

Cross-reactivity of anti-phosphorylcholine antibodies to neuromuscular blockers in a murine model of immunization

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Abstract

Hypersensitivity reactions to curare-like neuromuscular blocking agents (NMBA) used in anesthesiology are more frequent in females and often occur at the first exposure to these drugs. To evaluate the antibody response to a hapten sharing the same allergenic epitope with NMBA in a murine model of immunization with Nitrophenylphosphorylcholine (NPPC) coupled with Keyhole Limpet Hemocyanin (KLH). BALB/c mice from both sexes were intraperitoneally immunized with NPPC–KLH with alum and boosted twice, on 7 and 14 days. The antibodies were tested for specificity to PC and for cross-reactivity to the haptens, NPPC and PC, as well as to the NMBAs. Mice immunized with NPPC–KLH produced anti-PC antibodies mainly of IgM, IgG1 and IgG3 isotypes, at similar levels in both sexes. Different affinities for the haptens were detectable between isotypes, with anti-PC IgG3 antibody reactivity mostly related to the choline portion of the NPPC hapten. When comparing among sexes, females developed greater IgG2 affinity to the hapten than males. Cross-reactivity to NMBAs was predominant among the anti-PC IgG3 antibodies, mainly in females achieving 75% of inhibition with 16 mM of suxamethonium, while other isotypes achieved up to 30% and was absent for IgE. The investigation of antibody isotypes regarding sensitization to choline-derived structures could contribute to understanding the differential ability of females to produce antibodies that are cross-reactive with NMBAs.

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1. Introduction

Allergic reactions to drugs are a common problem in clinical practice. Immediate reactions are those associated with greater risk. IgE-mediated reactions occur after prior patient sensitization by the drug or by a structural ana-

logue and are named anaphylactic reactions. Anaphylactic-type reactions that occur in the perianesthetic period, despite their rarity, are associated with high mortality, being one of the main causes of death attributable to secondary reactions to drugs [1,2]. These anaphylactic reactions to NMBAs have been reported in 1/6000–1/20,000 of general anesthetics [3]. Considerable differences in prevalence exist between countries; in France, the estimated incidence of IgE-mediated anaphylactic incidence is 1/13,000 anesthetic procedures [4].

The majority of anaphylactic reactions are thought to be IgE-mediated [5,6]. These reactions are mostly (70% to 75%) due to the use of NMBAs and are nine times

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more frequent in females than in males [7]. Of note is that patients sensitive to NMBAs frequently present adverse reactions at the first exposure to these drugs and a high rate of cross-reactivity to all compounds of the group [8,9]. The quaternary ammonia group of the choline structure functionally characterizes the NMBAs and is probably the sensitizing epitope [10]. The chemical structures of the NMBAs are at least bivalent for the quaternary ammonium epitope. They must present the capacity to cross-link the IgE antibody on sensitized mast cells and basophils in order to initiate an anaphylactic reaction. Therefore, patients who present IgE antibodies to quaternary ammonium should be considered individuals at risk when subjected to general anesthesia with NMBAs.

IgE antibodies that cross-react with the quaternary ammonium ion of NMBAs were usually evaluated by a radioimmunoassay based on *p*-amino-phenylphosphoryl-choline bound to agarose or morphine sulphate coupled to Sepharose [10–12]. Specific IgE antibodies to NMBAs usually react with the other drugs of the same group, remaining detectable in RAST or ELISA assays [11,13].

The frequency of sensitization to quaternary ammonium ions in the absence of previous anesthesia is reported as 9.3% in the adult population with no identified risk factor [14]. The possibility that sensitization occurs through contact with ammonia quaternary compounds present in antiseptics like Benzalkonium chloride, Cetylpyridinium hydrochloride, or other industrial products such as cosmetics, have been reported [10,15,16]. These products are preferentially used by women and may explain the occurrence of reactions after the first exposure to NMBAs. Conversely, it has been suggested that the sensitization could occur by contact with self or infectious agent antigens containing choline structures [17].

Despite the existence of diagnostic tests for NMBAs, the rarity of these reactions and the lack of knowledge regarding other risk factors besides gender raise some difficulties for assessing these tests for large population screening. Improving current knowledge of the situations that determine the production of antibodies directed to phosphorylcholine analogs, associated with a presurgical questionnaire discriminating the individuals at risk, appears to be an adequate approach.

To address the profile of reactivity of anti-phosphorylcholine (PC) antibodies between males and females, a murine model of immunization with a NPPC-carrier was evaluated. The isotype profile of the antibodies and their cross-reactivity with choline derivatives, such as the NMBA, were studied.

2. Materials and methods

2.1. Animals

BALB/c mice, male and female, between 15 and 18 weeks of age were provided by the animal facilities of the São Paulo University Medical School. Lewis rats (RT1^{LI}), from both sexes, between 8 and 12 weeks of age, bred in our own laboratory's animal facilities were used for passive cutaneous anaphylaxis reaction.

2.2. Hapten diazotation and protein haptenization

Initially the hapten NPPC (Sigma) was submitted to a diazotation method [18]. For haptenization with KLH, 100 mg of KLH (Sigma, St Louis MO, USA) was added to the equivalent of 80 mg of diazotated NPPC. The solution of NPPC–KLH in borate buffer, pH 9.0, was maintained for 12 h at 4 °C, neutralized with 1 N HCl and dialyzed against cacodylate buffer for 72 h. For haptenization with bovine serum albumin (BSA, Sigma), 120 mg of diazotated NPPC were added to 62.7 mg of BSA diluted in phosphate buffer, pH 9.0, and the solution maintained for 12 h at 4 °C. The solution was neutralized with 1 N HCl and dialyzed against phosphate buffer pH 7.0–7.2 for 72 h. Haptenization was evaluated by spectrophotometry. The NPPC–KLH molecule was used for immunization of BALB/c mice and NPPC–BSA for the detection of specific antibodies.

2.3. Immunization protocol

BALB/c mice were immunized with 50 µg of NPPC–KLH in Al(OH)₃ solution by intraperitoneal route, after 7 days the same antigen dose in alum was administered and another boost was performed 1 week later with antigen and without adjuvant. Serum was obtained 7 days later (28 days after immunization) by retro-orbital bleeding and the samples were frozen at –70 °C until use. A similar immunization protocol was performed for immunization with non-haptenized KLH.

2.4. Passive cutaneous anaphylaxis (PCA)

The anaphylactic activity of IgE was evaluated by PCA reaction in rats as described by Mota and Wong [19]. Dilutions of serum pools were inoculated by subcutaneous route with 0.1 mL on the shaved backs of the rats. After 16 h, these rats were challenged with NPPC–BSA or suxamethonium (Sigma) by intravenous route, along with 0.5% Evans Blue. The reactions were evaluated 30 min later by measuring the diameter of the reaction on the inverted skin and the reciprocal of the highest serum dilution that produced a spot larger than 5 mm in diameter was taken as the PCA titer.

2.5. ELISA for measurement of anti-PC antibody levels

The detection of antibodies from PC–KLH-immunized mice was performed by ELISA. Briefly, 96-well polystyrene

high binding plates (Costar, Cambridge, MA, USA) were sensitized with 10 µg/mL of NPPC–BSA or non-haptenized BSA in carbonate–bicarbonate buffer 0.01 M, pH 9, for 1 h at 37 °C. The wells were blocked with PBS/1.0% BSA for 1 h at 37 °C before the incubation of the serum samples for 1 h at 37 °C. After washing in PBS–0.1% Tween, peroxidase-conjugated goat anti-mouse IgA, IgG1, IgG2 or IgG3 antibodies (Nordic Immunological Labs, Tilburg) were added for 2 h. After several washings, the reaction was developed with *ortho*-phenylenediamine (OPD, Sigma) and the enzymatic reaction was stopped with H₂SO₄ 1N. The reactions were read at 492 nm in an ELISA microplate reader (Bio-Rad, Hercules, CA, USA).

2.6. Evaluation of the reactivity of anti-PC antibodies with haptens and neuromuscular blocking agents (NMBA)

To analyze the reactivity of anti-PC antibodies with the haptens NPPC and PC or the cross-reactivity to NBMA, dilutions of serum pools of NPPC–KLH-immunized mice were incubated with 0.0612 to 32 mM of the haptens or with Succinylcholine chloride (Sigma), Pancuronium bromide (Pavulon®, Organon Teknika), Vecuronium bromide (Norcuron®, Organon Teknika) and Gallamine triethyliodide (Flaxedil®, Rhodia), respectively. A non-related drug, sodium thiopental, was used as control for these assays. Serum was incubated for 2 h at room temperature with the competitor and the ELISA assay was performed as above. The results were expressed as inhibition percentage of anti-PC reactivity, considering as reference (100%) the values of serum samples without haptens or drugs or by inhibition quotients (IQ₅₀) as a ratio of 50% inhibitory molar concentration of NPPC/50% inhibitory molar concentration of PC.

2.7. Statistical analysis

Difference between groups was considered statistically significant when *p* value ≤ 0.05, using the unpaired *t* test.

3. Results

3.1. Profile of antibody production in NPPC–KLH-immunized mice

To assess the cross-reactivity of anti-PC antibodies with the NMBAs, we first evaluated the profile of antibodies to nitrophenylphosphorylcholine (NPPC) coupled with Keyhole Limpet Hemocyanin (KLH) in immunized BALB/c mice. Fig. 1 shows that quaternary ammonium ion in the structures of PC and NPPC is also shared by the SUX structure.

Immunized BALB/c mice with NPPC–KLH preferentially induced IgM, IgG1 and IgG3 anti-PC antibody production compared to non-haptenized KLH-immunized group and non-immunized mice (Fig. 2). The reactivity of IgM, IgG1 and IgG3 against PC was detectable up to 1/32,000 serum dilution, while achieving only 1/2000 for IgG2 antibodies. In addition,

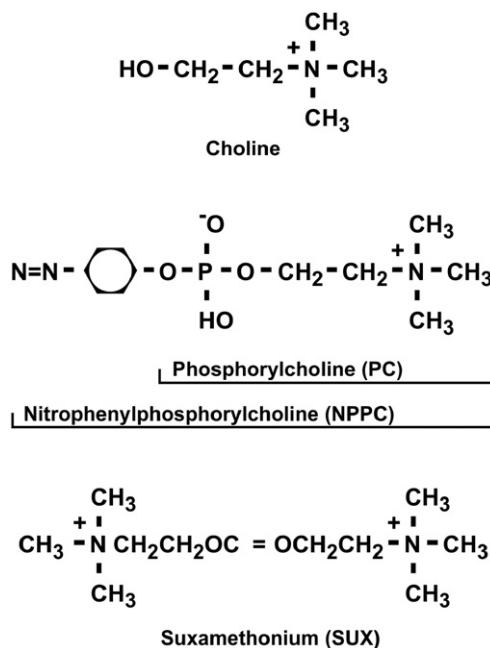


Fig. 1. Schematic illustration of the structures of choline, phosphorylcholine (PC), nitrophenylphosphorylcholine (NPPC) and a representative neuromuscular blocking agent, succinylcholine (SUX).

anti-PC IgA antibody production was detectable up to 1/2000 dilution, whereas reactivity to this hapten was observed at low levels in the control mice group. Mice immunized with PC–KLH developed high titers of anaphylactic anti-PC IgE antibodies, evaluated by the passive cutaneous anaphylactic reaction. Similarly, antibody production was verified in either female or male NPPC–KLH-immunized mice.

3.2. Differential affinity of anti-PC antibody isotype to the haptens nitrophenylphosphorylcholine (NPPC) and phosphorylcholine (PC)

To assess the reactivity of anti-PC antibody isotypes generated by immunization with NPPC–KLH, to the haptens PC and NPPC, ELISA inhibition assays were performed with the sera pool in the presence of inhibitors, haptens or non-related drug (thiopental) in increasing molar concentrations. The use of haptens could discriminate antibody reactivity to PC or NPPC between the isotypes. Sera dilution with anti-PC reactivity greater than optical density (OD) 0.8 was chosen for the inhibition assays.

Fig. 3 shows great affinity for all isotype anti-PC antibodies derived from NPPC–KLH-immunized mice to NPPC. The degree of inhibition was related to the hapten molar concentrations. Thus, 1 mM of inhibitor induced a 28% decrease in the anti-PC IgM reaction of female mice, in contrast to 70% inhibition for IgA, IgG1, IgG and 94% inhibition for the IgG3 antibodies. In addition, female mice showed higher affinity to IgG3 and IgG2 anti-PC antibodies directed to the

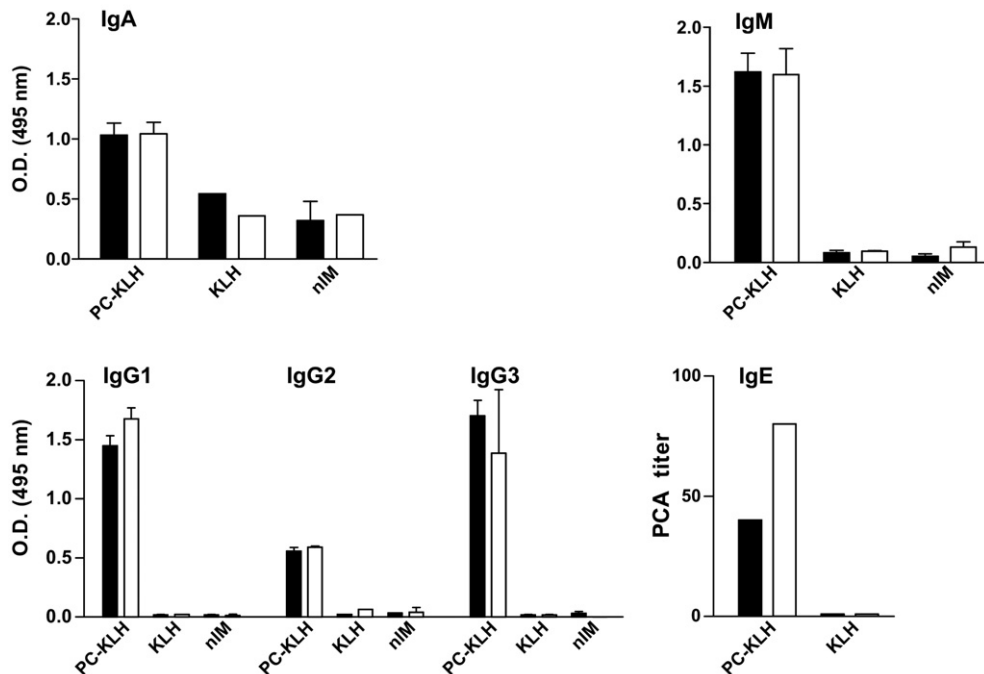


Fig. 2. Profile of antibody production in the NPPC–KLH immunization. Sera from BALB/c mice immunized with NPPC–KLH or KLH after 28 days of immunization and non-immunized (nIm) female (black bar) or male (white bar) mice were assessed for anti-PC IgA (1/1000), IgM (1/4000) and IgG1 (1/4000), IgG2 (1/1000) and IgG3 (1/4000) by ELISA. Data represents the mean $\pm$ S.E.M. of optical density (OD) of individual serum of male and female mice ($n=20$ /each group) (a); pool of sera ($n=20$) was assayed for IgE anti-PC antibodies by passive cutaneous anaphylaxis reaction (b).

haptens than male mice. An increased inhibition percentage was observed in females at 6 M concentration of NPPC by IgG3 (0.12–4.0 mM) and at three concentrations (0.5–2.0 mM) by IgG2 antibodies in comparison with male mice.

A different profile for anti-PC isotype reactivity was verified using PC haptens as an inhibitor. For the anti-PC IgA and IgG1 antibodies, irrespective of the PC molar concentration, a constant 20–40% inhibition was detected. High affinity to PC was revealed for IgM, IgG3 and IgG2; again, female mice showed higher affinity than male mice. A remarkable IgG3 affinity for PC was verified, even at the lowest PC concentration of 0.06 mM, which achieved 68% inhibition in the antibody reactivity. Female mice showed increased IgG3 inhibition at lower PC molar concentrations (0.06–1.0 mM) than male mice and for IgG2 at higher inhibitor concentrations (2.0–16 mM). The anti-PC IgG3 antibodies were inhibited with lower quantities of inhibitor, indicating higher affinity to PC than IgG2.

In addition, antibody reactivity to the haptens was verified by the NPPC:PC ratio, in order to verify the relation of the individual haptens concentrations capable of inhibiting 50% of the antibody reaction (Table 1). The ratio for IgM was greater than 1 and for IgG1 was 0.0625. When comparing between sexes, the IgG2 ratio was 0.0625 in females, but could not be determined in males, because no inhibition was seen even at 32 mM of PC, consequently the ratio was probably lower than 1. Similar results were obtained for IgA antibodies. Regarding anti-PC IgG3 antibodies, the ratio was probably greater than 1.

3.3. Evaluation of the cross-reactivity of anti-PC antibodies to neuromuscular blocking agents

To assess the cross-reactivity of anti-PC antibodies with the NMBAs, inhibition assays with sera from NPPC–KLH-immunized mice were performed with 1 mM of Vecuronium, Pancuronium, Gallamine or Suxamethonium by ELISA.

IgE anti-PC antibody cross-reaction with the neuromuscular blocking agent Suxamethonium was investigated. In these experiments, inhibition of the anti-PC antibody reactivity with suxamethonium was not detected, indicating absence of cross-reactivity.

The inhibition percentage of the sera from NPPC–KLH-immunized mice was equivalent for all NMBAs analyzed (Fig. 4). IgG3 anti-PC antibodies presented a greater affinity for the NMBAs, achieving up to 60% inhibition compared to other isotypes. NPPC–KLH-immunized mice, both male and female, showed a similar cross-reactivity percentage to the NMBAs. The data showed that between the IgG subclasses, cross-reactivity to curare was predominant for the IgG3 isotype. Thus, to further verify the cross-reactivity of anti-PC antibodies with the NMBAs, suxamethonium or thiopental were chosen as representative drugs in varying molar concentrations for these tests. Fig. 5 shows the inhibition of anti-PC IgG3 antibodies according to the drug dose. Sex-related differences showed that anti-PC IgG3 antibodies of female mice presented a significantly increased inhibition percentage, at 0.5–8.0 mM of

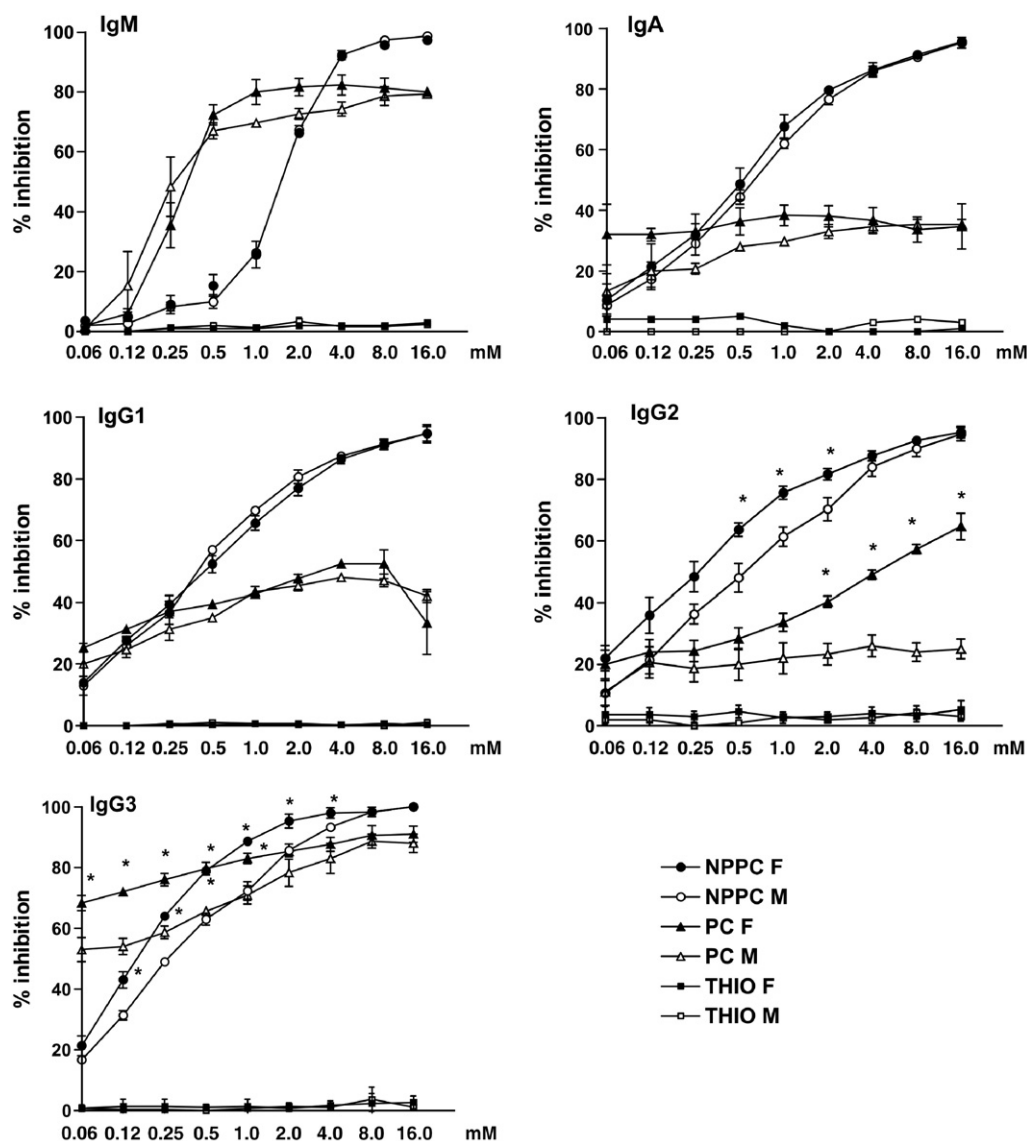


Fig. 3. Reactivity of anti-phosphorylcholine antibodies to the haptens NPPC and PC. Sera from NPPC–KLH immunized female (F) and male (M) mice were incubated with molar concentrations (0.06–16 mM) of nitrophenylphosphorylcholine (NPPC), phosphorylcholine (PC) or with a non-related drug, sodium thiopental (THIO) to assess anti-PC reactivity by means of ELISA. Sera dilution: IgM and IgG1 (1/8000), IgA and IgG2 (1/1000) and IgG3 (1/4000). Inhibition percentage represents the mean $\pm$ S.E.M. values obtained from 3 experiments. * $p \leq 0.05$ when compared to male mice.

suxamethonium, than male mice. Other curares resulted in a similar inhibition pattern of anti-PC IgG3 reactivity (data not shown).

Table 1

Inhibition quotient of anti-PC antibodies by the haptens NPPC and PC

	Inhibition quotient (IQ ₅₀) ^a				
	IgA	IgM	IgG1	IgG2	IgG3
Female	$\ll 1$	> 1	0.0625	$\ll 1$	$\gg 1$
Male	$\ll 1$	> 1	0.0625	0.0625	$\gg 1$

^a Ratio of NPPC:PC that inhibited 50% of the reaction.

Cross-reactivity of other isotypes to suxamethonium was also evaluated, demonstrating a maximal inhibition percentage of 25–30% for IgA, IgG1 and IgG2 at greater drug concentrations (16–32 mM).

4. Discussion

In this study, it was demonstrated that nitrophenylphosphorylcholine–carrier protein (NPPC–KLH) immunization in mice was able to preferentially induce IgM, IgG1 and IgG3 antibody production to phosphorylcholine

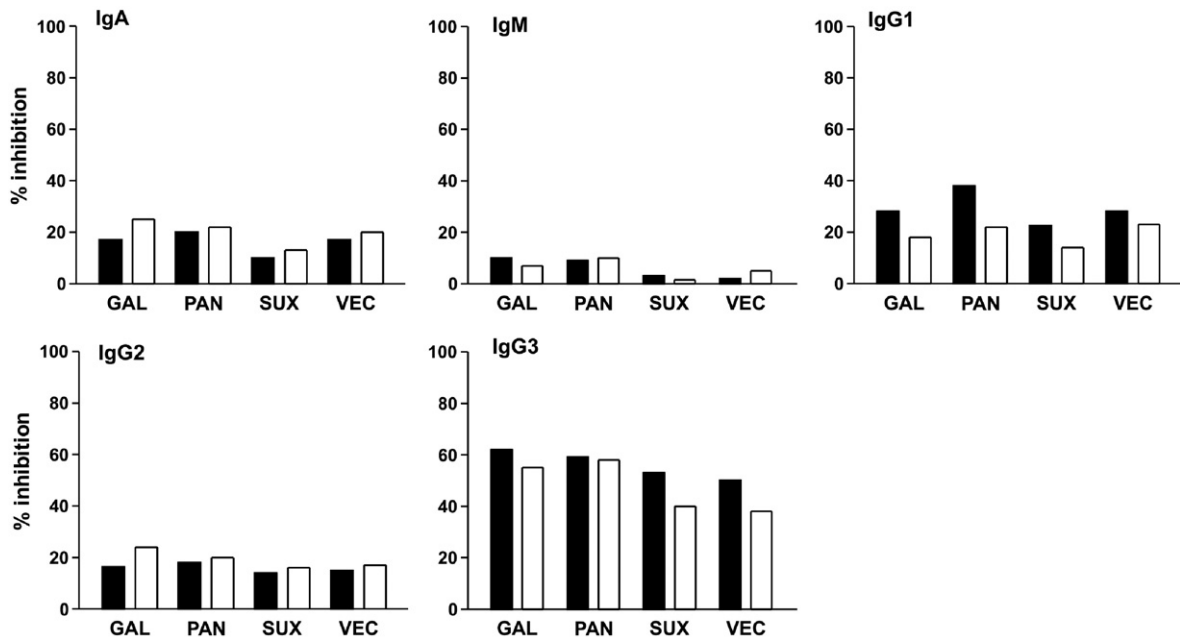


Fig. 4. Cross-reactivity of anti-PC antibodies to neuromuscular blocking agent. Sera from NPPC–KLH immunized mice were incubated with 1 mM of gallamine (GAL), pancuronium (PAN), suxamethonium (SUX) or vecuronium (VEC). Sera dilution of female (black bar) and male (white bar) mice were assessed for IgM and IgG1 (1/8000), IgA and IgG2 (1/1000) and IgG3 (1/4000) anti-PC reactivity by means of ELISA. Inhibition percentage represents one representative experiment out of two.

(PC). Comparison between the sexes revealed that female immunized mice showed greater affinity mediated by IgG3 and IgG2 antibodies to the PC hapten than males. Antibodies developed by the immunization process also showed cross-reactivity to NMBAs was exerted mainly by IgG3 antibodies, whereas it was absent for IgE antibodies.

The high cross-reactivity to NMBAs detected by IgG3 anti-PC antibodies seems to be directed mainly to the choline portion of the NPPC hapten, the only structure that NMBAs share with the antigen used for

immunization. In mice, IgG3 isotype switching does not appear to require Th₂-cytokines and is T-independent [20,21]. The equivalent in humans is the IgG2 isotype, that presents an overwhelming response to PC, comprising more than 80% of all immunoglobulins [22]. Phosphorylcholine is a component of several bacterial, mycotic and parasitic antigens [23–25] and antibodies induced by PC–KLH immunization in mice could mediate protection against *Streptococcus pneumoniae* and *Neisseria meningitidis* infections [26,27]. This isotype is also represented among natural anti-PC

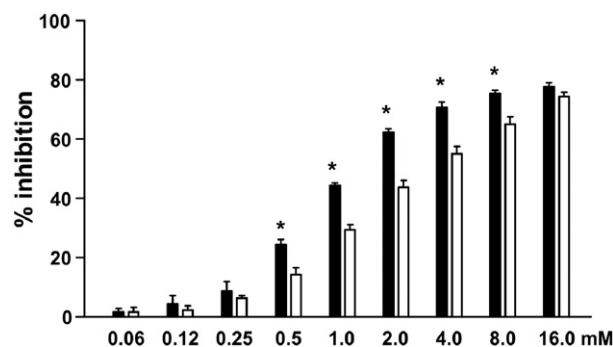


Fig. 5. Inhibition of anti-PC IgG3 antibodies by suxamethonium. Sera from NPPC–KLH immunized mice were incubated with suxamethonium or sodium thiopental (control) at concentrations ranging from 0.0612 to 16 mM. Sera dilution (1/4000) of female (black bars) and male (white bars) mice were assessed for IgG3 anti-PC reactivity by means of ELISA. Inhibition percentage represents the mean $\pm$ S.E.M. values obtained from 3 experiments. * $p \leq 0.05$ when compared to male mice.

antibodies, as well as in antipneumococcal vaccination-induced antibodies [22,28]. Natural autoantibodies are encoded by non-mutated germline V_H or V_L genes [29] and they react with a variety of self-determinants, such as carbohydrates and glycolipids, and often cross-react with bacterial to provide a fundamental tier of the adaptive immune system. It has been demonstrated that natural antibodies may recognize oxidation-specific epitopes contributing to the physiological homeostasis of apoptotic and necrotic cells, as well as senescent cells (such as erythrocytes) and lipoproteins (and similar lipid-rich membranes) that have undergone oxidative changes [30]. This B-cell clonal set is identical to classic T15 natural autoantibodies that dominate anti-PC responses and provide optimal protection from bacterial infection from virulent *S. pneumoniae* [31]. Presumably, natural antibodies participate in housekeeping roles in health, while also providing the advantages of natural immunity from certain common infectious agents.

The present findings related to the cross-reactivity of IgG3 anti-PC antibodies to NMBAs have not been previously described and suggest that an innate response to choline, or even antibodies triggered by the infections, could be reactive to NMBAs. The existence of natural antibodies that are reactive to the hapten and to choline induced by exposure to pathogens bearing abundant PC molecules that can cross-react with NMBAs, indicate a possible role in the protection of allergic reaction. The high levels of anti-PC IgG antibodies generated by immunization to choline in mice, which recognize NMBA components, may compete with IgE antibodies by blocking the interaction of anaphylactic antibodies and mast cell degranulation. In this context, it has been described that allergen-specific IgG produced during the course of an immune response, or administered passively, can suppress IgE-mediated anaphylaxis in immunized mice with a goat Ab against mouse IgD [32]. Nevertheless, in mice, IgG–allergen interactions can also induce anaphylaxis by activating macrophages through Fc γ RIII [33]. The condition of IgG blocking Abs to inhibit IgE-mediated anaphylaxis without inducing Fc γ RIII-mediated anaphylaxis appear to occur only when IgG Ab concentration is high and the challenging allergen concentration is low. Allergen epitope density correlates inversely with the allergen dose required to induce both IgE- and Fc γ RIII-mediated anaphylaxis.

In immunized mice with NPPC–KLH, absence of IgE cross-reactivity to suxamethonium occurs, as assessed by the passive cutaneous anaphylactic reaction. PCA reaction has long been used as a functional assay to measure the anaphylactic ability of allergen-specific IgE

antibodies [19,34,35]. The lack of cross-reactive IgE anti-PC antibodies appears to be related to the monomeric form of the haptens PC, NPPC and NP, since at least 2 epitopes are necessary to link the IgE molecules adsorbed by the mast cell membrane. Although the immunization protocol with NPPC–KLH in mice induced anti-PC IgE antibodies, these Ab presented no cross-reactivity to NMBAs, revealing this as an inadequate model for the induction of NMBA-induced hypersensitivity reactions. The absence of IgE cross-reactivity was probably related to the difficulty of measuring the anaphylactic ability of allergen-specific IgE antibodies, using a radioimmunoassay normally used for human diagnosis of NMBA allergies. In allergic individuals the cross-reactivity of IgE anti-NMBA antibodies with the hapten seem to react predominantly with the choline determinant of the NMBAs and hydrogenated NPPC (*p*-amino-phenylphosphoryl-choline), has been used for the laboratorial diagnosis of NMBA allergies [36].

Furthermore, the IgE antibodies produced by the immunization of BALB/c mice with hydrogenated NPPC linked to KLH seem to react with structures besides choline, involving other chemical groups in its composition. It is possible that the aromatic residue used for coupling the hapten to the carrier protein might be the complementary structure eliciting the specificity of these antibodies. It has been described that BALB/c mice immunized with PC–KLH induce IgE production capable of recognizing the integral hapten, which necessarily includes the nitrophenyl group [37,38]. IgE antibody specificity to the nitrophenyl portion was controlled by high mutation rates at the genomic sequences in other anti-PC antibodies, or by the use of genetic combinations in antibodies that react exclusively with the nitrophenyl portion [38].

Allergic reactions to NMBAs do not appear to be related to atopy or to other known factors that could enhance IgE production. A prospective study of surgical patients showed the occurrence of 4.7% who presented a positive prick test to anesthetic drugs [39]. Only prior history of allergy to medications was shown to predict positive prick tests to anesthetic drugs. One of the only known factors for allergic reactions to NMBAs is gender.

In comparisons between the sexes, the results showed a qualitative difference in the anti-PC response to the hapten PC, particularly for the IgG3 and IgG2 isotypes. Female mice essentially produced both IgG subclasses, especially IgG3, with greater affinity than males. The degree of IgG3 inhibition in the competition assay with PC or even to NPPC in female mice, verified that this isotype is inhibitable at lower inhibitor concentrations

than IgG2. This finding may suggest that IgG3 in female mice presented greater affinity than IgG2 antibodies. In humans, considering that the equivalent isotype IgG2 is the major IgG subclass raised by PC [22], it is still unknown whether they are cross-reactive to NMBAs. In addition, few studies exist that demonstrate IgG2 involvement in allergy. It has been reported that high titers of dextran-reactive IgG were regularly found in the sera of patients with severe dextran-induced anaphylactoid/anaphylactic reactions, in which, among IgG subclasses, IgG2 was considered the most important due to its high titers during severe reactions [40].

Furthermore, IgG2 antibodies in mice include both IgG2a and IgG2b subclasses, which were induced by different mechanisms. IgG2b is usually related to a T-independent antigen response and TGF- β is one of the factors that induce LPS-stimulated cultures to induce the switching to IgG2b and IgA [41]. IgG2a is an isotype that requires IFN- γ , a Th₁-type cytokine IFN- γ . Further information related to the IgG2 subclass and the influence of IFN- γ or TGF- β could contribute to understanding why females present certain qualitative differences to male mice regarding their anti-PC response. It is also possible that the females develop antibodies with a greater reactivity to the incomplete hapten (PC), or due to a restriction of this reactivity in the male response. The preferential reactivity of anti-PC antibodies to PC or NPPC involves the use of genomic combinations of heavy chain variable segments in BALB/c mice [38]. Consideration should also be given to whether the females are capable of producing antibodies that can mediate anaphylaxis by activating macrophages through Fc γ RIII.

The factors that lead to allergic sensitization and the preferential incidence in females [4] are not known, although domestic use of chemical products carrying quaternary ammonia, which may be found in cosmetics, over-the-counter medications and cleaning products [42], are claimed to be responsible for the prior sensitization of these patients. In the absence of a detectable IgE specific for the relevant Ag [43], Ag-mediated anaphylaxis suggests that IgG-mediated anaphylaxis may occur in humans. It has been suggested that an IgG anaphylaxis pathway may occur in persons repeatedly exposed to large quantities of antigen [32].

Improved comprehension of natural antibodies that are cross-reactive to NMBAs, including the discrimination of the IgG subclasses involved in the recognition of these drugs, should contribute further understanding regarding sensitization to choline-derived structures, such as NMBAs.

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