Antibody Responses in Mice to Particles Formed from Adsorption of a Murine Monoclonal Antibody onto Glass Microparticles

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ABSTRACT: Immunogenicity of therapeutic monoclonal antibodies (mAbs) is a concern because of the effects of anti-drug antibodies (ADAs) on therapeutic efficacy. Particulate matter has been suggested as a potential contributing factor to immunogenicity. In this study, we investigated ADA levels in mice in response to administration of a murine immunoglobulin G (IgG)2c/κ mAb (mAb1) that was generated in C57BL/6J mice. Particles of mAb1 were formed by adsorbing the protein to glass microparticles. Formulations containing microparticles were administered subcutaneously to mice of either the syngeneic strain, C57Bl/6J, or the allogeneic strain, BALB/c. ADA levels were measured using an isotype-specific enzyme-linked immunosorbent assay method. Whereas BALB/c mice showed strong IgG1 and IgG2b responses against both the particulate and native mAb1 samples, adsorption of mAb1 to particles rendered it slightly more immunogenic than its native, soluble form. In BALB/c mice, immunoglobulin M (IgM) was produced after the first week of injections and then faded gradually. In contrast, C57BL/6J mice showed moderate IgM, IgG1, IgG2b, and IgG3 responses to injections of glass particle-adsorbed mAb1. ADA responses were higher in the allogeneic BALB/c mice, which do not produce mAbs of the IgG2c/κ isotype. Thus, the presence of both foreign epitopes and particles may be important in inducing ADA responses. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:78–89, 2014

Keywords: protein; aggregates; immunogenicity; microparticles; adsorption; adjuvant effect; anti-drug antibody (ADA); monoclonal antibody (mAb); ELISA assay; infrared spectroscopy

INTRODUCTION

Therapeutic monoclonal antibodies (mAbs) are used for treatment of numerous diseases and conditions. A major concern with these types of drugs is that they may stimulate immune responses in patients, leading to the formation of anti-drug antibodies (ADAs).[1,2] Despite the development of fully humanized mAbs, there can be a high frequency of ADAs in patients.[3–5] ADAs may block a drug's activity and decrease its efficacy and/or lead to rapid clearance from the blood.[1,6–11] The causes for immunogenicity of mAbs and other therapeutic proteins are not well understood, but it has been suggested that protein aggregates or other particulate contaminants may play a critical role.[1,12–15]

For decades, the immunogenic potential of aggregated proteins has been studied in human clinical studies and in animal experiments. For example, patients receiving antibody therapies from non-human sources typically show an immune response that leads to rapid clearance of the therapeutic. However, Weksler et al.[16] demonstrated that patients could be tolerized to horse anti-human lymphocyte immunoglobulin G (IgG) if particles and aggregates were removed prior to administration by preparative ultracentrifugation. Similarly, Ring et al.[6] showed that removal of aggregates reduced immune response in humans. They showed that heat-aggregated human serum albumin could induce anaphylactic reaction in dogs, whereas ultracentrifuged solutions were well-tolerated.[6] They also showed that ultracentrifuged horse anti-human lymphocytes globulin were well-tolerated in human patients, whereas the aggregate-containing solutions were not.[6] They also observed the same result when patients were administered solutions containing aggregated human serum albumin.[6]

Also, numerous cross-species studies in animals have documented that immunogenicity to IgG's could be modulated by increasing or decreasing the aggregate and particle contents of samples. Dresser[17] succeeded in inducing immunological tolerance in adult CBA mice by injection of bovine gamma-globulin (BGG) that had been centrifuged to remove particulate matter. Biro and Garcia[18] observed the same result when they tested aggregated and aggregate-free (obtained by preparative ultracentrifugation) human gamma-globulin (HGG) in rabbits. They reported that heat-aggregated HGG is an excellent antigen for rabbits, whereas aggregate-free HGG is not capable of inducing the primary response. Gamble[19] and Sassen et al.[20] studied

Abbreviations used: mAb, monoclonal antibody; ADA, anti-drug antibody; ELISA, enzyme-linked immunosorbent assay; BGG, bovine gamma-globulin; PBS, phosphate-buffered saline; Trp, tryptophan; TD, T-dependent.

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the immunogenicity of HGG in mice for the initiation of primary and secondary antibody responses. They reported that heat-treated, but not untreated, HGG has potential to initiate a primary antibody response in mice. In the work of Sassen et al.,[20] no difference was reported between heat-treated and untreated HGG for their capacity to induce a secondary response; however, at limited antigen dose levels, heat-treated HGG was observed to induce larger secondary responses than untreated HGG.[20]

One mechanism by which particles of therapeutic proteins may form is by adsorption to microparticulate contaminants; these may derive from several sources. For example, Tyagi et al.[21] showed that stainless steel nanoparticles shed from filling pumps can nucleate particles of mAbs. Also, glass nanoparticles and microparticles have been shown to induce protein particles from mAbs and other therapeutic proteins.[22] Glass particles shed from containers (vials, syringes, and cartridges) have been responsible for numerous recent product recalls because of the visible particulate matter in drug products, including therapeutic proteins.[23,24] Moreover, one recent study showed that glass particles can be potent adjuvants. Fradkin et al.[25] demonstrated that murine growth hormone adsorbed to microparticles obtained from ground glass syringes (nonsiliconized) stimulated a robust immune response in mice, and that particle-free samples were not immunogenic. Van Beers et al.[26] found that human interferon-beta adsorbed to the same glass particles slightly enhanced immunogenicity of the protein in transgenic mice.

Considering the immunogenicity potential of therapeutic antibodies and the potential role of pharmaceutically relevant glass particles as adjuvants, our first goal in this study was to test the hypothesis that immunogenicity of a monoclonal antibody would be enhanced by adsorption to glass microparticles. For comparison, the mAb1 was adsorbed onto aluminum hydroxide, an adjuvant commonly used in vaccine formulations. To conduct this investigation, we chose a murine monoclonal IgG2c/κ (mAb1) generated in C57BL/6J mice, which would be expected to be minimally immunogenic in its soluble native form when administered to mice of this same strain.

The second goal of this study was to investigate the immunogenicity of mAb1 in BALB/c mice, which do not produce IgG2c/κ antibodies. It has been shown to be very challenging to make BALB/c mice unresponsive to ultracentrifuged human or BGG compared with C57BL/6J mice.[27–29] It was reported by Golub and Weigle[29] that when even an extremely small amount of ultracentrifuged HGG (as low as 50 μg) was introduced to C57BL/6J mice, it could induce immunological tolerance, whereas to make BALB/c mice tolerant, a dose as high as 10 mg was needed. They postulated BALB/c mice may process the trace amount of aggregates remaining in samples efficiently, whereas C57BL/6J mice may not.[29] However, when the trace amount of aggregates was removed by salt fractionation, mice from both strains became unresponsive to small doses of HGG. Similar results were observed by Das and Leskowitz[28] when single doses of ultracentrifuged BGG were administered to BALB/c and DBA/2 mice. In that study, they showed that DBA/2 mice became tolerant at doses of 0.2 mg, whereas BALB/c mice required more than 20 mg to induce tolerance. Also, BALB/c mice do not have a gene to produce the IgG2c immunoglobulin isotype.[30,31] There is a 16% difference in amino acid sequences between IgG2c and IgG2a.[30] Therefore, we should be able to avoid interference between injected IgG2c and the endogenous immunoglobulin in the bioanalytical assay.

Samples of adsorbed mAb1 on glass microparticles were administered by subcutaneous injections in the two mouse strains. Sera were collected and evaluated for levels of immunoglobulin M (IgM) and G (IgG1, IgG2b, and IgG3) ADAs against mAb1. An enzyme-linked immunosorbent assay (ELISA) coupled with an acid-dissociation step[22] was used for ADA detection.

MATERIALS AND METHODS

Materials

All chemicals used in this work were of reagent grade or higher quality. Sterile water for injection was used entirely and all materials used for injection were of USP grade. mAb1, a mouse monoclonal antibody (IgG2c/κ, 145 kDa) against tumor necrosis_factor alpha (TNF-a), was provided by AbbVie Bioresearch Center (Worcester, Boston, Massachusetts). HRP-goat anti-mouse IgM, IgG1, and IgG2b were purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, Pennsylvania) and HRP-rabbit anti-mouse IgG3 was purchased from Fitzgerald Industries International Inc. (Acton, Massachusetts). Alum adjuvant (Alhydrogel®) was purchased from Brenntag Biosector (Frederikssund, Denmark), L-Histidine was obtained from RPI (Prospect, Illinois), sucrose was obtained from Sigma–Aldrich (St. Louis, Missouri), citric acid, tri-sodium salt dehydrate was obtained from ACROS ORGANICS (Fair Lawn, New Jersey), and sulfuric acid was obtained from Mallinckrodt (Hazelwood, Missouri). The other chemical reagents were purchased from Fisher: acrylamide from Fisher BioReagents (Pittsburgh, Pennsylvania), urea from Fisher Scientific (Fair Lawn, New Jersey), sodium chloride, polysorbate20, and phosphate-buffered saline (PBS; 10× solution, DNase–RNase- and protease-free, 1.37 M sodium chloride, 0.027 potassium chloride, and 0.119 phosphate buffer) from Fisher Scientific (Pittsburgh, Pennsylvania), and TMB from Thermo Scientific (Rockford, Illinois).

METHODS

Sample Preparation and Characterization

Model mAb

Monoclonal IgG2c/k antibody (mAb1) was received as a frozen solution at a concentration of 24 mg/mL in 15 mM histidine buffer (pH 6.0). Size-exclusion chromatographic analysis of the thawed solution showed that the mAb1 preparation contained 97.3% monomer, 2.1% low-molecular-weight species, and 0.6% high-molecular-weight species. The original thawed sample was aliquotted into 1-mL tubes under aseptic conditions and stored in a freezer at −80°C until further use.

Preparation and Characterization of Ground Glass Microparticles

Glass shards from vials (5cc, Type 1 Glass, USP/PhEur, nontreated; Schott Inc., Syracuse, New York) were ball-milled with zirconia (ZrO_2) medium, and the resulting powders were sieved through a 45-μm screen. The sieved particles were washed with 1 M ammonium sulfate and thoroughly rinsed with purified water to dissolve and wash away exposed oxides.[32] Finally, the particles were dried under vacuum at 100°C for 1 h, and the

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