Use of a Human Recombinant Immunoglobulin G1 CH3 Domain as a Probe for Detecting Alternatively Folded Human IgG in Intravenous Ig Products

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ABSTRACT: It has been previously reported that intravenous immunoglobulin (IVIg) contains alternatively folded (aggregation-prone) monomeric immunoglobulin (Ig) G molecules. These alternatively folded IgG molecules may act as precursors for Fc–Fc-mediated dimerization and/or aggregation in IVIg. To study this phenomenon, we set up a fluid-phase binding assay using an acid-shocked (pH 2.5) recombinant human IgG1 CH3 domain as a probe in combination with size-exclusion chromatography. Three IVIg products and a recombinant IgG1 antibody were analyzed. Besides CH3 probe binding to monomeric IgG derived from all IVIg products, the CH3 probe also bound to IgG4 half-molecules. This IgG4 binding could be distinguished from binding to IgG molecules on the basis of molecular weight. In contrast, no CH3 probe binding to IgG from the recombinant IgG1 antibody was observed. After acid-induced aggregation of either IVIg or a recombinant IgG1 antibody, CH3 probe binding to oligomeric complexes was observed, but no longer to monomeric IgG, demonstrating that the alternatively folded monomeric IgG molecules had oligomerized. Our results indicate that the tested IVIg products contain traces of alternatively folded IgG molecules within the "normal" monomeric IgG fraction. Furthermore, we conclude that the fluid-phase binding assay is sensitive to detect these alternatively folded IgG molecules in IVIg. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:978–986, 2012

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INTRODUCTION

The first generation of therapeutic immunoglobulin (Ig) products derived from pooled plasma was initially developed as a replacement therapy for treating patients with primary antibody deficiencies. Because of the presence of a wide range of antibody specificities, the patients were protected against common antigens by administering these Ig products intramuscularly.[1] Intravenous administration sometimes resulted in immediate severe side effects due to activation of the complement system. Barandun et al.[1] discovered that IgG aggregates were causing these severe side effects. By extending the purification process, the IgG aggregates were removed, resulting in the current generation of Ig products [intravenous immunoglobulin (IVIg)].

Despite this adaptation of the IVIg manufacturing process, dimers and larger oligomers (polymers) may still form due to various types of self-interaction.[2] The current generation of IVIg products contain between 5% and 15% dimeric IgG.[3] The most abundant dimer type in IVIg is the Fab–Fab (idiotype–anti-idiotype) dimer,[4,5] yet other types of dimer are also present in IVIg.[6] The origin of these other types of dimer is less clear, but the conditions of the IVIg manufacturing process might play a role. Identification of the origin will allow studying the biochemical and biological properties of these other types of dimer.

Abbreviations used: IVIg, intravenous immunoglobulin; SEC, size-exclusion chromatography; GSH, glutathione

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Ahrer et al.[7] reported that the amount of dimers and aggregates in IVIg depends on the variations of manufacturing process conditions such as pH, flow rate, and mixing speed. Another study showed that at a pH between 3.8 and 4, most aggregates present in IVIg dissociate, whereas a pH treatment below 3.8 results in IgG aggregation due to acid denaturation of IgG.[8] By using a monoclonal mouse anti-rat antibody, Vermeer and Norde[9] demonstrated that this pH-induced denaturation is mainly seen in the Fc region. At a pH below 3, dissociation of CH3 domains in the Fc region is induced, thereby exposing a hidden surface.[10–12] Further analysis revealed that after low pH treatment, the IgG molecules may not all fully return to their native conformation at neutral pH, resulting in a an increased tendency for Fc–Fc-mediated dimerization and/or aggregation.[13] Although the pH during IVIg manufacturing usually remains above pH 4, a recent study by Sakamoto et al.[14] demonstrated that the tested IVIg contains alternatively folded IgG molecules. In their study, a peptide bound specifically to IgG derived from IVIg and to serum IgG that was treated at pH 2.7, indicating the presence of alternatively folded monomeric IgG molecules. In a previous study we demonstrated that pepsin-generated Fc-like fragments (pFc′) of IgG that form upon digestion with pepsin may inhibit acid-induced aggregation of IgG.[15] Such pFc′ fragments comprise the CH3 domains and a small portion of the CH2 domains of IgG.

The aim of this study was to determine whether the scavenging action of these fragments may also be used for the detection of "alternatively folded" monomeric IgG molecules in IVIg, which may act as precursors for (Fc–Fc-mediated) dimerization and/or aggregation. To this end, we used an acid-denatured recombinant human IgG1 CH3 domain as a probe that may bind preferentially to these alternatively folded IgG molecules.

MATERIALS AND METHODS

Cell Line and Media

Escherichia coli strain BL21 (DE3) single competent cells (Novagen, Gibbstown, New Jersey) were grown in Lysogeny broth (LB) medium (Bacto pepton 10 g/L, Gibco Yeast extract 5 g/L, sodium chloride 10 g/L) or on LB agar plate (LB medium + 1.5% Gibco agar). Kanamycin was added to the medium at a final concentration of 30 μg/mL.

Construction of Human IgG1 CH3 Domain

From the human receptor-Ig chimera (sdα; kindly provided by L. Vangelista), a human recombinant IgG1 CH3 domain was generated by using an upstream primer (5′-CGCGGTAGTACTGGGCAGCCCCGAG AACCACAG-3′) and a downstream primer (5′-CCCATGAGATCTTCATTTACCCGGAGACAGGGA-3′). The PCR product was cloned into the pET-40b(+) vector (Novagen). This vector was used for the transformation of *E. coli* strain BL21 (DE31; Novagen) and contained a T7 promoter, a gene for resistance to kanamycin, and a His6-Tag sequence. Selected clones were grown in LB medium containing kanamycin at 37°C. Cells with absorbance at 600 nm of 0.5 were induced with 0.1 mM isopropyl 1-thio-β-D-galactopyranoside followed by culture for 3 h at 37°C. Cells were harvested by centrifugation, resuspended in 50 mM Tris–HCl, pH 8, 50 mM NaCl, 0.1 mg/mL lysozyme (Calbiochem, Gibbstown, New Jersey), and 40 mM imidazole (Biomedicals Inc., Solon, Ohio), incubated at 30°C for 15 min, and sonicated five times on ice for 1 min in a Branson Sonifier 250 (hold, 50% power; VWR, Leuven, Belgium). After centrifugation for 30 min (20,000*g* at 4°C), the supernatant was 0.2-μm filtered and NaCl was added to the final concentration of 0.5 M. The filtered supernatant was applied to a 3-mL column of nickel–nitrilotriacetic acid (NTA)–agarose (Qiagen, Hilden, Germany) and incubated overnight at 4°C. After washing with 20 mM Tris–HCl/300 mM NaCl/10 mM imidazole (pH 8) and elution with 20 mM Tris–HCl/150 mM NaCl/200 mM imidazole (pH 8), the histidine tail was removed from the CH3 protein using the thrombin CleanCleave Kit (Sigma–Aldrich, St.Louis, Missouri). The cleaved His-Tag was removed by nickel–NTA chromatography, and the purified CH3 was stored at–20°C. The purified CH3 domain contained three additional N-terminal amino acids (Gly–Ser–Thr), resulting in a CH3 domain with the following amino acid sequence: GSTGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPS- DIAVEWES-NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR-WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK. The expected molecular weight is approximately 12 kDa for a single CH3 domain.

Ig Products

In this study, three IVIg products were studied: Nanogam® (product A), a 5% IgG solution (Sanquin, Amsterdam, the Netherlands), Multigam® (product B), a 5% IgG solution (CAF-DCF, Brussels, Belgium), and Gamunex (product C), a 10% IgG solution (Talecris, Frankfurt am Main, Germany).

Adalimumab (product D), a monoclonal IgG1 antibody, was obtained from Humira (Abbott, Abbott Park, Illinois). Natalizumab (product E), a monoclonal IgG4 antibody, was obtained from Tysabri (Biogen Idec, Cambridge, Massachusetts). Before use, product E was incubated with 0.5 mM glutathione (GSH) for 30 min at 37°C to obtain a representative fraction of intrachain isomers.[16] The products were stored at 4°C.

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