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Short communication

Possible allelic structure of IgG2a and IgG2c in mice

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ABSTRACT

Earlier publication suggested that IgG2a and IgG2c (coding for Igh-1a and Igh-1b) are organized in tandem on the same chromosome as two distinct loci in mice. Our data suggest that IgG2a and IgG2c are not physically linked on the chromosome and are allelic – single locus in majority strains of mice. In another word, IgG2b–IgG2c–IgG2a haplotype proposed by Morgado et al. (1989) may exist in some strains of mice, but IgG2b–IgG2a and IgG2b–IgG2c are likely to be most common haplotypes in mice. Therefore, inbred mice may produce different IgG2a isotypes dependent on their origin (strain); C57B/6 and SJL mice secrete IgG2c while NMRI and DBA/2 mice secrete IgG2a only. The situation is more complicated for Swiss Webster mice (outbred) and Alzheimer's disease transgenic (AD/Tg) mice with multi-genetic backgrounds; mice may secrete only IgG2a, or IgG2c, or both IgG2a and IgG2c. IgG2a and IgG2c likely have different immune profile (response, immune-decoration) in mice due to their divergence of protein sequence. If antibodies based on IgG2a (or IgG2c) are used in chronic studies for preclinical evaluation of antibody efficacy, characterization of IgG2a isotypes in advance becomes critical in the design of such biopharmaceutical projects in order to avoid immune response.

1. Introduction

Mouse immunoglobulin heavy chain constant region are encoded by several tightly linked genes which are inherited as Igh haplotypes (Sikorav et al., 1980; Yamawaki-Kataoka et al., 1981). All of these isotypic genes are present in multiple allelic forms. An earlier publication (Morgado et al., 1989) suggested that Igh-1a and Igh-1b could derive from two distinct isotypes, IgG2a and IgG2c, which are organized in tandem on the same chromosome as two distinct loci in MAI strain (a in Fig. 1) and BALB/c and C57B/6 mice carry different IgG2a isotypes. Furthermore, expression of only IgG2c in C57B/6 and SJL mice was discovered and was assumed to be caused by a deletion of IgG2a gene in those strains (Martin et al., 1997, b in Fig. 1). Information from Ensembl database (http://www.ensembl.org) is inconclusive due to the complexity of the cluster and confusing names. IgG2c (coding for immunoglobulin heavy chain 1b-serum, Igh-1b) was located to the cluster where immunoglobulin heavy chain 1a (Igh-1a, coding by IgG2a) was given as official name and official symbol (Ensembl database, http://www.ensembl.org/index.html). However, only IgG2c genomic sequence, not the IgG2a, is shown in the cluster from Ensembl database. That is likely due to the strain of mice used for establishing the database (C57BL/6J). The data here indicate that in addition to the previous report of a tandem duplication involving IgG2c–2a, some mice have single copies of the IgG2a or IgG2c gene on individual alleles.

2. Materials and methods

In total, 28 APP/PS1 transgenic mice (Dave Morgan, University of South Florida) and 36 different strains of mice (4 of each C57B/6, NMRI, SJL, DBA/2 and 20 of Swiss Webster mice from Taconic) are collected for analysis. Genomic DNA was isolated using Maxwell 16 tissue DNA purification kit (Promega Corporation, Madison, USA). APP/PS1 transgenic mice (double transgenic mice) are obtained by crossing heterozygous APP and PS1 mice (single transgenic mice) which are originally generated from C57BL6 mice. To avoid high incidence of death rates in new offspring, APP/PS1 transgenic mice from Dave Morgan lab in this study were bred with other strains DBA/2 or Swiss Webster.

2.1. Polymerase chain reaction (PCR)

Allelic specific PCR based on divergences between IgG2a and 2c genes were designed to amplify all the coding region of IgG2a and 2c gene selectively (Morgado et al., 1989, Fig. 2). 50 ng of genomic DNA were used for PCR amplification. PCR was performed in a total volume of 25 μl, including 100 nM of each PCR primer, 80 μmol dNTP, 2.5 mM Mg and 1 unit AmpliTaq Gold in 1× PCR

Abbreviations: AD, Alzheimer's disease; PCR, polymerase chain reaction.

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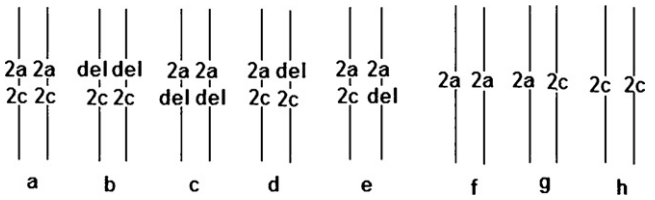

Fig. 1. Physical position of IgG2a and IgG2c on chromosome 12 in mouse. (a–e) Originally proposed position of IgG2a and IgG2c and possible combination in mice. (f–h) IgG2a and IgG2c are allelic (suggested by us).

buffer. Reaction conditions were the following: 95 °C for 5 min for activation of AmpliTaq Gold, followed by 40 cycles of amplification (95 °C for 40 s, 55–62 °C for 40 s and 72 °C for 1 min).

2.2. Sequencing

PCR products were purified with Qiaquick Multiwell PCR purification kit. 10–25 ng of purified PCR products were used for cyclic sequencing with BigDye terminator kit version 3.1 and followed by 30 cycles (96 °C for 30 s, 50 °C for 15 s and 60 °C for 4 min) on PCR machine. Sequencing reactions were purified with DyeEx 96 kit according to the protocol providing by manufacture and were run on an ABI 3130xl Genetic analyzer. Sequence traces were analyzed for polymorphisms after assembly using DNA Star version 5 packages.

3. Results and discussion

Allelic specific PCR based on divergences between IgG2a and 2c genes were designed to amplify the coding region of IgG2a and 2c gene selectively (Morgado et al., 1989, Fig. 2). In total, 64 mice were collected for sequence analysis, including 28 APP/PS1 transgenic mice (Dave Morgan, University of South Florida) and 36 mice from five different strains of mice (4 C57B/6, 4 NMRI, 4 SJL, 4 DBA/2 and 20 Swiss Webster from Taconic). In this way, all the coding regions of IgG2a and 2c genes were amplified and sequenced completely. Sequencing data showed that the IgG2a gene is highly polymorphic between the NMRI, DBA/2 and Swiss Webster strains. No coding polymorphism within the IgG2c gene was identified in all our analyzed mice, which is in concordance with the previous report (Martin et al., 1997). Only the IgG2a gene was found in DBA2 and NMRI strains while the IgG2c gene was present in C57B/6 and SJL strains (inbred mice, Table 1). Both IgG2a and IgG2c were found to be present in Swiss Webster and APP/PS1 transgenic mice (Table 1). Out of 20 Swiss Webster mice, 13 mice had IgG2a/IgG2a genotype, 6 mice had IgG2a/IgG2c and 1 mouse had IgG2c/IgG2c (Table 1). If the IgG2a haplotype origin was considered for, three alleles of IgG2a: NMRI_IgG2a, DBA/2_IgG2a and Swiss Webster_IgG2a, were found. In this way, the 28 APP/PS1 transgenic mice can be divided into five groups. 13 APP/PS1 transgenic mice had NMRI_IgG2a/IgG2c genotype, 6 mice had NMRI_IgG2a/DBA/2_IgG2a, 3 mice had DBA/2-IgG2a/IgG2c, 3 mice had NMRI-IgG2a/NMRI-IgG2a and 3 mice had IgG2c/IgG2c (Table 1). Heterozygosity of IgG2a was observed only in the mice with IgG2a/IgG2a genotype while no heterozygosity of IgG2a was detected in any mice with IgG2a/IgG2c genotype (6 Swiss Webster outbred mice and 16 APP/PS1 transgenic mice). These data strongly suggest, only one allele of IgG2a is present in the mice with IgG2a/IgG2c genotype. If the IgG2a and IgG2c are linked in tandem on the same chromosome as previous report (Morgado et al., 1989, a in Fig. 1), heterozygous IgG2a (i.e. two copies of IgG2a) should be observed in some mice with IgG2a/IgG2c genotype (a and e in Fig. 1). However, only two alleles of IgG2a or IgG2c or IgG2a and IgG2c (IgG2a/IgG2a, IgG2a/IgG2c and IgG2c/IgG2c) were detected in the 64 investigated mice including outbred strains. These findings are hardly explained by IgG2a deletion in C57B/6 and SJL strains and IgG2c deletion in other strains of mice, such as NMRI and DBA/2 strains, occurring in parallel by chance (Petrushina et al., 2003, b and c in Fig. 1). A likely explanation for this observation is that IgG2a and IgG2c are not physically linked on the chromosome and are allelic-single locus (f–h in Fig. 1) in majority strains of mice. In another word, IgG2b–IgG2a and IgG2b–IgG2c are likely to be most common haplotypes (physical position) in mice. IgG2b–IgG2c–IgG2a haplotype proposed by Morgado et al. (1989) as evolution model may exist in some strains of mice since tandem IgG2a genes generated by spontaneous, unequal sister chromatid exchange in somatic cells during mitotic recombination between homologous chromosome can be a germ line events as well (Tilley and Birshtein, 1985).

Since antibodies as potential therapeutics, e.g. for Alzheimer's disease (AD), have gained intense attention in recent years, chronic studies in mice have become more and more valuable as preclinical research in order to evaluate antibody efficacy (Seabrook et al., 2004; Pul et al., 2011). Divergences of protein sequence between IgG2a and IgG2c (15% in protein sequence) indicated that IgG2a

Table 1
Genotyping information of IgG2a isotypes in different strains of mice.

Strains	Genotype	Number of mice
DBA2	IgG2a/IgG2a	4
NMRI	IgG2a/IgG2a	4
C57B6	IgG2c/IgG2c	4
SJL	IgG2c/IgG2c	4
Swiss Webster	IgG2a/IgG2a	13
	IgG2a/IgG2c	6
	IgG2c/IgG2c	1
APP/PS1 transgenic	N_IgG2a/D_IgG2a[a]	6
	N_IgG2a/N_IgG2a	3
	N_IgG2a/IgG2c	13
	D_IgG2a/IgG2c	3
	IgG2c/IgG2c	3

[a] N_IgG2a: NMRI strain origin; D_IgG2a: DBA/2 strain origin. BALB/c mice are not investigated in this study, but they likely have IgG2a gene only according to earlier publications (Martin et al., 1997; Morgado et al., 1989).

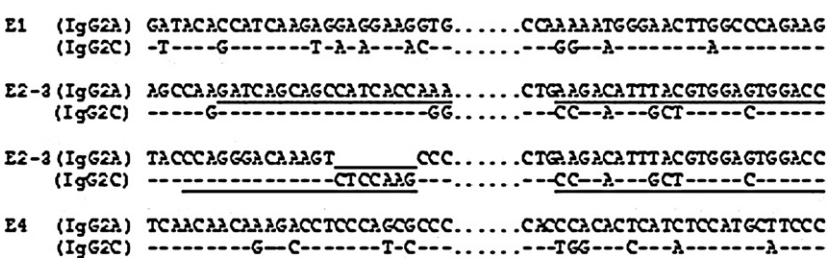

Fig. 2. Sequence comparison of IgG2a and 2c in the regions where allelic specific PCR primers are underlined (forward primers for E2–3 are located in different regions of IgG2a and IgG2c).

and IgG2c likely have different immune profile (response, immune-decoration) in mice. Mice lacking IgG2a could theoretically lack tolerance to IgG2a and mount an immune response to injected IgG2a antibodies, neutralize and increase plasma clearance, which will interfere with the conclusion of study. The presence of IgG2a isotypes in various mouse strains has practical implications for chronic treatment with murine antibodies. It is important to consider whether the Ig-haplotype of the receiving mice match to the isotype of the injected antibodies in order to avoid an immune response in a preclinical testing model. Inbred mice, such as C57B/6, NMRI, SJL and DBA/2 strains, produce different IgG2a isotypes; C57B/6 and SJL mice secrete IgG2c while NMRI and DBA/2 mice secrete IgG2a. The situation becomes more complicated for Swiss Webster mice (outbred); mice may secrete only IgG2a, or IgG2c, or both IgG2a and IgG2c. Most of transgenic mice models for AD (APP/Tg2576, APP/PS1, etc.) have multi-genetic backgrounds, therefore, AD/Tg mice may secrete only IgG2a, or IgG2c, or both IgG2a and 2c as Swiss Webster mice. If antibodies based on IgG2a (or IgG2c) are used in chronic studies for preclinical evaluation of antibody efficacy, characterization of IgG2a isotypes in advance becomes critical in the design of such biopharmaceutical projects in order to avoid immune response.

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