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Importance of IgG2c isotype in the immune response to β-amyloid in amyloid precursor protein/transgenic mice

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Abstract

A careful analysis of the immune response to immunization of amyloid precursor protein/transgenic (APP/Tg) mice with β-amyloid (Aβ) may provide insights into why a subset of the patients in a clinical trial receiving Aβ-immunotherapy developed encephalomyelitis. Characterization of isotypic immune responses have been reported in different APP/Tg models. In these studies the relative ratios of IgG1 to IgG2a anti-Aβ antibodies has been used as an indirect measure of T helper 1 (Th1) and Th2 types immune responses. However, it has previously been shown that certain strains of mice, C57Bl/6, C57Bl/10, SJL, and NOD, have an IgG2c rather than an IgG2a gene. Since a substantial number of Aβ-immunization studies rely on APP/Tg mice that have at least one parental C57Bl/6 strain, we have investigated whether antibodies specific for IgG2a can be used for characterization of antibody isotypes in APP/Tg2576 mice. Our results suggest that APP/Tg2576 and major histocompatibilty complex-matched parental strains are not expressing IgG2a, producing instead IgG2c anti-Aβ antibodies.

Keywords: Amyloid precursor protein/transgenic mice; IgG2a and IgG2c genes; Ig isotypes; $A\beta_{42}$

Recently, immunotherapy as a possible treatment for Alzheimer's disease (AD) has received considerable attention. It has been demonstrated that active immunization of amyloid precursor protein/transgenic mice (APP/Tg) mice with fibrillar $A\beta_{42}$, as well as passive immunization with anti-Aβ antibodies significantly reduce amyloid plaque deposition, neuritic dystrophy, and astrogliosis in APP/Tg mouse models of AD [1,3,8,13,14]. These data indicate that anti-Aβ antibodies play a major role in the clearance of Aβ deposition from the brains of APP/Tg mice.

Recently, analyses of the relative levels of IgG1, IgG2a, IgG2b, and IgG3 anti-Aβ antibodies have been reported for several APP/Tg mouse models, as well as in a presenilin-1 transgenic (PS1/Tg) and wildtype C57Bl/6 or Balb/c mice immunized with fibrillar $A\beta_{42}$ [4,10,12,16,17]. In many of these studies commercial antibodies raised against Balb/c IgG2a myeloma proteins were used to detect the levels of anti-Aβ IgG2a antibody. However, it has been previously reported that the IgG2a gene is deleted in C57Bl/6, C57Bl/10, SJL, and NOD mice, which have an IgG2c gene instead [9,11]. Accurately measuring the level of IgG2a production in the Aβ-immunized mice is critical because the ratio of IgG1 to IgG2a is routinely used as an indirect measure of the relative contribution of T helper 1 (Th1) versus T helper 2 (Th2) immune responses. The significance of the type of cell-mediated response induced (Th1 versus Th2) can be appreciated by comparing the functional outcomes of these two distinct pathways [5]. Importantly, in a substantial number of immunological studies APP/Tg2576 (strain background of C57Bl/6 and SJL) or PDAPP mice (strain background of SW and C57Bl/6xDBA/2) have been used. Accordingly, we examined a production of IgG2c anti-Aβ antibodies in APP/Tg2576 mice and their parental strains. As a positive control for the IgG2a isotype we have used Aβ immunized Balb/c mice that have IgG2a gene.

Six to eight week-old APP/Tg2576 (UCI Transgenic Mouse Facility), Balb/c, C57Bl/6, SJL and B6SJLF1 mice (Jackson Lab.) were housed in the animal facility at UCI in a

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temperature and light-cycle controlled facility, and their care was under the guidelines of the NIH and UCI. In order to investigate the differences in the immune response in mice of different major histocompatibilty complex (MHC) haplotypes, we immunized APP/Tg2576 mice (H2 bxs), as well as MHC-matched B6SJLF1 non-transgenic strain, and mice of the two parental wildtype strains - C57Bl/6 (H2b) and SJL (H2s), with $A\beta_{42}$ peptide synthesized at UCI. Balb/c (H2d) mice were included to experiment as a control. The fibrillar $A\beta_{42}$ peptide was prepared as previously described [14], and this immunogen was mixed with Alum or CFA/IFA (Complete/Incomplete Freund's Adjuvant). Each mouse was injected subcutaneously with 100 μg (100 μl) of peptide. After the first immunization, three boosts were performed at 2 weeks intervals. Individual mice from each experimental group were bled 8 days after last boost, and sera were used in enzyme-linked immunosorbent assay (ELISA) for detection of binding to $A\beta_{42}$ peptide. Briefly, 96-well plates (Immunolon II, Dynatech) were coated with 2.5 μM of soluble $A\beta_{42}$ in Bicarbonate coating buffer (pH 9.7) and incubated overnight at 4 °C. Then wells were washed and blocked with 3% non-fat dry milk in Tween-20–Tris buffer (TTBS) solution for 1–2 h at 37 °C. After washing the wells, primary sera from experimental and control mice (individual animals or pooled, as designated below) were added in duplicate at the indicated dilutions. After incubation (2 h, 37 °C) and washing, anti-mouse IgG conjugated with horseradish peroxidase (HRP) antibodies (Jackson Lab.), were added to detect total Ig. To detect mouse IgG1, IgG2a, IgG2b, and IgM isotypes, we have used appropriate rabbit anti-mouse Ig-subclass-specific secondary antibodies (Zymed) followed by incubation with goat anti-rabbit IgG HRP-conjugated antibodies (Zymed). To detect mouse IgG2c isotype, we used biotin-conjugated mouse-anti-mouse $IgG_{2a}b$ (*Igh-1b*) monoclonal antibody (PharMingen), followed by incubation with HRP-conjugated streptavidin (Vector Laboratories). To develop the reactions, OPD substrate solution (0-phenylendiamine in 0.05 M phosphate-citrate buffer, pH5.0, Sigma) was added, and plates were read at an emission wavelength of 405 nm using in a ThermoMax Microplate Reader (Molecular Devices, CA).

First, we analyzed total Ig production of anti-$A\beta_{42}$ antibodies in individual mice (Fig. 1a,b). Immunization of APP/Tg mice ($n = 6$) with $A\beta_{42}$ plus Alum induced the lowest, whereas MHC-matched non-transgenic B6SJLF1 animals induced the highest production of anti-$A\beta_{42}$ antibodies (Fig. 1a). Parental SJL strain ($n = 4$) and Balb/c animals ($n = 8$), which are not MHC-matched with APP/Tg or B6SJL mice, generated anti-$A\beta_{42}$ antibody response levels similar to that of B6SJLF1. At the same time, immune response was significantly impaired not only in APP/Tg 2576 animals, as was demonstrated earlier [12], but also in wildtype C57Bl/6 mice ($n = 8$). Thus, two parental strains of APP/Tg mice C57Bl/6 and SJL responded differently to immunization with $A\beta_{42}$ formulated in Alum. Immunization of APP/Tg ($n = 6$), C57Bl/6 ($n = 8$), B6SJL ($n = 5$) and Balb/c ($n = 8$) mice with antigen formulated in CFA/IFA induced higher anti-$A\beta_{42}$ antibody production compared to immunization with antigen plus Alum (Fig. 1b). However, the enhancement of antibody production was statistically significant only in C57Bl/6 animals ($P = 0.0002$).

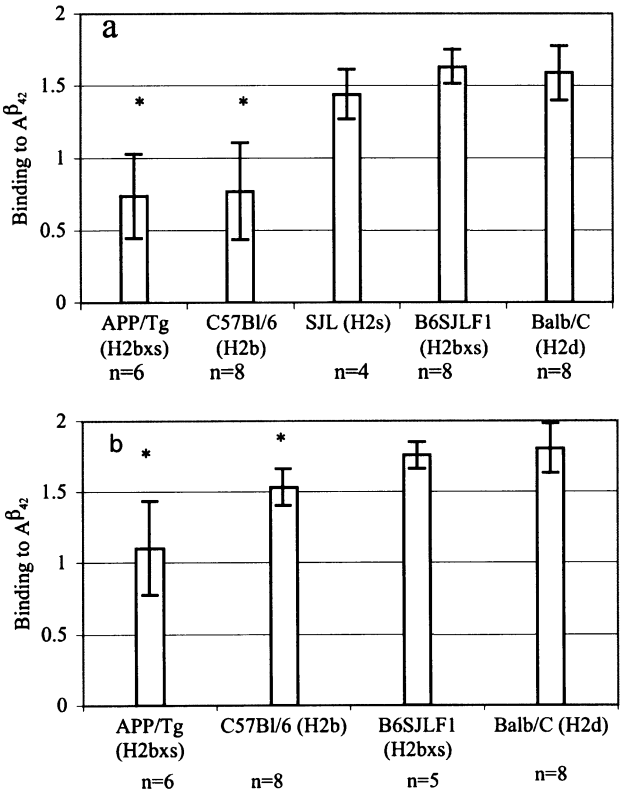

Fig. 1. (a,b) Anti-$A\beta_{42}$ immune responses in APP/Tg 2576 animals and wildtype mice of different haplotypes immunized with fibrillar Aβ peptide formulated in Alum (a) or CFA/IFA (b). Total Ig was detected in serum (dilution 1:500) from individual mice, (n = number of animals) after three boosts. *The Student's unpaired t-test analysis showed with statistical significance ($P < 0.01$) that APP/Tg 2576 and C57Bl/6 mice antibody responses were lower than that of mice of other haplotypes in case of Alum (a) and CFA/IFA (b). C57Bl/6 mice responded significantly ($P = 0.0002$) better after immunization with CFA/IFA (b) than with Alum (a).

Previously, it was demonstrated that $A\beta_{42}$ immunization generated mostly IgG1, IgG2a, and IgG2b antibodies in APP/Tg2576, PDAPP, APP-PS1/Tg, PS1/Tg, and C57Bl/6 animals [4,10,12,16,17]. Yet, since IgG2a gene is deleted in C57Bl/6 and SJL mice, which instead have IgG2c gene [9,11], we used both anti-γ2a antiserum and anti-$\gamma 2a^b$ monoclonal antibodies for detection of IgG2a and IgG2c isotypes, respectively. Anti-γ2a antiserum recognized IgG2a antibodies in pooled sera from Balb/c mice immunized with $A\beta_{42}$ formulated either in Alum or CFA/IFA. Again, CFA induced much higher level of IgG2a anti-$A\beta_{42}$ antibody production (Fig. 2a,b). More importantly, this antiserum almost did not recognize IgG2a antibodies in the pooled sera from APP/Tg 2576, C57Bl/6, SJL, and B6SJLF1 mice regardless of adjuvant used. Conversely,

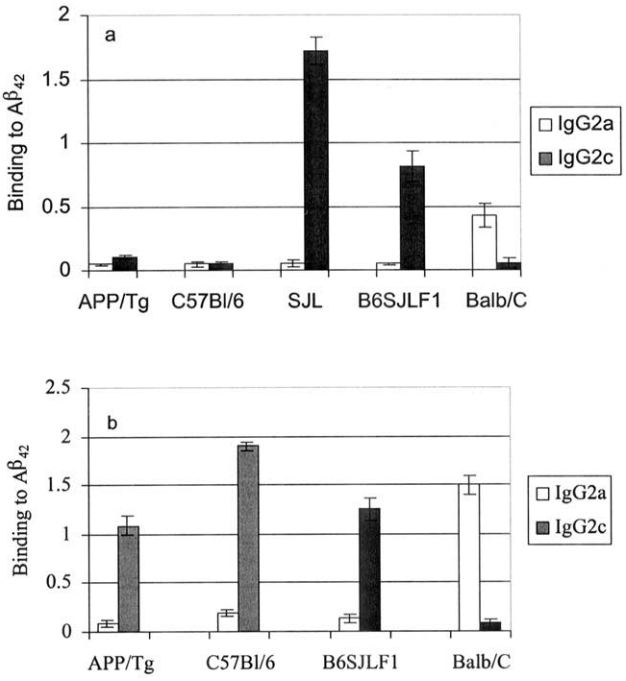

Fig. 2. (a,b) Anti-$A\beta_{42}$ IgG2a and IgG2c antibody responses in APP/Tg 2576 animals and wildtype mice of different haplotypes immunized with fibrillar Aβ peptide formulated in Alum (a) or CFA/IFA (b). Pooled serum diluted 1:500 have been used for ELISA. This experiment was repeated twice with similar results.

anti-$\gamma 2a^b$ monoclonal antibodies recognized anti-$A\beta_{42}$ antibody of IgG2c isotype in C57Bl/6, SJL, B6SJLF1, and APP/Tg2576 mice, but not in Balb/c mice immunized with immunogen mixed with Alum or CFA/IFA (Fig. 2a,b).

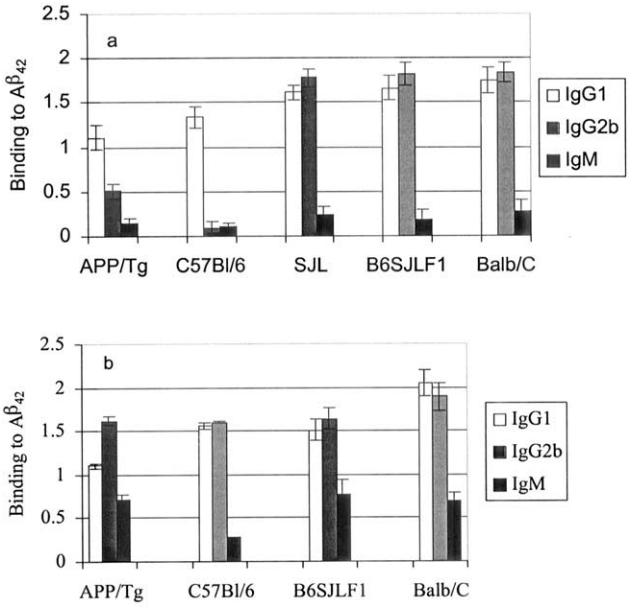

Fig. 3. (a,b) Anti-$A\beta_{42}$ IgG1, IgG2b, and IgM antibody responses in APP/Tg 2576 animals and wildtype mice of different haplotypes immunized with fibrillar Aβ peptide formulated in Alum (a) or CFA/IFA (b). Pooled serum diluted 1:500 have been used for the ELISA. This experiment was repeated twice with similar results.

In our experiments, we also measured production of IgG1, IgG2b and IgM antibodies after immunization with $A\beta_{42}$ formulated in CFA/IFA or Alum (Fig. 3a,b). Both CFA/IFA and Alum have induced significant levels of IgG1 anti-$A\beta_{42}$ antibodies in all types of mice. Both adjuvants also enhanced IgG2b anti-$A\beta_{42}$ antibody production in immunized B6SJLF1, Balb/c, and SJL mice. However, the level of IgG2b antibodies in APP/Tg 2576 and C57Bl/6 mice were substantially different, dependent on adjuvants. Immunization with antigen mixed with Alum did not induce IgG2b in C57Bl/6 and induced low level of antibodies of this isotype in APP/Tg 2576 mice. On the contrary, immunization with fibrillar $A\beta_{42}$, emulsified in CFA/IFA, induced very potent IgG2b immune responses in these mice. In addition, all animals immunized with antigen formulated in CFA/IFA but not in Alum, generated prolonged low levels of IgM antibodies to $A\beta_{42}$ (Fig. 3a,b).

Our results demonstrate that anti-IgG2a antiserum is not suitable for analysis of antibody isotypes in APP/Tg2576 and MHC-matched wildtype animals with the *Igh1-b* allele because there was very little crossreactivity with IgG2c isotype. Therefore it is necessary to use anti-IgG2c specific antibodies for detection of isotypic immune responses to $A\beta_{42}$ immunogen in APP/Tg2576 mouse model of AD [7]. In addition, because other mouse models of AD, such as PDAPP [6], APP Swedish [15] and PS1-APP/Tg [2] mice have C57Bl/6 background along with other haplotype/s, it will be essential to use both anti-IgG2a and anti-IgG2c antibodies in order to measure correctly isotypic immune responses to Aβ immunization.

The subclass of Ig that is induced after immunization is an indirect measure of the relative contribution of Th2-type cytokines versus Th1-type cytokines in the immune response [5]. More specifically, the production of IgG1-type antibodies is primarily induced by Th2-type cytokines, whereas production of IgG2a-type antibodies reflects the involvement of Th1-type cytokines. Therefore, higher IgG1/IgG2a ratio points toward Th2-type of immune response, whereas lower IgG1/IgG2a ratio serves as an indicator of the Th1-type immune response. In our current study, as well as in our previous experiments (paper submitted), immunizations of Balb/c with Alum induced primarily a Th2-type of immune response, whereas antigen mixed with CFA/IFA induced a predominately Th1-type of humoral response (Fig. 2,3). Further studies will clarify whether or not IgG1/IgG2c ratio will also be indicative for Th1 and Th2 immune responses in APP/Tg2576 mice.

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