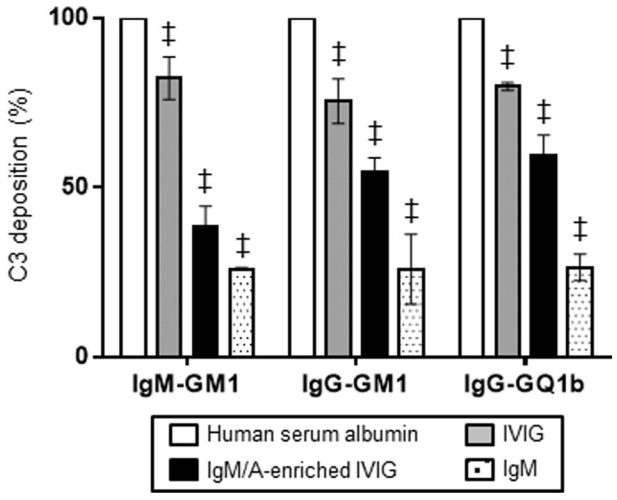

Fig. 2. Comparison of C3 deposition with constant amounts (1 mg/ml) of standard intravenous immunoglobulin (IVIG), IgM/A-enriched IVIG or polyclonal IgM. Each experiment was performed at least 3 times for each serum sample with anti-GM1 IgM (n = 5), anti-GM1 IgG (n = 5) and anti-GQ1b IgG (n = 5) antibodies. The values are normalized to the treatment of human serum albumin, and data are showed as mean values ± SD. ‡P < 0.001 *vs.* treatment with human serum albumin (open bar).