ALLERGY



# An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis

**Friederike Jönsson[1]*, Luc de Chaisemartin[2,3]*, Vanessa Granger[2,3†], Aurélie Gouel-Chéron[1,4†], Caitlin M. Gillis[1], Qianqian Zhu[1,3], Fadia Dib[5], Pascale Nicaise-Roland[2], Christelle Ganneau[6], Maria Hurtado-Nedelec[7], Catherine Paugam-Burtz[8,9], Skander Necib[8,9], Hawa Keita-Meyer[10,11], Matthieu Le Dorze[12], Bernard Cholley[13,14], Olivier Langeron[15,16], Laurent Jacob[17], Benoit Plaud[17], Marc Fischler[18], Caroline Sauvan[19], Marie-Thérèse Guinnepain[20], Philippe Montravers[4,21], Michel Aubier[19,21], Sylvie Bay[6], Catherine Neukirch[19,21], Florence Tubach[22‡], Dan Longrois[2,4‡], Sylvie Chollet-Martin[2,3‡§], Pierre Bruhns[1‡§]**

**Anaphylaxis is a systemic acute hypersensitivity reaction that is considered to depend on allergen-specific immunoglobulin E (IgE) antibodies and histamine release by mast cells and basophils. Nevertheless, allergen-specific IgG antibodies have been proposed to contribute when the allergen is an abundant circulating large molecule, e.g., after infusions of therapeutic antibodies or dextran. Data from animal models demonstrate a pathway involving platelet-activating factor (PAF) release by monocytes/macrophages and neutrophils activated via their Fc gamma receptors (FcγRs). We hypothesized that such a pathway may also apply to small drugs and could be responsible for non–IgE-mediated anaphylaxis and influence anaphylaxis severity in humans. We prospectively conducted a multicentric study of 86 patients with suspected anaphylaxis to neuromuscular-blocking agents (NMBAs) during general anesthesia and 86 matched controls. We found that concentrations of anti-NMBA IgG and markers of FcγR activation, PAF release, and neutrophil activation correlated with anaphylaxis severity. Neutrophils underwent degranulation and NETosis early after anaphylaxis onset, and plasma-purified anti-NMBA IgG triggered neutrophil activation ex vivo in the presence of NMBA. Neutrophil activation could also be observed in patients lacking evidence of classical IgE-dependent anaphylaxis. This study supports the existence of an IgG-neutrophil pathway in human NMBA-induced anaphylaxis, which may aggravate anaphylaxis in combination with the IgE pathway or underlie anaphylaxis in the absence of specific IgE. These results reconcile clinical and experimental data on the role of antibody classes in anaphylaxis and could inform diagnostic approaches to NMBA-induced acute hypersensitivity reactions.**



## INTRODUCTION

Anaphylaxis is an immune-mediated systemic acute hypersensitivity reaction (AHR) occurring rapidly upon contact with an allergen in sensitized individuals. Clinical signs include bronchoconstriction, severe vasodilatation, and hypovolemia that can lead to multiorgan dysfunction and death. The most prominent cause of anaphylaxis in adults is drugs, notably antibiotics and neuromuscular-blocking agents (NMBAs). Anaphylaxis is classically considered to rely on immunoglobulin E (IgE) antibodies against the allergen/antigen, and to involve massive histamine liberation by mast cells and basophils, after antigen-induced aggregation of IgE receptor (FcεRI)–bound specific IgE antibodies (*1*). The clinical diagnosis of anaphylaxis to NMBAs is based on this IgE paradigm. Unexpectedly, 10 to 20% of patients who experience an AHR consistent with an anaphylactic reaction do not present with any biological signs of IgE-dependent immune activation (positive skin test, presence of allergen-specific IgE, elevated histamine, or tryptase) (*2*, *3*), suggestive of other potential mechanisms. Among these alternative mechanisms, IgG antibodies have been proposed to trigger IgE-independent anaphylaxis induced by some protein antigens or large molecules (e.g., therapeutic

[1]Unit of Antibodies in Therapy and Pathology, Institut Pasteur, UMR 1222 INSERM, 75015 Paris, France. [2]Département d'Immunologie et d'Hématologie, UF Auto-immunité et Hypersensibilités, HUPNVS, Hôpital Bichat, 75018 Paris, France. [3]Inflammation Chimiokines et Immunopathologie, INSERM UMR S996, Faculté de Pharmacie, Université Paris-Sud, Université Paris-Saclay, 92290 Châtenay-Malabry, France. [4]AP-HP, Hôpital Bichat, Département d'Anesthésie-Réanimation, HUPNVS, 75018 Paris, France. [5]AP-HP, Hôpital Bichat, Department of Epidemiology and Clinical Research, INSERM, 75018 Paris, France. [6]Unité de Chimie des Biomolécules, Institut Pasteur, UMR 3523 CNRS, 75015 Paris, France. [7]Département d'Immunologie et d'Hématologie, UF Dysfonctionnements Immunitaires, HUPNVS, Hôpital Bichat, 75018 Paris, France. [8]Département d'Anesthésie-Réanimation, Hôpital Beaujon, AP-HP, 92110 Clichy, France. [9]Université Paris Diderot, 92110 Paris, France. [10]Service d'anesthésie, Hôpital Louis Mourier, AP-HP, 92700 Colombes, France. [11]Université Paris Diderot, Sorbonne Paris Cité, EA Recherche Clinique coordonnée ville-hôpital, Méthodologies et Société (REMES), 75010 Paris, France. [12]Département d'Anesthésie-Réanimation, Hôpital Lariboisière, AP-HP, 75010 Paris, France. [13]Service d'Anesthésie-Réanimation, Hôpital Européen Georges Pompidou, AP-HP, UMR 1140 INSERM, 75015 Paris, France. [14]Université Paris Descartes, Sorbonne Paris Cité, 75006 Paris, France. [15]Department of Anesthesia and Critical Care, Hôpital Pitié Salpêtrière, AP-HP, Sorbonne Université, 75013 Paris, France. [16]Infection and Epidemiology Department, Institut Pasteur Human Histopathology and Animal Models Unit, 75015 Paris, France. [17]Département d'Anesthésie-Réanimation, Hôpital Saint Louis, AP-HP, 75010 Paris, France. [18]Service d'Anesthésie, Hôpital Foch, 92150 Suresnes, France. [19]AP-HP, Hôpital Bichat, Service de Pneumologie A, HUPNVS, 75018 Paris, France. [20]Hôpital Foch, Service de médecine interne, 92150 Suresnes, France. [21]INSERM 1152, DHU FIRE, Labex Inflamex, Université Paris Diderot Paris 7, 75018 Paris, France. [22]Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, PEPITES, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière–Charles Foix, Département Biostatistique Santé Publique et Information Médicale, Centre de Pharmacoépidémiologie (Cephepi), CIC-1421, 75013 Paris, France.
*These authors contributed equally to this work.
†These authors contributed equally to this work.
‡Co-senior authors.
§Corresponding author. Email: bruhns@pasteur.fr (P.B.); sylvie.martin2@aphp.fr (S.C.-M.)

antibodies, protamine, and dextran) (*3*–*9*). There is also a lack of understanding why, for some patients, the severity of the AHR varies widely, despite similar degrees of activation of the IgE pathway.

Supporting a paradigm of non–IgE-dependent pathways of anaphylaxis, studies in mice have demonstrated that anaphylactic reactions may occur in the absence of IgE (*10*), FcεRIs (*11*, *12*), or mast cells (*12*, *13*). Antibodies of the IgG class are often responsible for these non-IgE reactions, via the generation of IgG antigen/allergen immune complexes (ICs). These complexes engage Fc gamma receptors (FcγRs) on various myeloid cells (*14*–*16*), including monocytes/macrophages (*17*, *18*), neutrophils (*19*), basophils (*20*), or mast cells (*12*), depending on the model of anaphylaxis used. FcεRI or FcγR engagement leads to internalization and down-regulation of these receptors (*21*), which has been proposed as means to distinguish types of anaphylaxis, i.e., FcεRI down-regulation on mast cells and basophils as a marker of IgE-induced anaphylaxis (*22*) and FcγR down-regulation on neutrophils and/or monocytes as a marker of IgG-induced anaphylaxis (*23*, *24*). Our previous work showed that neutrophils play a dominant pathogenic role in mouse models of severe anaphylaxis (*19*) including models driven by human IgG and human FcγR (*24*, *25*), suggesting that neutrophils could also contribute to anaphylaxis in humans. Neutrophils may even be sufficient to induce anaphylactic symptoms, because they can rapidly release a number of potent lipid and protein mediators upon activation and can also extrude their genomic and mitochondrial DNA decorated by potent enzymes in the form of neutrophil extracellular traps (NETs), a process termed NETosis (*26*). Neutrophils are major producers of platelet-activating factor (PAF) (*27*, *28*) and release PAF in vitro upon FcγR triggering (*19*, *25*), which also implicates neutrophils in anaphylaxis pathogenesis. IgG-dependent experimental anaphylaxis in animal models has been repeatedly reported to rely on PAF as a mediator, alone or in combination with histamine (*15*, *19*, *20*, *24*, *29*, *30*). Elevated PAF concentrations can be detected in the serum of human patients after anaphylaxis and correlate with reaction severity (*31*, *32*). To investigate the putative contribution of an IgG antibody–neutrophil-PAF pathway to human anaphylaxis to small drug molecules, we performed a prospective multicentric study on patients with suspected NMBA-induced AHR during anesthesia.

**Table 1. Characteristics of the patients.** Values are expressed as percentages or as median [25th to 75th percentile]. ACEI, angiotensin-converting enzyme inhibitor; ARA, angiotensin II receptor antagonist; BB, beta blocker; CI, calcium channel inhibitor.

| Variable | Cases ($n$ = 86) | Controls ($n$ = 86) | $P$ |
|---|---|---|---|
| Female gender | 62% | 62% | 1* |
| Age (years) | 57 [40;66] | 58 [42;66] | 0.66† |
| Previous general anesthesia | 84% | 87% | 0.66* |
| Medication (ACEI/ARA/BB/CI) | 38% | 29% | <0.0001* |
| History of allergy | | | |
| Drugs | 13% | 13% | 1* |
| Latex | 2% | 8% | 0.17* |
| Food | 7% | 0% | 0.03* |
| Hymenoptera venom | 3% | 0% | 0.09* |
| Pollen/moth/animals/mold | 15% | 13% | 0.83* |
| Asthma | 12% | 5% | 0.16* |
| Atopy | 21% | 19% | 0.85* |
| Type of surgery | | | |
| Cardiothoracic, vascular | 20% | 20% | 1* |
| Maxillofacial | 8% | 8% | 1* |
| Orthopedic neurosurgery | 20% | 20% | 1* |
| Visceral, urologic, gynecologic | 52% | 52% | 1* |
| Scheduled surgery | 92% | 98% | 0.17* |
| Surgery with a context of infection | 2.3% | 2.3% | 1* |
| NMBA used during surgery | | | |
| Suxamethonium | 56% | 56% | 1* |
| Atracurium | 51% | 51% | 1* |
| Rocuronium | 3% | 3% | 1* |

*Fisher's exact test. †Student's $t$ test.

## RESULTS

### NMBA-specific IgE and IgG concentrations associate with anaphylaxis severity

Because of the unpredictability and low incidence of anaphylactic events to NMBAs (1 of 10,000 anesthesia procedures), we prospectively recruited a multicentric cohort of patients with AHRs to NMBA injection, along with matched controls [Tables 1 and 2; additional parameters of the cohort described in (*33*)]. Reaction severity was retrospectively classified as mild (grades 1 and 2) or severe (grades 3 and 4) according to the Ring and Messmer (*34*) classification. Patients ($n$ = 86) received suxamethonium ($n$ = 39), atracurium ($n$ = 35), rocuronium ($n$ = 3), or a mixture of atracurium and suxamethonium ($n$ = 9) (Fig. 1, A and C, and table S1). Among those, the frequency of severe (grade 3 or 4) AHR was 72, 37, 33, and 22%, indicating that severe reactions can occur in response to any of the NMBAs used.

All NMBAs contain quaternary ammonium groups, which are considered the main antigenic epitope (*35*). Circulating anti-NMBA antibodies were quantified using the quaternary ammonium morphine (QAM) compound (Fig. 1D) as a surrogate molecule. Antibodies against this shared epitope were detected as serum anti-QAM IgE and anti-QAM IgG. As expected (*36*), anti-QAM IgE was elevated in patients with AHR compared to controls ($P < 0.0001$) but was also higher in patients with reactions graded as severe versus those with reactions graded as mild ($P < 0.0001$) (Fig. 1E and table S1). Anti-QAM IgE is considered the most sensitive biomarker in NMBA anaphylaxis (*37*) and was not detected in 98% of controls. Unexpectedly, it was also undetectable in 72% of patients with anaphylaxis. Conversely, circulating anti-QAM IgG was detected in all controls and patients but, nevertheless, was found at significantly higher concentrations in patients with severe AHR compared to mild ($P < 0.0001$) or to paired controls ($P = 0.007$) (Fig. 1F). Patients with very low (<0.5 μg/ml) anti-QAM IgG had undetectable anti-QAM IgE (Fig. 1G). Detectable

**Table 2. Clinical signs and therapeutic interventions during AHRs, according to severity grade.** Values are expressed as percentages or as median [25th to 75th percentile] when appropriate. The numbers of observations and missing values varied according to the numeric value analyzed: maximum heart rate (HR) (43/17), minimum HR (14/6), minimum PAS (70/21), minimum $SpO_2$ (24/9), and minimum end-tidal $CO_2$ ($etCO_2$) (33/7). ICU, intensive care unit; IV, intravenous; NA, not available; SAP, systolic arterial pressure; $SpO_2$, photoplethysmographic oxygen saturation.

| Clinical/hemodynamic signs | Severe ($n$ = 43) | Mild ($n$ = 43) | $P$ |
|---|---|---|---|
| Erythema (%) | 65 | 79 | 0.2* |
| Tachycardia (%) | 58 | 44 | 0.3* |
| Maximum HR ($min^{-1}$) | 130[125;133] | 127[111;136] | 0.3† |
| Bradycardia (%) | 26 | 7 | 0.04* |
| Minimum HR ($min^{-1}$) | 42[40;49] | 52[51;54] | 0.1† |
| Arrhythmia (%) | 23 | 2 | 0.007* |
| Arterial hypotension (%) | 100 | 63 | <0.0001* |
| Minimum SAP value (mmHg) | 50[43;60] | 65[58;73] | 0.01† |
| Bronchospasm (%) | 37 | 28 | 0.5* |
| Hypoxemia (%) | 49 | 7 | <0.0001* |
| Minimum $SpO_2$ (%) | 82[82;88] | 91[90;91] | 0.1† |
| Hypocapnia (%) | 63 | 14 | <0.0001* |
| Minimum $etCO_2$ (mmHg) | 18[13;21] | 26[24;29] | 0.002† |
| Cardiac arrest (%) | 23 | 0 | 0.001* |
| **Delay of resuscitation** | | | |
| Delay between anesthesia induction and first signs of the reaction (min) | 5[5;16] | 5.5[5;10] | 0.6† |
| Delay between first signs of the reaction and treatment initiation (min) | 1[0;2] | 0[0;0.75] | 0.008† |
| **Therapeutic interventions** | | | |
| Epinephrine IV alone (%) | 35 | 0 | <0.0001* |
| Cumulative doses of epinephrine (mg) | 2.5[1;6.9] | NA | NA |
| Norepinephrine IV alone (%) | 5 | 0 | 0.5* |
| Cumulative doses of norepinephrine IV (mg) | 1.75[1.62;1.87] | NA | NA |
| Epinephrine IV and norepinephrine IV (%) | 12 | 0 | 0.06* |
| Cumulative doses of epinephrine IV (mg) | 5[2;5.4] | NA | NA |
| Ephedrine and/or phenylephrine IV alone (%) | 0 | 44 | <0.0001* |
| Ephedrine and/or phenylephrine IV, then epinephrine, and/or norepinephrine IV (%) | 49 | 2 | <0.0001* |
| Cumulative doses of epinephrine IV (mg) | 0.6[0.3;4.1] | 0.1 | NA |
| Atropine IV (%) | 12 | 5 | 0.4* |
| Cumulative doses of atropine IV (mg) | 1[1;1] | 1[1;1] | NA |
| $\beta_2$-Adrenergic agonist IV/inhaled (%) | 14 | 12 | 1* |
| Glucocorticoids (%) | 23 | 23 | 1* |
| Histamine receptor antagonists (%) | 0 | 2 | 1* |
| Fluid resuscitation with crystalloids (%) | 70 | 42 | 0.02* |
| Cumulative volume of crystalloids (liters) | 1[1;2] | 0.87[0.5;1.37] | 0.03† |
| Fluid resuscitation with colloids (%) | 83 | 9 | 0.008* |
| Cumulative volume of colloids (liters) | 0.5[0.5;1] | 0.65[0.5;0.85] | 0.8† |
| **Evolution** | | | |
| Surgery canceled (%) | 81 | 19 | <0.0001* |
| Admission to ICU (%) | 76 | 26 | <0.0001* |

*Fisher's exact test. †Mann-Whitney $U$ test.

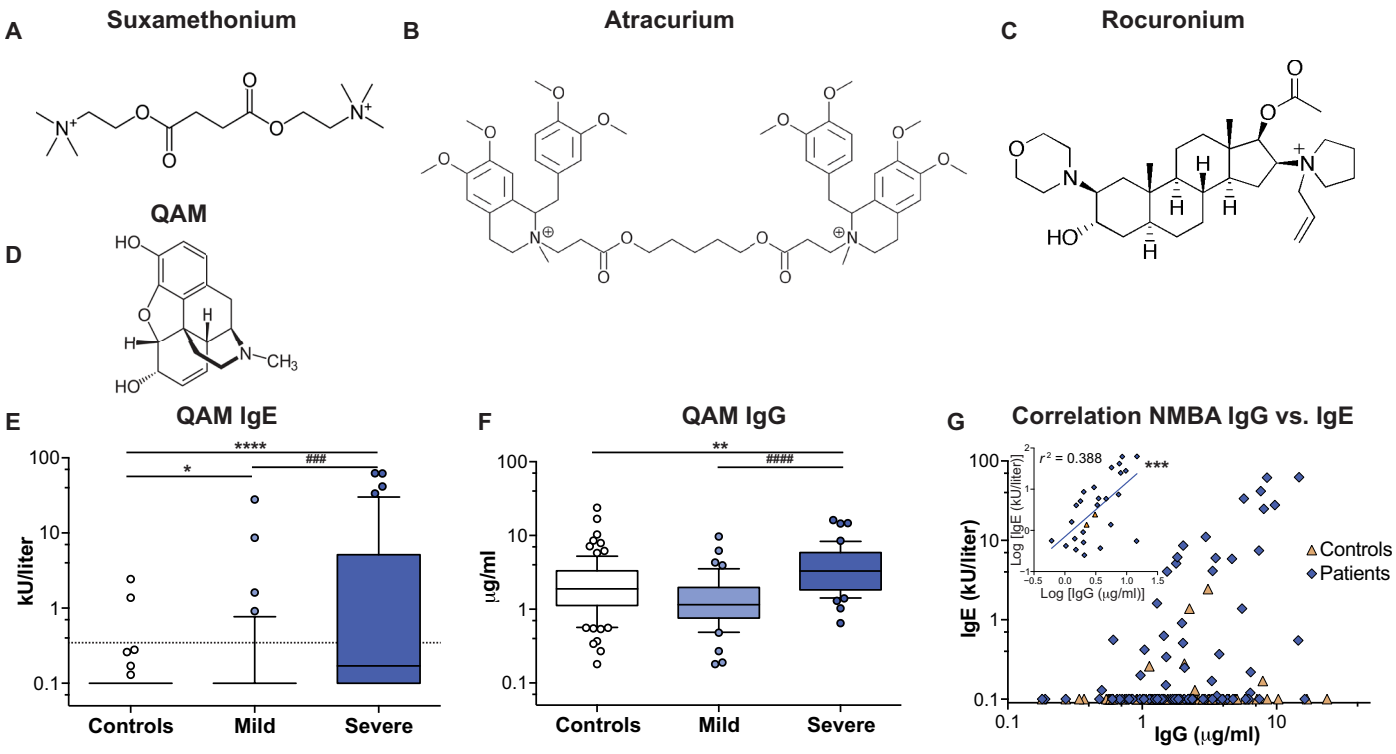


**Fig. 1. NMBA-specific antibodies concentrations are elevated in severe AHR.** (**A** to **D**) Schematic representation of (A) quaternary ammonium morphine (QAM) antigen, (B) suxamethonium, (C) atracurium, and (D) rocuronium. (**E** and **F**) Measurements of (E) IgE and (F) IgG against QAM in patients presenting with mild AHR ($n = 43$), severe AHR ($n = 43$), and matched controls ($n = 86$). Wilcoxon signed-rank test, ****$P < 0.0001$, **$P < 0.01$, *$P < 0.05$. Mann-Whitney $U$ test, ####$P < 0.0001$, ###$P < 0.001$. (D and E) Results are represented as boxplots; whiskers indicate the 10th and 90th percentiles. (**G**) Plotting of individual data of anti-QAM IgE and IgG concentrations for controls (orange triangles) and patients (blue diamonds). Inset: Correlation plot including only patients and controls with anti-QAM IgE of >0.3 kU/liter. Spearman's correlation, ***$P < 0.001$.

anti-QAM IgE concentrations correlated significantly with anti-QAM IgG ($P = 0.0009$; Fig. 1G, inset), indicating that whenever a patient had high anti-QAM IgE, they also had high anti-QAM IgG. The reverse was not true, because 49% of patients with above median anti-QAM IgG concentrations did not have detectable IgE. Irrespective of reaction severity, patients presenting with the highest anti-QAM IgE or IgG concentrations also tended to present with high NMBA-specific IgE and IgG, detected via both clinically validated and research-grade ImmunoCAP assays (fig. S1). Thus, both anti-QAM IgE and IgG concentrations correlated with NMBA-induced anaphylaxis severity.

## FcγR down-regulation on neutrophils is associated with anaphylaxis severity

Upon intravenous injection, NMBAs may immediately interact with anti-NMBA IgE-sensitized cells in circulation and ICs of [anti-NMBA IgG–NMBA] may instantaneously form to engage FcγRs on myeloid cells in patients with high circulating anti-QAM IgE and IgG. Our analyses revealed significantly lower FcεRI expression on basophils, but not on eosinophils, from patients during severe AHR compared to their baseline expression measured during the follow-up visit (V2) or compared to controls ($P = 0.02$ and $P = 0.01$, respectively; Fig. 2, A and B). Among FcγRs (Fig. 2, C to L), CD32A/FcγRIIA and CD16/FcγRIII, but not CD64/FcγRI, demonstrated lower expression on neutrophils from patients with severe AHR during AHR compared to controls (Fig. 2, E and K). This reduction was also apparent compared to baseline expression measured at V2, indicating that these FcγRs are not constitutively down-regulated in these patients. Down-regulation of CD32A and CD16 on neutrophils was significantly more pronounced in patients with severe reactions versus those with mild reactions ($P < 0.0001$ and $P = 0.0004$, respectively). Monocytes from patients with severe AHR also demonstrated modest CD32A down-regulation compared to controls but no modulation of CD64, CD16, or of the inhibitory receptor CD32B/FcγRIIB (Fig. 2, D, H, J, and L). Basophils may have lower CD32B expression during severe AHR compared to baseline expression (V2), patients with mild AHR, or controls (Fig. 2I), but this difference did not reach statistical significance.

When we performed similar analyses after segregating patients according to the NMBA administered, we confirmed a down-regulation of CD32A and CD16 on neutrophils and CD32A on monocytes during suxamethonium-induced AHR and of CD32A on neutrophils during atracurium-induced AHR (fig. S2, A to D). There were too few rocuronium-induced severe AHR cases to permit statistical comparisons in this particular subgroup. Together, these data reveal down-regulation of specific activating FcγRs on neutrophils and monocytes in patients with severe AHR. These data are consistent with the hypothesis that FcγR are engaged by circulating IgG ICs after NMBA injection in patients with AHR. Further supporting this hypothesis, concentrations of complement components C3 and C4, as well as complement activity (50% hemolytic activity, CH50), were significantly reduced during severe AHR compared to baseline

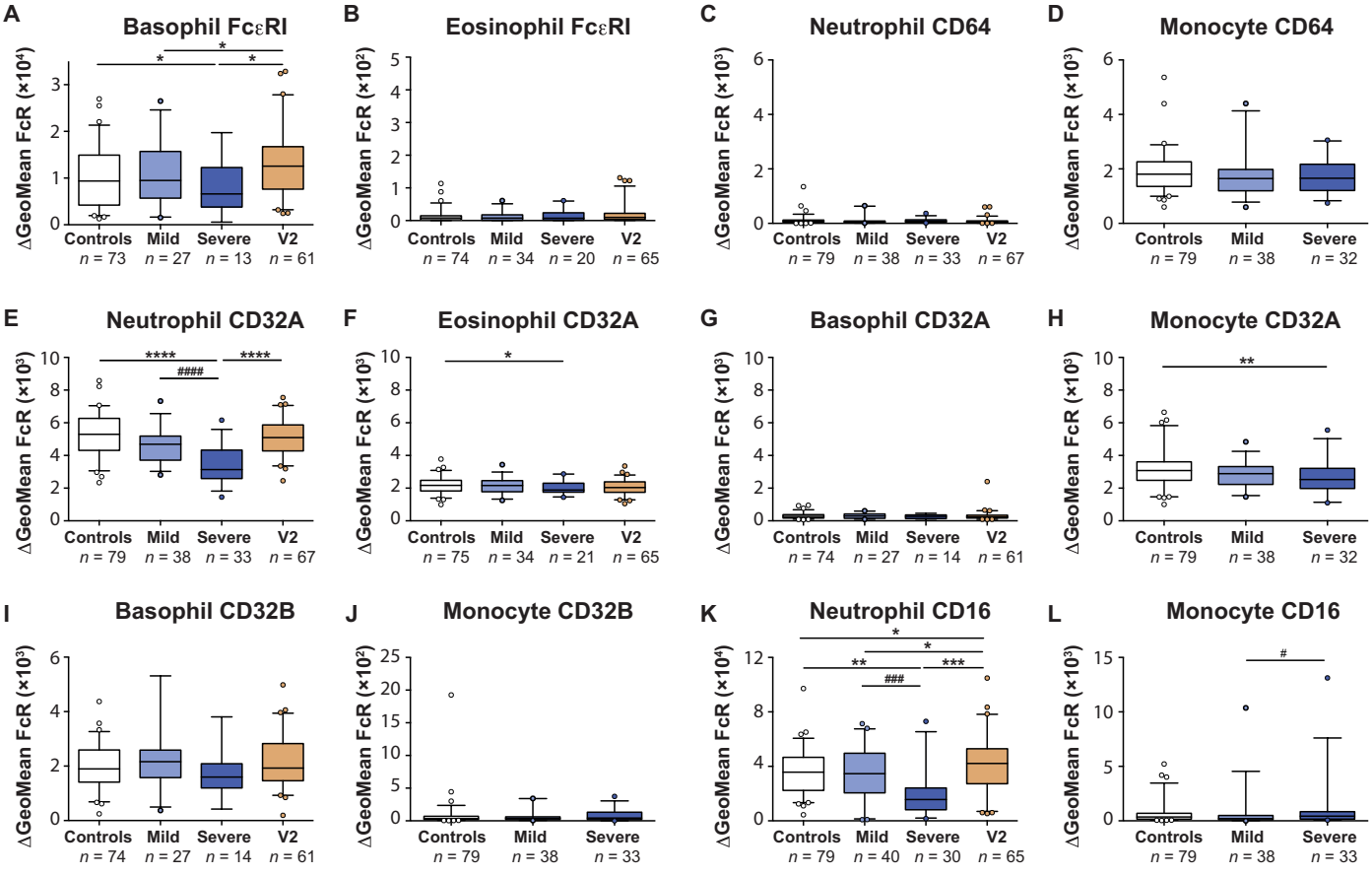


**Fig. 2. Modulation of IgE and IgG receptor expression on myeloid cells during AHR.** (**A** and **B**) FcεRI, (**C** and **D**) CD64 (FcγRI), (**E** to **H**) CD32A (FcγRIIA), (**I** and **J**) CD32B (FcγRIIB), and (**K** and **L**) CD16 (FcγRIII) expression on circulating basophils (A, G, and I), eosinophils (B and F), neutrophils (C, E, and K), and monocytes (D, H, J, and L) in the blood from patients 30 min after onset of AHR during the follow-up visit (V2) or from matched controls. Values represent the delta geometric mean (ΔGeoMean) between specific staining and corresponding isotype or fluorescence-minus-one control. Wilcoxon signed-rank test, ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Mann-Whitney *U* test, ####$P < 0.0001$, ###$P < 0.001$, #$P < 0.05$. All boxplot whiskers represent 5th and 95th percentiles.

(V2) ($P < 0.0001$; fig. S3), suggesting activation of the classical complement pathway by IgG ICs during severe NMBA anaphylaxis.

### Neutrophils are activated during human anaphylaxis

Down-regulation of activating FcγRs during AHR was predominant on neutrophils compared to other myeloid cells (Fig. 2). We therefore investigated the activation status of neutrophils during AHR by measuring surface markers CD11b, CD18, and CD66b, circulating neutrophil elastase, DNA-myeloperoxidase (DNA-MPO) complexes as a measure of circulating NETs, and PAF-acetylhydrolase (PAF-AH) activity as an indirect measure of PAF concentrations. Expression of CD11b, CD18, and CD66b were all significantly increased in neutrophils from patients with severe AHR compared to controls ($P = 0.0003$, $P = 0.001$, and $P = 0.02$, respectively; Fig. 3, A and C). Neutrophil elastase was significantly increased in the plasma during severe AHR compared to controls or to baseline (V2) concentrations ($P < 0.0001$ for both; Fig. 3D). DNA-MPO complexes detected in the serum were also significantly increased in severe AHR compared to controls ($P < 0.0001$) or patients with mild AHR ($P < 0.0001$; Fig. 3E). PAF-AH activity was significantly lower in patients with severe AHR compared to controls or patients with mild AHR ($P < 0.0001$ and $P = 0.01$, respectively; Fig. 3F), which is indicative of elevated plasma PAF concentrations. Expression of both CD11b and CD18, as well as circulating concentrations of DNA-MPO complexes, was also significantly higher in patients with mild AHR compared to controls ($P = 0.004$, $P = 0.007$, and $P = 0.001$), indicating that evidence of an IgG pathway of immune activation is not only associated with severe AHR (Fig. 3, A, B, and E). Moreover, CD11b, CD18, and DNA-MPO complexes remained elevated in patients with severe AHR even 6 to 8 weeks after the reaction (V2; Fig. 3, A, B, and E). Similar analyses after separating patients according to the NMBA used supports and sustained up-regulation of CD11b and CD18 on neutrophils and NET release during and after suxamethonium-induced severe AHR (fig. S4, A and C).

To ascertain that the link between neutrophil activation and anaphylaxis severity was not biased by treatments given during AHR (epinephrine, fluids, or other drugs), we incubated neutrophils from healthy donors with a wide range of concentrations of these compounds in vitro and assessed neutrophil responsiveness. Epinephrine, ephedrine, atropine, and resuscitation fluids had no effect on neutrophil activation. Only methylprednisolone at concentrations far above therapeutic range could induce a weak neutrophil activation as measured by NET release (fig. S5). Together, these data provide evidence of neutrophil activation in patients with intra-anesthetic AHR and demonstrate that the degree of neutrophil activation associates with AHR severity.

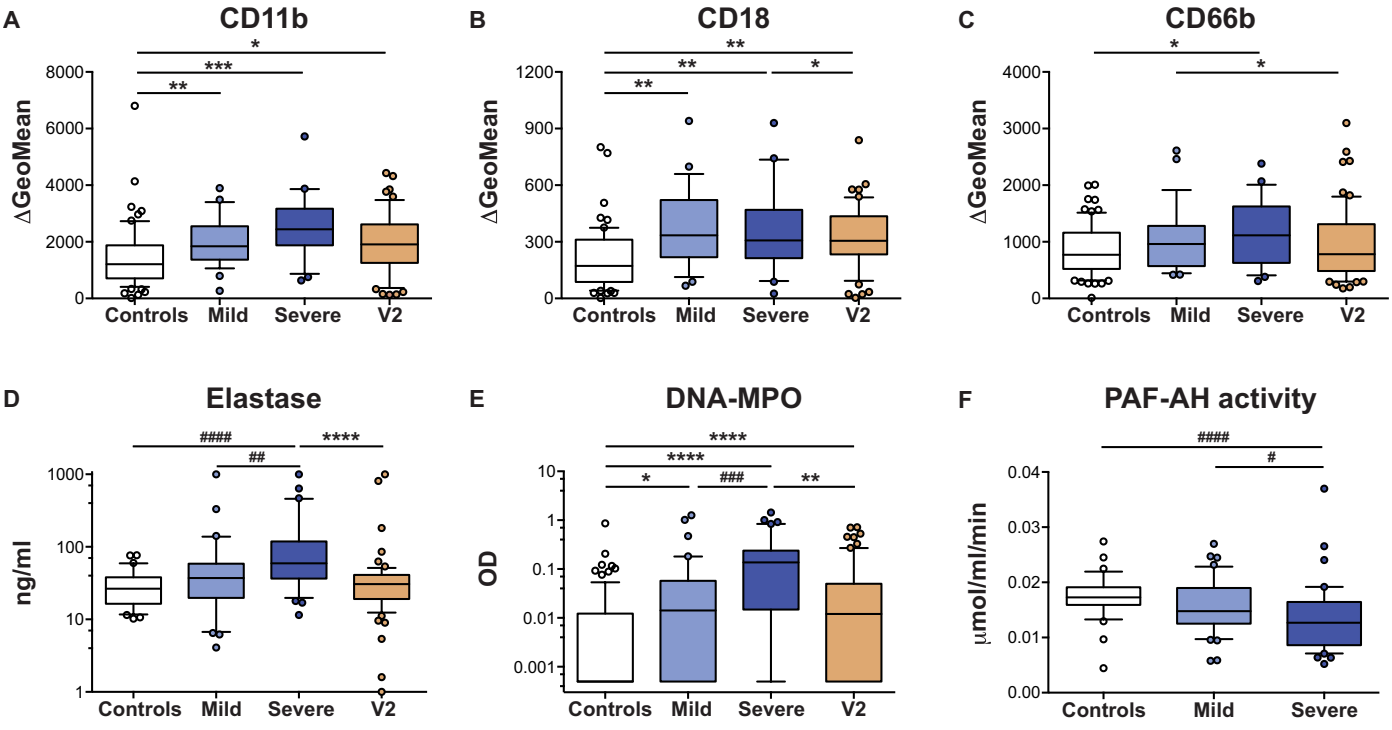


**Fig. 3. Circulating neutrophils are activated during AHR.** (**A** to **C**) Surface expression on circulating neutrophils of activation markers (A) CD11b, (B) CD18, and (C) CD66b expressed as geometric mean of fluorescence intensity after subtraction of isotype control. (**D**) Neutrophil elastase in the plasma of patients 30 min after AHR occurrence in patients with mild ($n = 37$) or severe ($n = 32$) AHR, at V2 ($n = 69$) or in healthy controls ($n = 30$). (**E**) Circulating NETs measured as DNA-MPO complexes in the serum of patients 30 min after AHR occurrence in patients with mild ($n = 43$) or severe ($n = 43$) AHR, at V2 ($n = 86$) or in matched controls ($n = 86$). OD, optical density. (**F**) Activity of PAF-AH in the plasma of patients 30 min after AHR occurrence in patients with mild ($n = 42$) or severe ($n = 42$) AHR or of healthy controls ($n = 33$). Wilcoxon signed-rank test, ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Mann-Whitney $U$ test, ####$P < 0.0001$, ###$P < 0.001$, ##$P < 0.01$, #$P < 0.05$. All boxplot whiskers represent 10th and 90th percentiles.

### IgG-dependent neutrophil activation can occur concomitantly with or independently of IgE-mediated anaphylaxis

Patients experiencing severe AHR had higher anti-QAM IgE and decreased FcεRI expression on basophils compared to controls (Figs. 1 and 2), as well as elevated histamine and tryptase concentrations (table S2). The same patients also had higher anti-QAM IgG concentration, decreased FcγR expression, and greater evidence of neutrophil activation and degranulation (Figs. 1 to 3) compared to controls. Patients with severe AHR thus presented markers typical of both IgE- and IgG-dependent anaphylaxis. To assess whether an IgG-dependent pathway could exist independently of the IgE-dependent pathway, we evaluated the presence of IgG pathway markers relative to the likelihood of IgE-dependent activation. To this end, we classified evidence for an IgE-dependent anaphylactic reaction as "absent" ($n = 27$, 31%), "equivocal" ($n = 19$, 22%), or "strong" ($n = 40$, 47%) for each patient based on clinical data, skin tests, and biological tests used in standard care by a consortium of experts blinded to the IgG status of the patients (*33*). Only the strong patient subgroup presented significantly reduced FcεRI expression on basophils compared to controls, absent or equivocal groups ($P = 0.001$, $P = 0.003$, and $P = 0.01$, respectively; Fig. 4A). The strong patient group had higher anti-NMBA IgG (Fig. 4B), lower CD32B expression on basophils (Fig. 4C), lower CD32A expression on monocytes (Fig. 4D), lower CD32A and CD16 expression on neutrophils (Fig. 4, E and F), and higher circulating elastase and DNA-MPO complexes concentrations (Fig. 4, G and H) compared to controls. This group also showed higher CD11b, CD18, and CD66b expression on neutrophils (Fig. 4, I to K) and lower PAF-AH activity (Fig. 4L). Furthermore, the strong patient group also had significantly higher anti-NMBA IgG, elastase, and NET release and lower CD32A and CD16 expression on neutrophils than the absent group ($P = 0.0001$, $P = 0.02$, $P = 0.03$, $P = 0.005$, and $P = 0.02$, respectively; Fig. 4, B and E to H). In addition, patients in the strong group presenting with severe AHR had higher anti-NMBA IgG ($P = 0.003$), circulating NETs ($P = 0.004$), neutrophil CD11b expression ($P = 0.03$), and lower neutrophil CD32A expression ($P = 0.0009$) than those with mild AHR (fig. S6). These results substantiate the coexistence of IgE- and IgG-dependent mechanisms in patients with AHR with evidence of IgE-dependent anaphylaxis, suggesting that both pathways may contribute to AHR induction and severity. Moreover, the absent group, which would usually be categorized as idiopathic in standard care, had significantly more circulating NETs, higher expression of CD11b and CD18 (all $P < 0.01$), but no difference in FcεRI expression compared to the control group (Fig. 4, A, E, and H to K). Thus, we document neutrophil activation during NMBA-induced AHR in patients for whom evidence of an IgE-dependent pathway is lacking despite clinical signs compatible with anaphylaxis. These results substantiate the existence of an autonomous IgG-dependent mechanism that could lead to severe reactions, because 26% of patients in the absent group developed severe AHR.

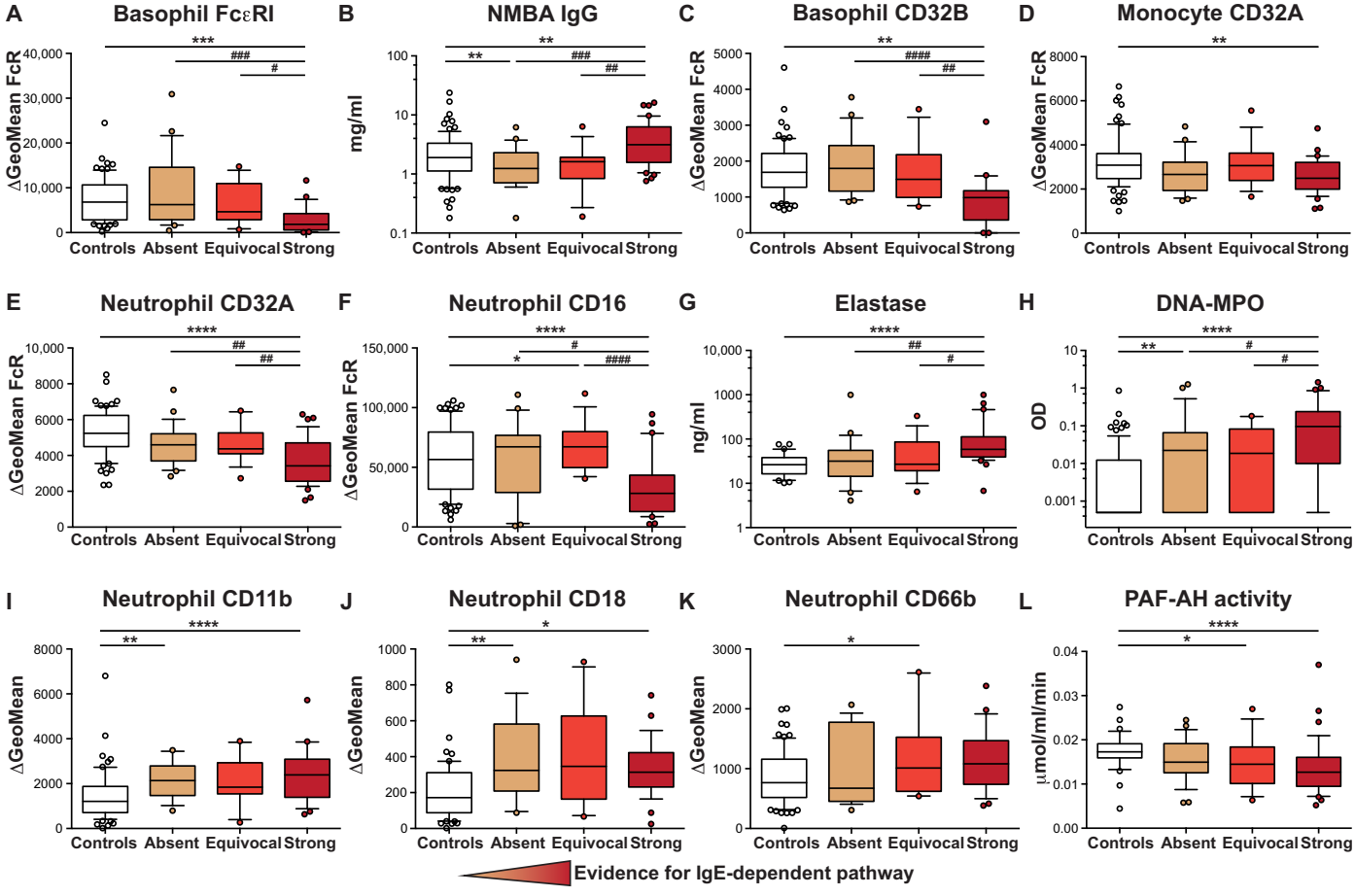


**Fig. 4. Evidence of an IgG-neutrophil-PAF pathway in the presence or absence of IgE-mediated anaphylaxis markers.** Evidence for IgE-dependent anaphylaxis was classified as absent ($n = 27$), equivocal ($n = 19$), or strong ($n = 40$) for each patient of the NASA cohort. Data from Figs. 1 to 3 were reanalyzed on the basis of these criteria, and (**A**) FcεRI expression on basophils, (**B**) anti-NMBA IgG, (**C**) CD32B expression on basophils, (**D**) CD32A expression on monocytes, (**E**) CD32A, (**F**) CD16 expression on neutrophils, circulating (**G**) neutrophil elastase, (**H**) NETs (DNA-MPO complexes), (**I**) CD11b, (**J**) CD18, (**K**) CD66b expression on neutrophils, and (**L**) plasma PAF-AH activity were compared between controls and patients with absent, equivocal, or strong evidence for IgE-dependent anaphylaxis. Wilcoxon signed-rank test, ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Mann-Whitney $U$ test, ####$P < 0.0001$, ###$P < 0.001$, ##$P < 0.01$, #$P < 0.05$. All boxplot whiskers represent 10th and 90th percentiles.

## Patient-derived anti-NMBA IgG activates neutrophils ex vivo in the presence of NMBA

ICs formed by specific antibodies binding directly to small chemical entities such as NMBAs (with molecular masses of 290, 530, and 929 Da for suxamethonium, rocuronium, and atracurium, respectively) are not expected by themselves to be sufficiently large or multivalent to activate cells through FcR triggering. Rather, larger molecules such as serum proteins may serve as substrates for NMBA haptenization, but the precise in vivo chemistry remains unknown (*38*). It has been reported that the free fraction of suxamethonium and rocuronium are reduced to 80 and 54%, respectively, after infusion (*39*). We therefore investigated the potential interaction of these NMBAs with abundant human serum proteins using microscale thermophoresis (MST). Rocuronium, but not suxamethonium, was found to interact with human serum albumin (HSA; a known carrier of basic compounds), orosomucoid (a known carrier of acidic and neutral compounds), and transferrin but not with human IgG1 (fig. S7, A to D). The affinity of all these interactions was very poor, however, necessitating NMBA concentrations in the range of 1 to 4 mM (fig. S7, E to H), suggesting that at standard intubation doses of rocuronium (0.6 to 1.2 mg/kg, estimated to correspond to ~18 to 36 μM in circulation), other molecules may be the partners for NMBA haptenization.

In the absence of an identified protein partner in human serum for rocuronium haptenization, we developed a set of tools, including purified human anti-rocuronium antibodies, rocuronium and succinylmonocholine-albumin bioconjugates, and an anti-rocuronium enzyme-linked immunosorbent assay (ELISA), to investigate the potential for anti-NMBA IgG ICs to activate FcγR-expressing neutrophils. Anti-rocuronium IgG ELISA permitted the identification of four patients with high serum concentrations of rocuronium-specific antibodies (Fig. 5A), of whom one patient (#7016; mild AHR after atracurium administration; refer to table S1) consented for additional blood sampling. Anti-rocuronium IgG purified from this patient's plasma (fig. S8A) was confirmed to bind to rocuronium and also cross-reacted with some other ammonium-containing molecules by ELISA, i.e., morphine antigen bioconjugates and suxamethonium bioconjugates, but not other NMBAs or unrelated antigens or allergens (Fig. 5B). In addition, the binding of these purified IgGs could be dose-dependently inhibited using free rocuronium or suxamethonium in solution but not by the rocuronium-analog molecule 5-α-dihydrotestosterone that lacks quaternary

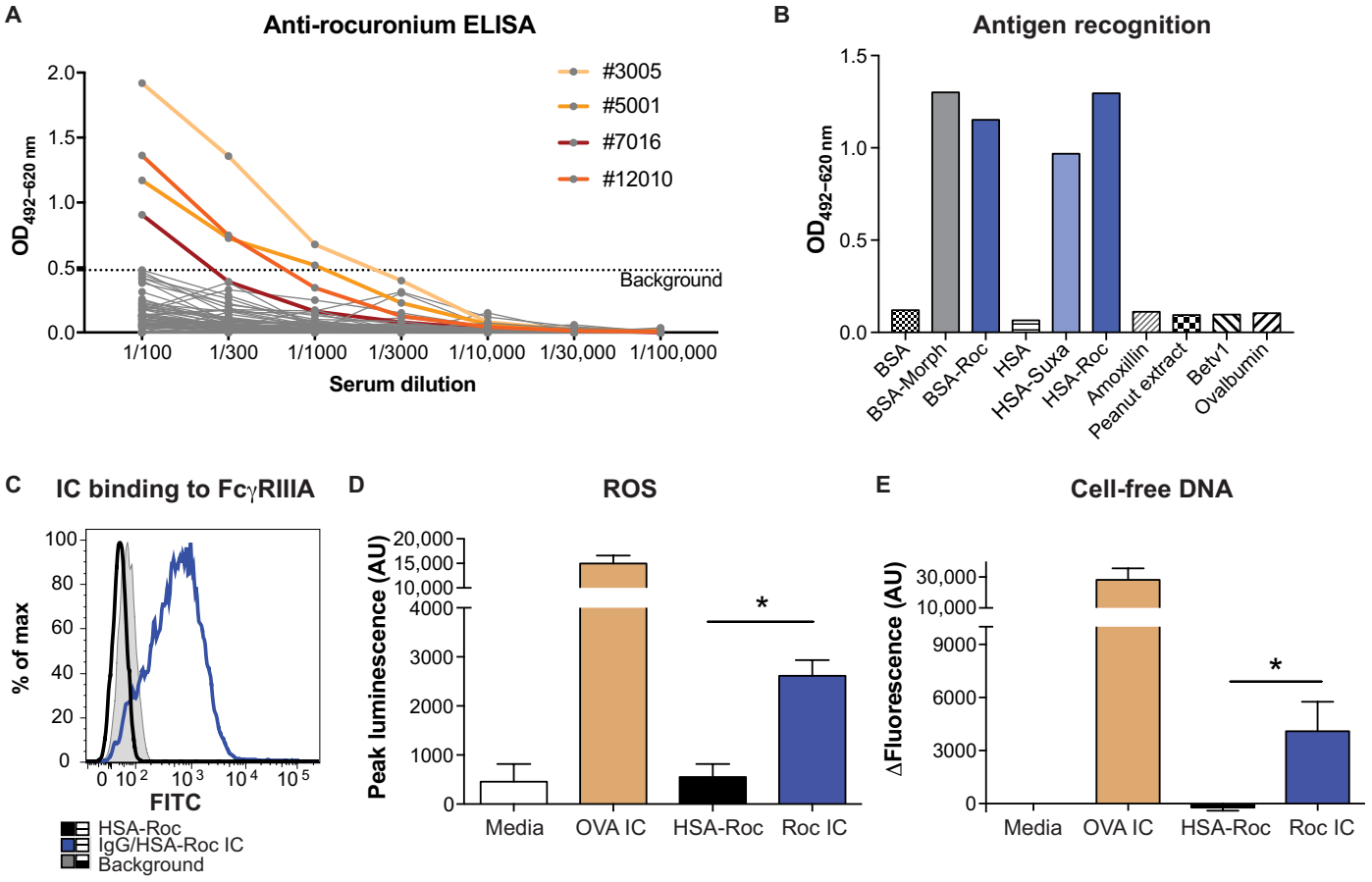


**Fig. 5. NMBA–anti-NMBA IgG complexes activate neutrophils ex vivo.** (**A**) Anti-rocuronium ELISA; four patients with high rocuronium-specific IgG antibody indicated with colored lines. (**B**) Specificity of purified anti-rocuronium IgG from patient #7016 measured by ELISA against indicated protein antigens and their morphine (Morph), suxamethonium (Suxa), and rocuronium (Roc) derivatives or against common allergens: amoxicillin, peanut extract, Bet v 1 (the major birch pollen antigen, *Betula verrucosa*) and ovalbumin (OVA). One of two independent experiments is shown. (**C**) Flow cytometry analysis of the binding of (anti-rocuronium IgG + rocuronium-HSA) ICs to CHO cell transfectants expressing human CD16A/FcγRIIIA variant $V_{176}$ (blue line), compared to rocuronium-HSA alone (black line), and unstained cells (gray-shaded histogram), one of three independent experiments. (**D** and **E**) Fluorescent-based measurement of (D) ROS or (E) cell-free DNA (NETs) released by purified neutrophils from healthy donors ($n = 4$), either left untreated (media) or incubated with control ICs (OVA IC), rocuronium-HSA, or (anti-rocuronium IgG + rocuronium-HSA) complexes (Roc IC). Error bars represent SD. Mann-Whitney *U* test, $^*P < 0.01$. AU, arbitrary units.

ammonium groups nor by atracurium, cis-atracurium, pancuronium, or vecuronium (fig. S8B). This confirms a binding preference of these purified IgGs to rocuronium and suxamethonium, but no cross-reactivity to the other four NMBAs tested. Purified anti-rocuronium IgG could form ICs with rocuronium bioconjugate and bind to Chinese hamster ovary (CHO) cell transfectants expressing CD16A (Fig. 5C). Last, human neutrophils from healthy controls produced reactive oxygen species (ROS) and released NETs when incubated on plate-bound rocuronium ICs (Fig. 5, D and E); moreover, they up-regulated CD11b and CD66b expression and produced ROS when incubated with soluble rocuronium ICs (fig. S9). Thus, anti-rocuronium IgG isolated from patients with AHR can directly activate human neutrophils in the presence of the culprit NMBA.

## DISCUSSION

This study provides several converging lines of evidence for the contribution of an IgG-FcγR-neutrophil–dependent pathway to human anaphylaxis using NMBA-induced AHR as a case study. Such a pathophysiological mechanism entails the presence of IgG antibodies against NMBAs in patients, leading, together with administered NMBA, to the formation of ICs that primarily engage two activating FcγRs on neutrophils, CD32A and CD16. Consequently, neutrophils become activated as shown by increased expression of activation markers, degranulation of neutrophil elastase, and elevated circulating NETs. Presumably, such an IgG-dependent pathway leads to the release of the highly potent lipid mediator PAF. The IgG pathway may worsen AHR in concert with IgE-dependent immune activation or could even be responsible for AHR induction in the absence of IgE-mediated reactions.

Anaphylaxis induction by pathogenic IgG rather than IgE antibodies has been demonstrated by many groups in animal models (*15*, *17*, *20*). Moreover, IgG has been implicated in rare clinical studies on anaphylaxis induced by high molecular weight or protein antigens, such as therapeutic antibodies, dextran, protamine, or von Willebrand factor (*3*–*8*, *40*). Patients with elevated anti-infliximab or anti-protamine IgG antibody concentrations have a greater risk of developing anaphylaxis during treatment (*7*, *8*, *41*), suggesting a direct role for IgG in anaphylaxis pathogenesis. This study reports a potential IgG pathway in human anaphylaxis caused by a low–molecular

weight (<1 kDa) antigen, NMBAs. The commonality between previous clinical studies and the current report is that the culprit antigen is administered intravenously in rather large quantities, favoring the formation of ICs in circulation. Our study reports low-affinity interactions between rocuronium and prominent human plasma proteins (HSA, orosomucoid, and transferrin), yet these millimolar range interactions cannot explain interactions that form when NMBAs circulate at micromolar concentrations in patients' plasma. Hence, the in vivo haptenization chemistry of NMBAs remains unknown, along with the identity of putative carrier molecule(s) that may enable NMBA IgG ICs to form and activate FcγR-expressing myeloid cells.

Thirty-one percent of patients with AHR in our study lacked biomarkers of IgE-dependent anaphylaxis, of which 26% had severe reactions: These data corroborate the existence of alternative, IgE-independent pathways of NMBA anaphylaxis. An entirely different, and antibody-independent, pathway of anaphylaxis has been reported after cationic drug administration, involving an interaction with Mas-related G-protein coupled receptor member X2 on mast cells (*42*, *43*). Nevertheless, such a phenomenon cannot explain the "non-IgE" reactions we observed: Among the three culprit NMBAs in our study, only atracurium and rocuronium, but not suxamethonium, could potentially induce such a response and, if so, would result in positivity for some IgE-dependent biomarkers, such as elevated histamine concentrations and positive skin tests (*43*).

Unexpectedly, concentrations of anti-QAM IgG were similar between the AHR population and the controls, although patients with severe AHR had higher anti-QAM IgG than those with mild AHR. The latter observation is in line with the principle of dose/concentration-dependent formation of ICs and may contribute to AHR severity. As quaternary ammonium groups and similar moieties are present in a large number of household products, hair dyes, and anticough medication (*44*), positivity for anti-QAM IgG likely reflects exposure of the general population to these compounds. It remains unclear, however, why controls with high anti-QAM IgG do not develop AHR after NMBA infusion. Anti-QAM IgG antibodies could have different affinities in patients versus controls, which could affect both their capacity to form ICs in the presence of NMBA and to compete with IgE for NMBA binding (*45*). Supporting this notion, this cohort demonstrates a positive correlation between high concentrations of anti-QAM IgE and IgG, both in patients and in the rare controls with positive anti-QAM IgE. Using a combination of clinically validated tests and research-grade assays not yet validated for clinical practice, our analyses revealed that specific IgE concentrations for suxamethonium, atracurium, and rocuronium also tended to be higher in samples initially determined as high for anti-QAM IgE. This also appears to be the case for IgG, particularly for suxamethonium-specific IgG, although no clinically relevant threshold of "positivity" for anti-NMBA IgG in these assays has ever been established.

Neutrophils from patients with severe AHR early after reaction onset demonstrated increased expression of activation markers CD18, CD11b, and CD66b compared to patients with mild AHR. These data reveal the activation of neutrophils during NMBA-induced AHR in humans. In addition, the severity of AHR correlated with elevated neutrophil-specific circulating markers: elastase and NETs. A role for neutrophils in anaphylaxis has previously been reported in animal models (*19*, *46*–*48*). Circulating NETs and expression of CD11b and CD18 were also higher in patients with mild AHR than controls, perhaps indicating that these are more sensitive markers of neutrophil activation than circulating elastase or CD66b expression. Considering patient subgroups according to the NMBA received an increase in CD18 and CD11b on neutrophils remained highly significant when examining suxamethonium-infused patients but was less apparent in atracurium-infused patients. Antibody-independent alternative mechanisms reported for AHR induction by atracurium (*49*) may be confounding factors in the latter analysis. The following two key findings strongly suggest the direct activation of neutrophils as a consequence of their interaction with NMBA-IgG ICs: first, the down-regulation of FcγR CD32A and CD16B on neutrophils from patients with AHR and, second, the in vitro demonstration that NMBA-IgG ICs induce neutrophils to produce ROS and NETs. Less pronounced CD32A down-regulation on monocytes from patients with severe AHR suggests concomitant monocyte activation directly by NMBA-IgG complexes, of lesser magnitude, which may also contribute to mediator release and AHR induction/severity. Stimulation of neutrophils and monocytes from CD32A transgenic mice via CD32A results in the production of PAF and other lipid mediators (*25*) and, more generally, engagement of CD32A by IgG ICs results in activation of cells in vitro and in vivo (*50*, *51*), including NET release (*52*). When analyzing suxamethonium-infused or atracurium-infused patients, only the down-regulation of CD32A on neutrophils remained highly significant. Together, these findings suggest CD32A as a marker for IgG-dependent severe AHR, at least those induced by suxamethonium and atracurium.

The relative contribution of cell subsets to anaphylaxis has been described using depletion studies in mouse models (*15*, *17*, *19*, *53*) and is highly dependent on the model used (*23*, *54*, *55*). Our previous reports using a mouse model expressing human FcγRs (CD32A, CD32B, CD16A, and CD16B) in place of mouse FcγRs showed neutrophils to be predominant (*24*), whereas using mice expressing only human CD32A showed neutrophils and monocytes to contribute equally (*25*). The very high coexpression of CD16B with CD32A on neutrophils may favor neutrophil activation over monocytes during the induction phase of anaphylaxis and explain both the results obtained in mice (*24*) and the clinical results presented herein. IgG-dependent pathways may thus preferentially trigger neutrophils, whereas IgE-dependent pathways may preferentially trigger mast cells (*9*, *56*). The main anaphylactogenic mediators reported for these two modalities are PAF (*19*, *20*) and histamine (*30*, *57*), respectively. Although neutrophils represent by far the largest cell population in circulation and a major source of PAF, most cell subsets that have been proposed to contribute to anaphylaxis can produce PAF, including IgG IC–stimulated monocytes and basophils, and IgE-stimulated mast cells (*58*–*60*). The reduction in PAF-AH activity we observed in patients with severe AHR indirectly substantiates elevated PAF concentrations during NMBA-AHR. In addition, we observed consumption of complement components during severe AHR, suggesting that complement may also contribute to the severity of anaphylaxis (*61*–*63*) by potentiating myeloid cell activation, in conjunction with PAF, histamine, and other mediators. This observation needs, however, to be confirmed in additional studies because a complement consumption could also be a consequence of general anesthesia (*64*).

Circulating DNA-MPO complexes are markers of NETs, and here, we identify that their concentrations in circulation are associated with AHR severity. Although NETs have been implicated in some

allergic contexts, such as asthma (*65*), they have not been described in anaphylaxis. NETs may contribute to AHR directly by toxic effects of their components on tissues and epithelia (*66*), thereby impeding organ function. Alternatively, the involvement of NETs in AHR may be via the activation of the coagulation contact system (*67*), which has been linked to the severity of anaphylaxis (*68*). Elevated circulating NETs were detected in patients during the acute phase of AHR and remained even 6 to 8 weeks later, whether considering all patients or subgroups infused with suxamethonium only or with atracurium only. Not only the NET concentrations but also the expression of neutrophil activation markers (CD11b and CD18) were elevated in patients at the time of the follow-up visit 6 to 8 weeks after AHR, a finding which was also significant when analyzing only patients infused with suxamethonium. Such a long-lasting effect of AHR on neutrophil activation status is in accordance with one study, which found an elevated transcription of neutrophil-related genes in patients with a previous episode of anaphylaxis (*69*).

Our study carries some limitations due to the design and observational nature. First, we report here a consistent correlation between several IgG-dependent markers and anaphylaxis severity, but we cannot prove direct causality between neutrophil activation and anaphylaxis. Second, basophils from patients with severe AHR demonstrated significantly lower FcεRI expression during the anaphylaxis event than during the allergology visit 6 to 8 weeks after AHR. Whereas fast down-regulation of FcεRI after IgE-allergen triggering has been reported on rat and mouse mast cells (*70*), reports do not support the existence of such a consequence on human basophils (*71*, *72*). This discrepancy might relate to a different behavior of human basophils in vivo than in vitro, to the nature of allergen aggregating the IgE-FcεRI complex, to FcγRs dragging IgE-bound FcεRI inside the basophil after interaction with IgG-allergen complexes, or even to an unknown bystander effect of anaphylaxis. Third, although we classified our patients according to the likelihood of an IgE-dependent reaction, we did not account for other reactions, e.g., pharmacological activation of mast cells (*43*) or nonspecific histamine release (*73*), which can represent alternative mechanisms of anaphylaxis induction in the absence of specific IgE. Fourth, our IC binding study was performed on a chemically modified drug that does not exist in vivo. Last, because of technical limitations, we could not measure directly PAF or circulating ICs in patients.

In conclusion, this work defines a clinically relevant pathway of NMBA-induced AHR, involving IgG, FcγRs, and neutrophils. This pathway may coexist with the classical IgE-dependent pathway and compound the severity of IgE-associated "allergic" AHR. It could also be responsible for AHR in patients with no markers of IgE-dependent immune activation, previously designated "nonallergic" AHR (*74*). Furthermore, in patients showing strong evidence for engagement of the IgE-dependent pathway, IgG markers remained strongly associated with anaphylaxis severity, indicating that simultaneous engagement of IgG- and IgE-dependent mechanisms may occur during human anaphylaxis. Our data suggest that these pathways can combine to worsen AHR. We predict that our findings can be extrapolated to most AHR wherein the culprit allergen(s) are present in the circulation. Evaluation of IgG pathway markers in the clinical AHR diagnostic process may clarify diagnosis and be a way to risk-stratify patients. Our results also pave the way for novel therapeutic interventions, particularly aiming at FcγR blockade.

## MATERIALS AND METHODS

### Study design

The multicentric Neutrophil Activation in Systemic Anaphylaxis (NASA) study involved 11 anesthesia and intensive care departments in the Ile-de-France region in France (NCT01637220). The study was approved for all centers by an institutional review board (ethical committee "Comité de Protection des Personnes Ile-de-France 1," reference 2012-avril-12880) and registered before the first inclusion at ClinicalTrials.gov (NCT0163722). The design of the study and detailed clinical characteristics of the patients are described elsewhere (*33*). Briefly, patients with clinical signs consistent with intra-anesthetic AHR to NMBA were prospectively included between November 2012 and June 2014. Venous blood samples were collected at 30 min and 2 hours after the onset of the AHR and during the allergology-anesthesia visit 6 to 8 weeks after AHR. At this visit, immediate reading intradermal tests against suspected culprit agents were performed. Clinical, biological, and demographic patient data are summarized in Tables 1 and 2. Patients were classified a posteriori in grades of severity according to the Ring and Messmer (*34*) classification by two independent intensive care unit experts. A severe AHR was defined as a grade 3 or 4 (severe organ failure or cardiac/respiratory arrest), and a mild AHR was defined as a grade 1 or 2. Classifications of AHR according to evidence of an IgE-mediated pathway, i.e., strong, equivocal, or absent, were established a posteriori by a consortium of six experts taking in account clinical symptoms, tryptase, histamine, specific IgE, and results of skin tests. For each patient included, a control was recruited: a patient undergoing surgery with NMBA without any signs consistent with intra-anesthetic AHR. Controls were matched to patients for age (±5 years), sex, NMBA used, type of surgical procedure, and infectious status. Written informed consent was obtained from the patients and controls or their legal representative before study inclusion. By French law, in case of impaired decision-making capacity without a legal representative available at the time of inclusion, the patient's informed consent could be obtained after enrollment ("emergency inclusion"). Venous blood samples were collected 30 min after anesthesia for controls. Primary data are reported in data file S1.

### Classical anaphylaxis parameters

As part of standard care procedures, the following circulating parameters were assessed as previously described (*37*): histamine (enzyme immunoassay, Immunotech, Beckman Coulter) and tryptase [fluorescence enzyme immunoassay (FEIA), Phadia ImmunoCAP 250, Thermo Fisher Scientific] in plasma and antiquaternary ammonium-specific IgE (FEIA, Phadia ImmunoCAP 250, Thermo Fisher Scientific) in serum. A value less than 0.35 kU/liter of specific IgE was considered negative, as recommended (*37*). Tryptase concentrations 2 hours after AHR were considered elevated when greater than (1.2 × [baseline tryptase] + 2 μg/liter) as recently recommended (*75*). A histamine concentration above 20 nM 30 min after AHR was considered elevated. Data are summarized in table S2.

In addition, specific IgE concentrations for suxamethonium were assessed using the clinically validated suxamethonium ImmunoCAP (Phadia ImmunoCAP c202). Moderately sensitive ImmunoCAPs for atracurium (*76*) and rocuronium (*77*) were obtained as experimental prototypes made for research use (Phadia, Thermo Fisher Scientific). Atracurium ImmunoCAP is prepared using tetrahydropapaverine modified with β-propiolactone, as a surrogate for atracurium, before conjugation to poly-L-lysine (atracurium ImmunoCAP conjugate) (*78*).

To avoid commonly reported problems with the diagnosis of IgE-dependent reactions (false-negative tryptase/histamine measurements), blood sampling was carefully and uniformly performed across the study, and there was no difference in delay before blood sampling between patients with a negative or a positive plasma tryptase (Table 2). Specific IgE was analyzed on a single instrument, and skin tests were performed and analyzed by only three allergologists.

### Human plasma protein interactions with rocuronium

Protein binding of rocuronium was studied by MST measurements on a Monolith NT.LabelFree (NanoTemper). Rocuronium predilutions were prepared by 16-fold 2:1 serial dilutions of compound in phosphate-buffered saline (PBS) buffer to yield a range of 8 mM to 18 μM in a final volume of 10 μl. Lower concentrations were studied in preliminary experiments and did not show any binding. Rocuronium dilutions were mixed with 10 μl of target proteins (HSA, transferrin, orosomucoid, and IgG1), at 2 μM final each. Samples were loaded in standard treated capillaries (NanoTemper). Experiments were performed with MST power medium (20%) and light-emitting diode intensity of 10 or 20%. Each experiment was performed in duplicate and reproduced three times. Data were analyzed using MO Affinity Analysis software (NanoTemper).

### Specific IgG measurement

Anti-QAM IgG concentrations were measured using an adaptation of the ImmunoCAP matrix used to measure anti-QAM IgE concentrations [considered the most sensitive marker for IgE-induced NMBA-AHR (*37*)] (Phadia, Thermo Fisher Scientific). Because no reference interval exists for this parameter, raw values were evaluated relative to an IgG calibration curve and expressed in micrograms per milliliter. The secondary antibody used to detect IgG in the ImmunoCAP is a mouse monoclonal IgG1 antibody binding all four human IgG subclasses (Phadia, Thermo Fisher Scientific). The human IgE detection reagent was replaced with the same monoclonal anti-human IgG reagent in the clinically validated suxamethonium ImmunoCAP (Phadia ImmunCAP c202) or research-grade atracurium ImmunoCAP and rocuronium ImmunoCAP (Phadia) to detect anti-suxamethonium, anti-atracurium, or anti-rocuronium IgG, respectively.

### Flow cytometry analysis of Fc receptors and activation markers

Heparinized blood sampled during NMBA-induced AHR and at the patient's allergy visit, as well as blood from anesthetized matched controls, was immediately transported from the site of blood collection to the Institut Pasteur (Paris, France). Upon receipt, red blood cells were lysed using Ammonium-Chloride-Potassium (ACK) buffer, and white blood cells (WBCs) were washed in PBS/1% bovine serum albumin (BSA)/2 mM EDTA and incubated with indicated antibodies for 30 min at 4°C (table S3). Cells were washed and acquired on a MACSQuant flow cytometer (Miltenyi Biotec) and analyzed using FlowJo software (Tree Star). Blood samples showing signs of clotting or hemolysis, as well as samples during which processing problems occurred, were excluded from the analysis. WBC populations were identified as neutrophils ($CD15^+/CD193^-$), eosinophils ($SSC^{hi}/CD193^+$), basophils ($CD123^+/CD203c^{int/+}$ or $SSC^{lo}/CD193^+$ for Fig. 2), monocytes ($CD14^+/CD3^-$), B cells ($CD19^+/CD14^-$), T cells ($CD3^+/CD56^-$), or natural killer cells ($CD56^+/CD3^-$). The expression value for each marker was determined as the difference between the geometric mean of the fluorescence intensity of the marker and the geometric mean of the matched isotype control [delta geometric mean (ΔGeoMean)].

### Circulating NETs quantification

To quantify circulating NETs, DNA-MPO complexes were measured in serum samples using a capture ELISA described elsewhere (*33*). Briefly, 96-well microtiter plates were coated overnight with rabbit anti-human MPO antibody (5 μg/ml; Millipore). After blocking with 2% BSA, serum samples were added together with a peroxidase-labeled anti-DNA monoclonal antibody (component 2 of the Cell Death ELISA Kit, Roche). After incubation, the peroxidase substrate was added for 40 min at 37°C. Results are expressed as optical absorbance measured at 405 nm in a spectrophotometer.

### Production and characterization of rocuronium and suxamethonium bioconjugates

Hapten-protein couplings were performed using active ester derivatives of rocuronium or suxamethonium and HSA or BSA as the carrier protein. For the rocuronium derivative, a carboxylate function was first introduced by adding succinic anhydride to rocuronium bromide in the presence of 4-(dimethylamino)-pyridine in pyridine. After stirring overnight at room temperature under argon, the solvent was removed under vacuum. The crude compound was purified by reverse-phase flash chromatography (linear gradient, 10 to 80% of $CH_3CN$ + 0.1% trifluoroacetic acid in $H_2O$ + 0.1% trifluoroacetic acid over 60 min), resulting in a rocuronium derivative with a carboxylic acid at the 3-position of the steroid scaffold (81% yield). The compound was characterized by mass spectrometry (Q-Tofmicro spectrometer, Micromass) with an electrospray ionization (positive mode) source (Waters) ($C_{36}H_{57}N_2O_7$ calculated 629.4166, 315.2122; mass/charge ratio, 629.4183 $[M + H]^+$, 315.2085 $[M + 2H]^{2+}$) and by nuclear magnetic resonance analysis (table S4).

All quantities of products used in the protocol were calculated relative to the carrier protein. The functionalized rocuronium (300 equivalent) was dissolved in 0.1 M MES + 0.5 M NaCl buffer (pH 5.6) and combined with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1000 equivalent) in the presence of *N*-hydroxysulfosuccinimide (400 equivalent). After 5-min stirring at room temperature, pH was adjusted to 7.2 with 2 N NaOH. This solution was progressively added to the carrier protein dissolved in PBS 1× (pH 7.2). After 3 hours of gentle agitation at room temperature and extensive dialysis in PBS, the conjugated proteins were obtained at a 50% yield.

For the suxamethonium derivative, choline chloride was treated as previously reported (*79*) to generate a carboxylated intermediate succinyl-monocholine (93% yield), which was then conjugated with the proteins as described above (but at a 600 equivalent relative to the carrier protein). The resulting conjugates were obtained at 80 to 85% yield.

The exact measurement of the concentration of the bioconjugates was achieved by quantitative amino acid analysis using a Beckman 6300 analyzer after hydrolysis with 6 N HCl at 110°C for 20 hours. The average density of conjugated rocuronium and suxamethonium derivatives was evaluated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and was found to be 22 ± 10 and 42 ± 10, respectively, for the HSA conjugates.

### Ex vivo neutrophil activation by [anti-rocuronium IgG–rocuronium HSA bioconjugate] ICs and drugs used for anaphylaxis treatment

Neutrophils from healthy blood bank donors were purified using MACSxpress Neutrophil isolation kit (Miltenyi Biotec), which gave a purity routinely over 98%. Purified neutrophils were suspended at 1.5 × 106 cells/ml in Hank's balanced salt solution, and 100 µl of cells were seeded in 96-well plates for stimulation with plate-bound ICs or soluble ICs. Plate-bound ICs were prepared by coating 96-well plates with rocuronium-coupled HSA (HSA-Roc; 200 µg/ml), followed by incubation with patient-derived purified anti-rocuronium human polyclonal IgG (50 µg/ml). Soluble ICs were prepared by mixing HSA-(20)-Roc (5 µg/ml) with anti-rocuronium IgG (100 µg/ml). Positive controls were obtained by coating with ovalbumin (200 µg/ml), followed by rabbit polyclonal anti-ovalbumin antibodies (50 µg/ml). Negative controls were obtained by coating antigen alone, without further incubation with antibodies. Oxidative burst (ROS release) and NETosis were measured as previously described (*33*). Briefly, oxidative burst was measured by chemoluminescence at 37°C for 1 hour after addition of luminol (final concentration, 50 µM). Results are expressed as peak luminescence value. NETosis was measured by cell-free DNA release using Sytox Green DNA probe (final concentration, 5 µM; Thermo Fisher Scientific) at 37°C for 300 min. Results are expressed as 530-nm fluorescence normalized for baseline and unstimulated controls. Both luminescence and fluorescence were read on a microplate reader (TriStar LB 941, Berthold). Neutrophil activation was also tested after incubation in solution with epinephrine, ephedrine, methylprednisolone, atropine, and various resuscitation fluids using serial dilutions from drugs vials obtained from an intensive care unit.

### Statistical analyses

Paired comparisons (between patients and matched controls or between inclusion and follow-up visits) were performed with a paired Wilcoxon signed-rank test. Subgroups of patients were compared with a Mann-Whitney *U* test. Statistical tests were bilateral, and a type I error was fixed at 5%. Statistical analyses were performed with GraphPad Prism versions 5.0 and 7.0 (GraphPad Software Inc.), R version 3.0.2 software (R foundation for Statistical Computing), and SAS 9.2 (SAS Institute).

## SUPPLEMENTARY MATERIALS

stm.sciencemag.org/cgi/content/full/11/500/eaat1479/DC1
Materials and Methods
Fig. S1. Anti-QAM, anti-suxamethonium, anti-atracurium, and anti-rocuronium IgE and IgG ImmunoCAP.
Fig. S2. NMBA-dependent analyses of FcR expression.
Fig. S3. Complement pathway CH50, C3, and C4 during AHR versus V2.
Fig. S4. NMBA-dependent analyses of neutrophil CD11b and CD18 expression and circulating NETs.
Fig. S5. Effect of resuscitation fluids and drugs used during AHR on neutrophil activation.
Fig. S6. IgG markers in patients with strong evidence of IgE pathway.
Fig. S7. MST analyses of rocuronium and suxamethonium protein partners.
Fig. S8. Characterization of patient-derived anti-rocuronium IgG.
Fig. S9. Human neutrophil activation by soluble NMBA–anti-NMBA IgG ICs.
Table S1. Individual values of QAM IgE (kilounits per liter) and QAM IgG (micrograms per milliliter) for case-control pairs.
Table S2. Tryptase and histamine measurements.
Table S3. Flow cytometry antibodies.
Table S4. Nuclear magnetic resonance data of carboxylated rocuronium in $D_2O$.
Data file S1. Primary data.
Reference (*80*)

## REFERENCES AND NOTES


1. J. H. Lee, J. W. Kim, D. K. Kim, H. S. Kim, H. J. Park, D. K. Park, A.-R. Kim, B. Kim, M. A. Beaven, K. L. Park, Y. M. Kim, W. S. Choi, The Src family kinase Fgr is critical for activation of mast cells and IgE-mediated anaphylaxis in mice. *J. Immunol.* **187**, 1807–1815 (2011).
2. S. G. A. Brown, K. E. Blackman, R. J. Heddle, Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg. Med. Australas.* **16**, 120–124 (2004).
3. A. Cheifetz, M. Smedley, S. Martin, M. Reiter, G. Leone, L. Mayer, S. Plevy, The incidence and management of infusion reactions to infliximab: A large center experience. *Am. J. Gastroenterol.* **98**, 1315–1324 (2003).
4. A. P. Schmidt, H. F. Taswell, G. J. Gleich, Anaphylactic transfusion reactions associated with anti-IgA antibody. *N. Engl. J. Med.* **280**, 188–193 (1969).
5. H. Hedin, W. Richter, K. Messmer, H. Renck, K. G. Ljungström, H. Laubenthal, Incidence, pathomechanism and prevention of dextran-induced anaphylactoid/anaphylactic reactions in man. *Dev. Biol. Stand.* **48**, 179–189 (1980).
6. L. Bergamaschini, P. M. Mannucci, A. B. Federici, R. Coppola, S. Guzzoni, A. Agostoni, Posttransfusion anaphylactic reactions in a patient with severe von Willebrand disease: Role of complement and alloantibodies to von Willebrand factor. *J. Lab. Clin. Med.* **125**, 348–355 (1995).
7. C. Steenholdt, M. Svenson, K. Bendtzen, O. Ø. Thomsen, J. Brynskov, M. A. Ainsworth, Acute and delayed hypersensitivity reactions to infliximab and adalimumab in a patient with Crohn's disease. *J. Crohns Colitis* **6**, 108–111 (2012).
8. M. E. Weiss, D. Nyhan, Z. Peng, J. C. Horrow, E. Lowenstein, C. Hirshman, N. F. Adkinson Jr., Association of protamine IgE and IgG antibodies with life-threatening reactions to intravenous protamine. *N. Engl. J. Med.* **320**, 886–892 (1989).
9. F. D. Finkelman, M. V. Khodoun, R. Strait, Human IgE-independent systemic anaphylaxis. *J. Allergy Clin. Immunol.* **137**, 1674–1680 (2016).
10. H. C. Oettgen, T. R. Martin, A. Wynshaw-Boris, C. Deng, J. M. Drazen, P. Leder, Active anaphylaxis in IgE-deficient mice. *Nature* **370**, 367–370 (1994).
11. D. Dombrowicz, V. Flamand, I. Miyajima, J. V. Ravetch, S. J. Galli, J. P. Kinet, Absence of Fc epsilonRI alpha chain results in upregulation of Fc gammaRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between Fc epsilonRI and Fc gammaRIII for limiting amounts of FcR beta and gamma chains. *J. Clin. Invest.* **99**, 915–925 (1997).
12. I. Miyajima, D. Dombrowicz, T. R. Martin, J. V. Ravetch, J. P. Kinet, S. J. Galli, Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J. Clin. Invest.* **99**, 901–914 (1997).
13. I. H. Choi, Y. M. Shin, J. S. Park, M. S. Lee, E. H. Han, O. H. Chai, S. Y. Im, T. Y. Ha, H.-K. Lee, Immunoglobulin E-dependent active fatal anaphylaxis in mast cell-deficient mice. *J. Exp. Med.* **188**, 1587–1592 (1998).
14. F. D. Finkelman, Anaphylaxis: Lessons from mouse models. *J. Allergy Clin. Immunol.* **120**, 506–515 (2007).
15. H. Beutier, C. M. Gillis, B. Iannascoli, O. Godon, P. England, R. Sibilano, L. L. Reber, S. J. Galli, M. S. Cragg, N. Van Rooijen, D. A. Mancardi, P. Bruhns, F. Jonsson, IgG subclasses determine pathways of anaphylaxis in mice. *J. Allergy Clin. Immunol.* **139**, 269–280.e7 (2017).
16. M. V. Khodoun, Z. Y. Kucuk, R. T. Strait, D. Krishnamurthy, K. Janek, C. D. Clay, S. C. Morris, F. D. Finkelman, Rapid desensitization of mice with anti-FcγRIIb/FcγRIII mAb safely prevents IgG-mediated anaphylaxis. *J. Allergy Clin. Immunol.* **132**, 1375–1387 (2013).
17. R. T. Strait, S. C. Morris, M. Yang, X. W. Qu, F. D. Finkelman, Pathways of anaphylaxis in the mouse. *J. Allergy Clin. Immunol.* **109**, 658–668 (2002).
18. D. Jiao, Y. Liu, X. Lu, B. Liu, Q. Pan, Y. Liu, Y. Liu, P. Zhu, N. Fu, Macrophages are the dominant effector cells responsible for IgG-mediated passive systemic anaphylaxis challenged by natural protein antigen in BALB/c and C57BL/6 mice. *Cell. Immunol.* **289**, 97–105 (2014).
19. F. Jönsson, D. A. Mancardi, Y. Kita, H. Karasuyama, B. Iannascoli, N. Van Rooijen, T. Shimizu, M. Daëron, P. Bruhns, Mouse and human neutrophils induce anaphylaxis. *J. Clin. Invest.* **121**, 1484–1496 (2011).
20. Y. Tsujimura, K. Obata, K. Mukai, H. Shindou, M. Yoshida, H. Nishikado, Y. Kawano, Y. Minegishi, T. Shimizu, H. Karasuyama, Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* **28**, 581–589 (2008).
21. P. Bruhns, F. Jonsson, Mouse and human FcR effector functions. *Immunol. Rev.* **268**, 25–51 (2015).
22. R. Molfetta, F. Gasparrini, A. Santoni, R. Paolini, Ubiquitination and endocytosis of the high affinity receptor for IgE. *Mol. Immunol.* **47**, 2427–2434 (2010).
23. M. V. Khodoun, R. Strait, L. Armstrong, N. Yanase, F. D. Finkelman, Identification of markers that distinguish IgE- from IgG-mediated anaphylaxis. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 12413–12418 (2011).
24. C. M. Gillis, F. Jonsson, D. A. Mancardi, N. Tu, H. Beutier, N. Van Rooijen, L. E. Macdonald, A. J. Murphy, P. Bruhns, Mechanisms of anaphylaxis in human low-affinity IgG receptor locus knock-in mice. *J. Allergy Clin. Immunol.* **139**, 1253–1265.e14 (2017).

25. F. Jönsson, D. A. Mancardi, W. Zhao, Y. Kita, B. Iannascoli, H. Khun, N. van Rooijen, T. Shimizu, L. B. Schwartz, M. Daëron, P. Bruhns, Human FcγRIIA induces anaphylactic and allergic reactions. *Blood* **119**, 2533–2544 (2012).
26. V. Papayannopoulos, Neutrophil extracellular traps in immunity and disease. *Nat. Rev. Immunol.* **18**, 134–147 (2018).
27. G. Z. Lotner, J. M. Lynch, S. J. Betz, P. M. Henson, Human neutrophil-derived platelet activating factor. *J. Immunol.* **124**, 676–684 (1980).
28. E. Jouvin-Marche, E. Ninio, G. Beaurain, M. Tence, P. Niaudet, J. Benveniste, Biosynthesis of Paf-acether (platelet-activating factor). VII. Precursors of Paf-acether and acetyl-transferase activity in human leukocytes. *J. Immunol.* **133**, 892–898 (1984).
29. S. Ishii, T. Kuwaki, T. Nagase, K. Maki, F. Tashiro, S. Sunaga, W.-H. Cao, K. Kume, Y. Fukuchi, K. Ikuta, J.-i. Miyazaki, M. Kumada, T. Shimizu, Impaired anaphylactic responses with intact sensitivity to endotoxin in mice lacking a platelet-activating factor receptor. *J. Exp. Med.* **187**, 1779–1788 (1998).
30. K. Arias, M. Baig, M. Colangelo, D. Chu, T. Walker, S. Goncharova, A. Coyle, P. Vadas, S. Waserman, M. Jordana, Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *J. Allergy Clin. Immunol.* **124**, 307–314.e2 (2009).
31. P. Vadas, M. Gold, B. Perelman, G. M. Liss, G. Lack, T. Blyth, F. E. Simons, K. J. Simons, D. Cass, J. Yeung, Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N. Engl. J. Med.* **358**, 28–35 (2008).
32. P. Vadas, B. Perelman, G. Liss, Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis. *J. Allergy Clin. Immunol.* **131**, 144–149 (2013).
33. A. Gouel-Cheron, L. de Chaisemartin, F. Jönsson, P. Nicaise-Roland, V. Granger, A. Sabahov, M.-T. Guinnepain, S. Chollet-Martin, P. Bruhns, C. Neukirch, D. Longrois; NASA study group, Low end-tidal $CO_2$ as a real-time severity marker of intra-anaesthetic acute hypersensitivity reactions. *Br. J. Anaesth.* **119**, 908–917 (2017).
34. J. Ring, K. Messmer, Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* **309**, 466–469 (1977).
35. B. A. Baldo, M. M. Fisher, Substituted ammonium ions as allergenic determinants in drug allergy. *Nature* **306**, 262–264 (1983).
36. H. Chou, M. F. Tam, S.-S. Lee, R.-B. Tang, T.-H. Lin, H.-Y. Tai, Y.-S. Chen, H.-D. Shen, Asp159 is a critical core amino acid of an IgE-binding and cross-reactive epitope of a dust mite allergen Der f 7. *Mol. Immunol.* **48**, 2130–2134 (2011).
37. D. Laroche, S. Chollet-Martin, P. Leturgie, L. Malzac, M.-C. Vergnaud, C. Neukirch, L. Venemalm, J.-L. Guéant, P. N. Roland, Evaluation of a new routine diagnostic test for immunoglobulin E sensitization to neuromuscular blocking agents. *Anesthesiology* **114**, 91–97 (2011).
38. B. A. Baldo, M. M. Fisher, N. H. Pham, On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: An immunochemical perspective. *Clin. Exp. Allergy* **39**, 325–344 (2009).
39. J. J. Roy, F. Varin, Physicochemical properties of neuromuscular blocking agents and their impact on the pharmacokinetic-pharmacodynamic relationship. *Br. J. Anaesth.* **93**, 241–248 (2004).
40. L. Bergamaschini, T. Santangelo, A. Faricciotti, N. Ciavarella, P. M. Mannucci, A. Agostoni, Study of complement-mediated anaphylaxis in humans. The role of IgG subclasses (IgG1 and/or IgG4) in the complement-activating capacity of immune complexes. *J. Immunol.* **156**, 1256–1261 (1996).
41. F. Baert, M. Noman, S. Vermeire, G. Van Assche, G. D'Haens, A. Carbonez, P. Rutgeerts, Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N. Engl. J. Med.* **348**, 601–608 (2003).
42. H. Subramanian, K. Gupta, H. Ali, Roles of Mas-related G protein–coupled receptor X2 on mast cell–mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. *J. Allergy Clin. Immunol.* **138**, 700–710 (2016).
43. B. D. McNeil, P. Pundir, S. Meeker, L. Han, B. J. Undem, M. Kulka, X. Dong, Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* **519**, 237–241 (2015).
44. E. Florvaag, S. G. Johansson, The pholcodine case. Cough medicines, IgE-sensitization, and anaphylaxis: A devious connection. *World Allergy Organ. J.* **5**, 73–78 (2012).
45. L. K. James, S. J. Till, Potential mechanisms for IgG4 inhibition of immediate hypersensitivity reactions. *Curr. Allergy Asthma Rep.* **16**, 23 (2016).
46. H. Z. Movat, T. Uriuhara, N. S. Taichman, H. C. Rowsell, J. F. Mustard, The role of PMN-leucocyte lysosomes in tissue injury, inflammation and hypersensitivity. VI. The participation of the PMN-leucocyte and the blood platelet in systemic aggregate anaphylaxis. *Immunology* **14**, 637–648 (1968).
47. B. Revenäs, G. Smedegård, T. Saldeen, The pulmonary reaction in aggregate anaphylaxis in the monkey. *Acta Chir. Scand. Suppl.* **499**, 161–170 (1980).
48. G. Smedegård, B. Revenäs, T. Saldeen, Aggregate anaphylaxis in the monkey: Haematological and histological findings. *Int. Arch. Allergy Appl. Immunol.* **61**, 117–124 (1980).
49. D. Spoerl, H. Nigolian, C. Czarnetzki, T. Harr, Reclassifying anaphylaxis to neuromuscular blocking agents based on the presumed patho-mechanism: IgE-mediated, pharmacological adverse reaction or "innate hypersensitivity"? *Int. J. Mol. Sci.* **18**, 1223 (2017).
50. S. E. McKenzie, S. M. Taylor, P. Malladi, H. Yuhan, D. L. Cassel, P. Chien, E. Schwartz, A. D. Schreiber, S. Surrey, M. P. Reilly, The role of the human Fc receptor FcγRIIA in the immune clearance of platelets: A transgenic mouse model. *J. Immunol.* **162**, 4311–4318 (1999).
51. N. Tsuboi, K. Asano, M. Lauterbach, T. N. Mayadas, Human neutrophil Fcγ receptors initiate and play specialized nonredundant roles in antibody-mediated inflammatory diseases. *Immunity* **28**, 833–846 (2008).
52. K. Chen, H. Nishi, R. Travers, N. Tsuboi, K. Martinod, D. D. Wagner, R. Stan, K. Croce, T. N. Mayadas, Endocytosis of soluble immune complexes leads to their clearance by FcγRIIIB but induces neutrophil extracellular traps via FcγRIIA in vivo. *Blood* **120**, 4421–4431 (2012).
53. B. Balbino, R. Sibilano, P. Starkl, T. Marichal, N. Gaudenzio, H. Karasuyama, P. Bruhns, M. Tsai, L. L. Reber, S. J. Galli, Pathways of immediate hypothermia and leukocyte infiltration in an adjuvant-free mouse model of anaphylaxis. *J. Allergy Clin. Immunol.* **139**, 584–596.e10 (2017).
54. R. Ahrens, H. Osterfeld, D. Wu, C.-Y. Chen, M. Arumugam, K. Groschwitz, R. Strait, Y.-H. Wang, F. D. Finkelman, S. P. Hogan, Intestinal mast cell levels control severity of oral antigen-induced anaphylaxis in mice. *Am. J. Pathol.* **180**, 1535–1546 (2012).
55. L. L. Reber, T. Marichal, K. Mukai, Y. Kita, S. M. Tokuoka, A. Roers, K. Hartmann, H. Karasuyama, K. C. Nadeau, M. Tsai, S. J. Galli, Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J. Allergy Clin. Immunol.* **132**, 881–888.e81-11 (2013).
56. S. G. Brown, S. F. Stone, D. M. Fatovich, S. A. Burrows, A. Holdgate, A. Celenza, A. Coulson, L. Hartnett, Y. Nagree, C. Cotterell, G. K. Isbister, Anaphylaxis: Clinical patterns, mediator release, and severity. *J. Allergy Clin. Immunol.* **132**, 1141–1149.e5 (2013).
57. Y. Makabe-Kobayashi, Y. Hori, T. Adachi, S. Ishigaki-Suzuki, Y. Kikuchi, Y. Kagaya, K. Shirato, A. Nagy, A. Ujike, T. Takai, T. Watanabe, H. Ohtsu, The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. *J. Allergy Clin. Immunol.* **110**, 298–303 (2002).
58. P. Gill, N. L. Jindal, A. Jagdis, P. Vadas, Platelets in the immune response: Revisiting platelet-activating factor in anaphylaxis. *J. Allergy Clin. Immunol.* **135**, 1424–1432 (2015).
59. J. M. Mencia-Huerta, R. A. Lewis, E. Razin, K. F. Austen, Antigen-initiated release of platelet-activating factor (PAF-acether) from mouse bone marrow-derived mast cells sensitized with monoclonal IgE. *J. Immunol.* **131**, 2958–2964 (1983).
60. L. B. Schwartz, Mediators of human mast cells and human mast cell subsets. *Ann. Allergy* **58**, 226–235 (1987).
61. J. Szebeni, F. M. Muggia, C. R. Alving, Complement activation by Cremophor EL as a possible contributor to hypersensitivity to paclitaxel: An in vitro study. *J. Natl. Cancer Inst.* **90**, 300–306 (1998).
62. M. R. Woolhiser, K. Brockow, D. D. Metcalfe, Activation of human mast cells by aggregated IgG through FcgammaRI: Additive effects of C3a. *Clin. Immunol.* **110**, 172–180 (2004).
63. M. Khodoun, R. Strait, T. Orekov, S. Hogan, H. Karasuyama, D. R. Herbert, J. Köhl, F. D. Finkelman, Peanuts can contribute to anaphylactic shock by activating complement. *J. Allergy Clin. Immunol.* **123**, 342–351 (2009).
64. R. E. Lewis Jr., J. M. Cruse, J. V. Richey, Effects of anesthesia and operation on the classical pathway of complement activation. *Clin. Immunol. Immunopathol.* **23**, 666–671 (1982).
65. M. Toussaint, D. J. Jackson, D. Swieboda, A. Guedán, T.-D. Tsourouktsoglou, Y. M. Ching, C. Radermecker, H. Makrinioti, J. Aniscenko, N. W. Bartlett, M. R. Edwards, R. Solari, F. Farnir, V. Papayannopoulos, F. Bureau, T. Marichal, S. L. Johnston, Host DNA released by NETosis promotes rhinovirus-induced type-2 allergic asthma exacerbation. *Nat. Med.* **23**, 681–691 (2017).
66. M. Saffarzadeh, K. Preissner, Fighting against the dark side of neutrophil extracellular traps in disease: Manoeuvres for host protection. *Curr. Opin. Hematol.* **20**, 3–9 (2013).
67. S. Oehmcke, M. Mörgelin, H. Herwald, Activation of the human contact system on neutrophil extracellular traps. *J. Innate Immun.* **1**, 225–230 (2009).
68. A. Sala-Cunill, J. Björkqvist, R. Senter, M. Guilarte, V. Cardona, M. Labrador, K. F. Nickel, L. Butler, O. Luengo, P. Kumar, L. Labberton, A. Long, A. Di Gennaro, E. Kenne, A. Jämsä, T. Krieger, H. Schluter, T. Fuchs, S. Flohr, U. Hassiepen, F. Cumin, K. McCrae, C. Maas, E. Stavrou, T. Renné, Plasma contact system activation drives anaphylaxis in severe mast cell–mediated allergic reactions. *J. Allergy Clin. Immunol.* **135**, 1031–1043.e6 (2015).
69. R. Muñoz-Cano, M. Pascal, J. Bartra, C. Picado, A. Valero, D.-K. Kim, S. Brooks, M. Ombrello, D. D. Metcalfe, J. Rivera, A. Olivera, Distinct transcriptome profiles differentiate nonsteroidal anti-inflammatory drug–dependent from nonsteroidal anti-inflammatory drug–independent food-induced anaphylaxis. *J. Allergy Clin. Immunol.* **137**, 137–146 (2016).
70. C. Uermösi, F. Zabel, V. Manolova, M. Bauer, R. R. Beerli, G. Senti, T. M. Kündig, P. Saudan, M. F. Bachmann, IgG-mediated down-regulation of IgE bound to mast cells: A potential novel mechanism of allergen-specific desensitization. *Allergy* **69**, 338–347 (2014).

71. D. MacGlashan Jr., M. Mogowski, L. M. Lichtenstein, Studies of antigen binding on human basophils. II. Continued expression of antigen-specific IgE during antigen-induced desensitization. *J. Immunol.* **130**, 2337–2342 (1983).
72. C. Kitzmuller, B. Nagl, S. Deifl, C. Walterskirchen, B. Jahn-Schmid, G. J. Zlabinger, B. Bohle, Human blood basophils do not act as antigen-presenting cells for the major birch pollen allergen Bet v 1. *Allergy* **67**, 593–600 (2012).
73. M. P. Hosking, R. L. Lennon, G. A. Gronert, Combined H1 and H2 receptor blockade attenuates the cardiovascular effects of high-dose atracurium for rapid sequence endotracheal intubation. *Anesth. Analg.* **67**, 1089–1092 (1988).
74. S. G. O. Johansson, J. O. Hourihane, J. Bousquet, C. Bruijnzeel-Koomen, S. Dreborg, T. Haahtela, M. L. Kowalski, N. Mygind, J. Ring, P. Van Cauwenberge, M. Van Hage-Hamsten, B. Wuthrich; EAACI (the European Academy of Allergology and Cinical Immunology) nomenclature task force, A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* **56**, 813–824 (2001).
75. J. Sprung, T. N. Weingarten, L. B. Schwartz, Presence or absence of elevated acute total serum tryptase by itself is not a definitive marker for an allergic reaction. *Anesthesiology* **122**, 713–714 (2015).
76. A. P. Uyttebroek, V. Sabato, C. H. Bridts, L. S. De Clerck, D. G. Ebo, Immunoglobulin E antibodies to atracurium: A new diagnostic tool? *Clin. Exp. Allergy* **45**, 485–487 (2015).
77. D. G. Ebo, L. Venemalm, C. H. Bridts, F. Degerbeck, H. Hagberg, L. S. De Clerck, W. J. Stevens, Immunoglobulin E antibodies to rocuronium: A new diagnostic tool. *Anesthesiology* **107**, 253–259 (2007).
78. S. G. O. Johansson, H. Öman, F. Degerbeck, J. Tunelli, E. Florvaag, A. Nopp, Anaphylaxis to atracurium – A non-QAI-dependent reaction? *Acta Anaesthesiol. Scand.* **56**, 262–263 (2012).
79. G. Doge, R. Pohloudek-Fabini, D. Kottke, Analysis and stability of suxamethonium chloride. 1. Detection and quantitative determination of the intact active agent with its degradation products. *Pharmazie* **37**, 708–711 (1982).
80. L. Fielding, $^{1}$H and $^{13}$C NMR studies of some steroidal neuromuscular blocking drugs: Solution conformations and dynamics. *Magn. Reson. Chem.* **36**, 387–397 (1998).


**Acknowledgments:** We are thankful to the team of the Pharmacoepidemiology Center of the Assistance Publique-Hôpitaux de Paris (AP-HP) and, in particular, N. Yelles, K. Chandirakumaran, and I. Younes at the Bichat Hospital for help with the NASA study logistics. We thank all clinicians from the NASA study group for help with patient recruitment, in particular, V. Faitot, A. Mebazaa, J. Mantz (deceased), S. Roche, C. Chahine, J. Bresson, and A. Mignon. From the Institut Pasteur, Paris, France, we thank B. Iannascoli for help with antibody purification, D. A. Mancardi for flow cytometry setups, F. Bonhomme for mass and nuclear magnetic resonance analyses of carboxylated rocuronium and suxamethonium, and B. Baron and P. England for MST. We thank V. Guérineau (Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France) for the mass analyses of bioconjugates and M. Parent, S. Peltier, L. Gaillanne, and C. S. Louis-Augustin (Immunology Laboratory, Bichat Hospital, Paris) for help with sample management and immunology diagnostic tests. We thank M. Pallardy (Toxicology department, UFR de Pharmacie de l'Université Paris-Sud and INSERM UMR996) for discussions on NMBA allergy. **Funding:** The NASA study was funded by AP-HP (Direction de la Recherche Clinique et de l'Innovation) through a "Contrat de Recherche Clinique 2011" to S.C.-M. by INSERM and the French Ministry of Health (Direction Générale de l'Offre de Soin, Paris, France) through a "Recherche Clinique translationnelle 2012" grant to P.B., by the European Research Council (ERC)–Seventh Framework Program (ERC-2013-CoG 616050) to P.B., by the Institut Pasteur, and by the INSERM. A.G.-C. benefited from a stipend provided by AP-HP, Paris, France and by the Institut Pasteur, Paris, France and from a grant provided by INSERM, SFAR (Société Française d'Anesthésie et de Réanimation), and SRLF (Société de Réanimation de Langue Française) through the "Bourse de Recherche du Comité d'interface INSERM-SFAR-SRLF 2012." C.M.G. was supported partly by a stipend from the Pasteur-Paris University (PPU) International PhD program and by the Institut Carnot Pasteur Maladies Infectieuses. P.B. benefited from an additional support from AP-HP through a "Contral Local d'Interface 2014" and the "Département Hospitalo-Universitaire" (DHU) FIRE. The sponsor of the NASA study was the Direction de la Recherche Clinique et de l'Innovation de l'AP-HP (France). F.J. is an employee of the CNRS. **Author contributions:** F.J., L.d.C., F.T., M.A., D.L., S.C.-M., and P.B. conceived and designed the study. F.J., L.d.C., S.C.-M., and P.B. wrote the manuscript. F.J., L.d.C., V.G., and A.G.-C. performed and analyzed most of the in vitro and ex vivo studies, with help from C.M.G., Q.Z., M.H.-N., and P.N.-R. C.G. and S.B. designed, produced, and analyzed bioconjugates. F.D. and F.T. assisted with the data analysis and statistical evaluation. C.N., C.S., and M.-T.G. assisted with the clinical allergology evaluation. C.P.-B., S.N., H.K.-M., M.L.D., B.C., O.L., L.J., B.P., M.F., and P.M. were responsible for including patients. S.C.-M. and P.B. obtained grants to fund and supervised the study. **Competing interests:** All authors declare that they have no competing interests. **Data and materials availability:** All data associated with this study are available in the paper or the Supplementary Materials.

Submitted 21 February 2018
Resubmitted 21 December 2018
Accepted 21 May 2019
Published 10 July 2019
10.1126/scitranslmed.aat1479

**Citation:** F. Jönsson, L. de Chaisemartin, V. Granger, A. Gouel-Chéron, C. M. Gillis, Q. Zhu, F. Dib, P. Nicaise-Roland, C. Ganneau, M. Hurtado-Nedelec, C. Paugam-Burtz, S. Necib, H. Keita-Meyer, M. Le Dorze, B. Cholley, O. Langeron, L. Jacob, B. Plaud, M. Fischler, C. Sauvan, M.-T. Guinnepain, P. Montravers, M. Aubier, S. Bay, C. Neukirch, F. Tubach, D. Longrois, S. Chollet-Martin, P. Bruhns, An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis. *Sci. Transl. Med.* **11**, eaat1479 (2019).

Science Translational Medicine

# An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis

Friederike Jönsson, Luc de Chaisemartin, Vanessa Granger, Aurélie Gouel-Chéron, Caitlin M. Gillis, Qianqian Zhu, Fadia Dib, Pascale Nicaise-Roland, Christelle Ganneau, Maria Hurtado-Nedelec, Catherine Paugam-Burtz, Skander Necib, Hawa Keita-Meyer, Matthieu Le Dorze, Bernard Cholley, Olivier Langeron, Laurent Jacob, Benoit Plaud, Marc Fischler, Caroline Sauvan, Marie-Thérèse Guinnepain, Philippe Montravers, Michel Aubier, Sylvie Bay, Catherine Neukirch, Florence Tubach, Dan Longrois, Sylvie Chollet-Martin, and Pierre Bruhns

*Sci. Transl. Med.* **11** (500), eaat1479. DOI: 10.1126/scitranslmed.aat1479

**A shocking contributor in drug-induced anaphylaxis**

Classic anaphylaxis is thought to depend on IgE engagement of Fc receptors on mast cells and basophils, but allergen-specific IgE is not always present. To resolve this conundrum, Jönsson and colleagues studied samples from people that experienced anaphylaxis after neuromuscular-blocking agent administration. They found that drug-specific IgG complexes could trigger neutrophils ex vivo and anaphylactic patients had increased circulating activated neutrophils. Not all of the patients had detectable antidrug IgE. Their results reveal that alternative pathways may be at play and could help develop tests to reduce risk in anesthetic drug administration.