Severe allergic and anaphylactic transfusion reactions in consecutive recipients from the same donor

Following multiple allergic/anaphylactic transfusion reactions (AATR) traced to a single donor, plasma-associated Fc-ε-receptor I (FcεRI)-specific immunoglobulin (Ig)G-autoantibodies were revealed as a rare but potent AATR trigger. AATR are a group of adverse reactions to blood product transfusions where combined donor and recipient factors enhance risk of reactions.[1] Donor-derived causes inducing AATR consist of a heterogenous group of immunoreactive molecules.[2] Several methods can detect these molecules[1] that clinically, are often focused on specific causative agents such as anti-IgA in IgA-deficient patients or other IgE-dependent pathways.[3-5] Also, AATR often occur in complex clinical settings e.g., trauma centers or perioperative settings involving concurrent drug administration that can cause allergy/anaphylaxis. Therefore, specialized allergy investigation is warranted to exclude drug allergy before consideration of AATR. Amongst newer diagnostic approaches to AATR-detection is the basophil activation test (BAT). Here, donor serum induces degranulation in basophils allowing rapid and sensitive functional assessment regardless of the underlying immunological mechanism.

AATR are rare but underreported, and improved blood establishment detection methods are needed for proper clinical assessment of AATR.[6] Applying functional assessment tools and characterization of antibodies/antigens involved in AATR could improve recipient outcomes. In addition to identifying multiple life-threatening AATR in recipients of a single donor's blood, revealed by BAT, we demonstrate how hemovigilance data, donor immunophenotyping, BAT, and combined immunoprecipitation and liquid chromatography mass spectrometry enables advanced AATR assessment.

In 2020, two cases of AATR were observed after transfusion of blood products from the same donor. The donor was A RhD^- with 125 prior donations, who, after assessment, was permanently deferred from further blood donation. The blood establishment initiated a 3-year look-back from 2020 to 2017 including assessment of reported AATR and clinical assessment of the donor. The study was done in accordance with General Data Protection Regulation, and the donor gave informed oral and written consent to publication of findings. The study was conducted in alignment with Danish law and adhered to the principles of the Declaration of Helsinki.

Adverse reactions in patients receiving transfusions from the AATR-inducing donor were assessed according to the International Society of Blood Transfusion (ISBT) Hemovigilance Working party including category, severity, and imputability. Patients experiencing AATR received either red blood cells (RBC), fresh frozen plasma (FFP), or platelet components from six donors with platelet additive solution (SSP+, Macopharma, France), but without pathogen reduction/inactivation. Assessment included evaluation of medication administered at the time of potential AATR. In two of the cases, severe anaphylaxis occurred during surgery and full allergy evaluation of all administered drugs was performed without identifying a causative drug. Presence of IgA/anti-IgA antibodies was applied as post-AATR serological assessment. Serum tryptase was available from time of AATR in seven cases, but only confirmed elevated by comparison with baseline samples in two perioperative AATR. For the remainders, imputability was therefore categorized as probable rather than definite.

Hematological profiling of the AATR-inducing donor was analyzed using a Sysmex® XN on 2020/2022 EDTA plasma and a clinically applied immunodeficiency flow cytometry (FC) panel that was previously reported.[7] The allergic profile of the donor was assessed with specific IgE assays using ImmunoCAP, BAT and confirmed by basophil histamine-release test.

BAT was performed on plasma and serum samples. Whole blood basophils from healthy individuals were stimulated with healthy donor serum, positive controls (positive: anti-IgE 1 μg/mL; non-releaser: N-formylmethionyl-leucyl-phenylalanine) with parallel analysis of the AATR-inducing donor's crude serum, IgG-depleted, and serum IgG fraction at varying concentrations. CD63-positive basophils were interpreted as activated. Purified IgG from the AATR-inducing donor was then coupled to beads and incubated with mast cell line (Laboratory of Allergic Diseases 2 [LAD2]) lysate. Precipitated proteins were eluted from antibodies by trypzination. The eluate was analyzed by a trapped ion mobility spectrometry and time-of-flight (timsTOF) Pro mass spectrometer. Raw mass spectrometry data was analyzed with MaxQuant (v1.6.15.0). Statistical analysis of label-free quantification derived protein expression data used the automated analysis pipeline of the Clinical Knowledge Graph.[8] Relative protein amounts were determined by the MaxLFQ algorithm with a minimum ratio count of two. Mass spectrometry analyses were performed by the Proteomics Research Infrastructure (PRI) at the University of Copenhagen, supported by the Novo Nordisk Foundation (grant number NNF19SA0059305). The mass spectrometry proteomics data was deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository[9] (data set identifier PXD045721).

Recipients and their reaction characteristics are presented in Table 1. The look-back revealed clinical signs of AATR

Table 1. Recipient allergic/anaphylactic hemovigilance overview.

Sex	Age group, years	Transfusion indication	Year/month	Component	Reaction	Previous allergy	Baseline tryptase	Tryptase after transfusion reaction	Serological assessment	Adverse reaction	Severity	Imputability
Female	70-80	Gastrointestinal bleeding	2017/SEP	RBC	None	Bandage		-		-	-	-
Male	70-80	Vascular surgery	2017/SEP	PFF	None	None	-	-	-	-	-	-
Female	50-60	Burn injury	2018/MAR	RBC	None	None	-	-	-	-	-	-
Male	70-80	Vascular surgery	2018/APR	PFF	None	None	-	-	-	-	-	-
Male	70-80	Hematological cancer	2018/JUN	RBC	None	Antibiotics	-	-	-	-	-	-
Male	60-70	Hematological cancer	2018/SEP	RBC	None	Grass	-	-	-	-	-	-
Male	60-70	Gastrointestinal bleeding	2018/SEP	PFF	Severe hypotension	None	N/A	5.72	Negative	Anaphylactic transfusion reaction	Severe (grade 3)	Probable
Female	20-30	Liver surgery	2019/DEC	PC	Severe respiratory problems and urticaria	Antibiotic and prednisolone	5.46	16.1	Not performed	Anaphylactic transfusion reaction	Severe (grade 3)	Definite
Male	0-10	Hematological cancer	2019/DEC	RBC	Hypotension and urticaria	None	N/A	6.07	Negative	Allergic transfusion reaction	Mild (grade 1)	Probable
Male	40-50	Hematological cancer	2020/MAR	PC	Hypotension and urticaria	None	N/A	N/A	Not performed	Anaphylactic transfusion reaction	Severe (grade 3)	Possible
Male	70-80	Hematological cancer	2020/MAR	PC	Hypotension and urticaria	Antibiotics	5.03	N/A	Negative	Anaphylactic transfusion reaction	Severe (grade 3)	Probable
Female	70-80	Sepsis	2020/MAR	RBC	None	Nickel	N/A	N/A	Not performed	-	-	-
Female	30-40	Hematological disease	2020/APR	FFP	Severe hypotension	None	N/A	28.5	Not performed	Anaphylactic transfusion reaction	Severe (grade 3)	Probable
Female	70-80	Hematological cancer	2020/JUN	PC	Severe respiratory problems, hypotension and urticaria	Antibiotics	N/A	19.9	Auto-antibody, DAT positive	Anaphylactic transfusion reaction	Severe (grade 3)	Probable
Male	50-60	Liver transplantation	2020/JUN	PFF	Severe respiratory problems and circulatory collapse	None	15.2	123	Negative	Anaphylactic transfusion reaction	Severe (grade 3)	Definite
Male	70-80	Anemia unspecified	2020/JUN	RBC	Respiratory problems and urticaria	None	N/A	11.1	Negative	Allergic transfusion reaction	Mild (grade 1)	Probable

DAT: direct antiglobulin test; FFP/PFF: plasma components; N/A: not available; PC: platelet component; RBC: red blood cell component; MAR: March; APR: April; JUN: June; SEP: September; DEC: December.

in nine of ten consecutive recipients from 2018 to 2020. Before the first case in 2018, six consecutive recipients experienced no AATR.

The AATR-inducing donor reported a distant history of urti-

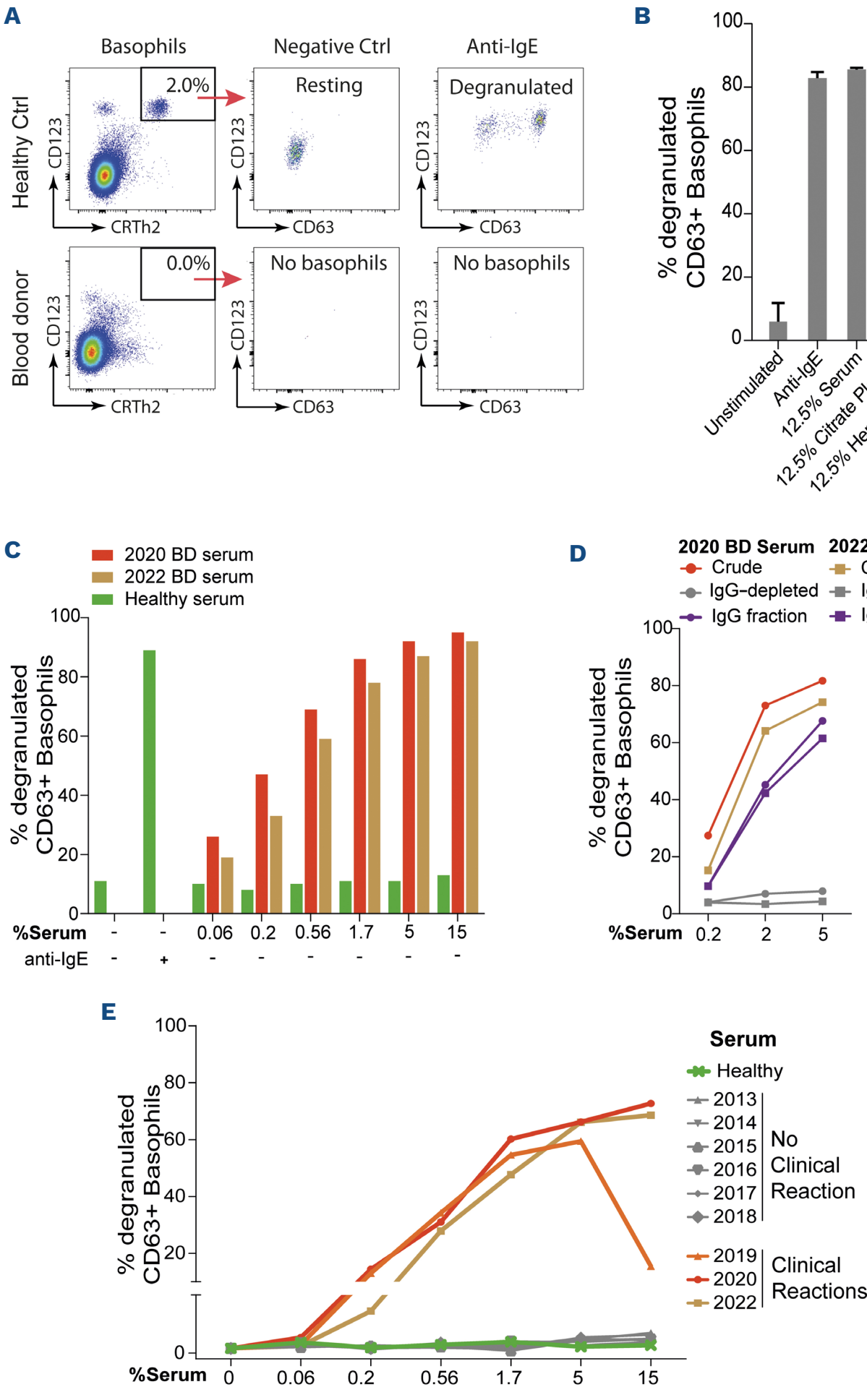

Figure 1. Serum from an AATR-inducing donor activates basophils from healthy controls. (A) Representative dot plots showing CD63⁺ basophils in healthy controls and the allergic/anaphylactic transfusion reactions (AATR)-inducing donor, unstimulated and after anti-immunoglobulin (Ig)E stimulation. (B) Degranulation of basophils from healthy controls following stimulation with serum or plasma from the AATR-inducing donor. (C, D) Degranulation of basophils from healthy control (Ctrl) stimulated with (C) serum from a healthy donor *versus* AATR-inducing donor (2020, 2022) (D) crude, IgG, and IgG-depleted serum from the AATR-inducing donor. (E) Degranulation of healthy control basophils following incubation with archival plasma (2013–2022) from the AATR-inducing donor, annotated with clinical reactions. BD: basophil degranulation.

caria on skin exposure to grass and had received subcutaneous grass allergy immunotherapy. There was no history of allergic reactions, unusual infections, surgery or transfusion in the period prior to 2018. Overall, the clinical work-up was unremarkable: normal overall T-, B-, and NK-cell concentration including subpopulations and major myeloid cell subpopulations; normal Ig concentrations and complement function. Screening for anti-HLA/-HPA/-HNA-antibodies was negative, but neutrophil agglutination after exposure to donor serum was observed with 1 of 4 neutrophil-assay donors. The only finding in donor's hematological profile was extreme basopenia between 2010 and 2022, which was confirmed using FC basophil markers CRTH2/CD123 (Figure 1A). Underlying hematological disease was excluded via a

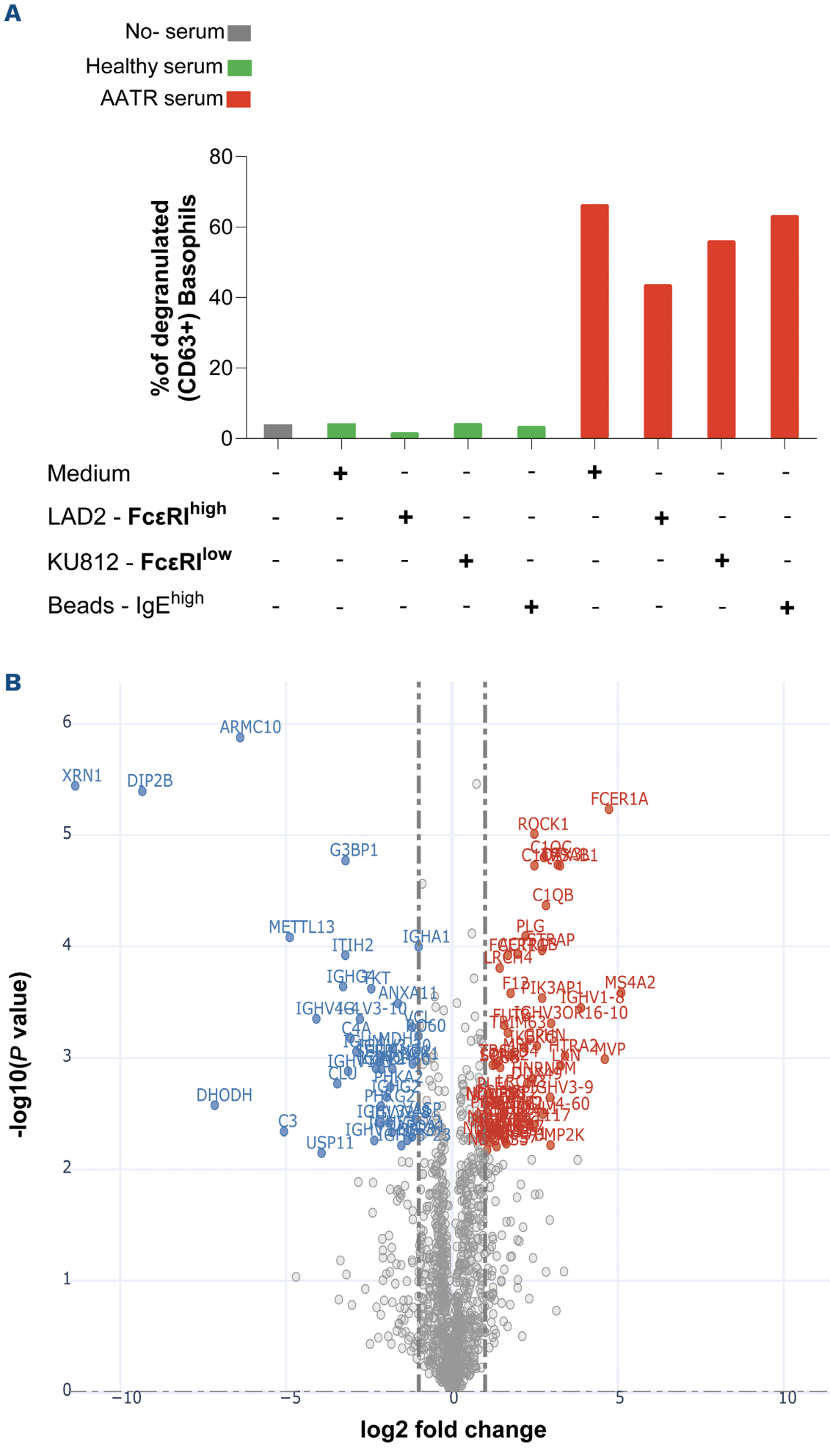

Figure 2. Functional and proteomic analyses indicate FcεRI as the target of AATR-donor autoantibodies. (A) Degranulation of basophils from healthy controls following stimulation with healthy and allergic/anaphylactic transfusion reactions (AATR)-sera depleted of autoantibodies by incubation/elution of sera with Fc-ε-receptor I (FcεRI)-expressing cells (LAD2, KU812) and immunoglobulin (Ig)E-coated beads. (B) Volcano plot of combined immunoprecipitation and mass spectrometry results showing relative abundance of proteins targeted by AATR-donor antibodies. Donor IgG was incubated with lysate of LAD2 cells followed by immunoprecipitation. Comparison of donor IgG to control IgG from healthy individual is displayed. X-axis shows log2-fold change (Log2FC) with red-labeled proteins denoting increased Log2FC of proteins targeted by donor IgG, while blue-labeled show decreased Log2FC. Y-axis shows the negative log10 *P* value. Proteins with no Log2FC differences between index donor sample and healthy controls or proteins that did not reach statistical significance are represented by grey dots.

diagnostic leukemia FC panel. The AATR-inducing donor's serum was highly positive using basophil histamine-release test. Skin prick test (SPT) with histamine and increasing morphine doses[10] was positive. Combined with normal baseline serum tryptase, this confirmed the presence of functional skin mast cells.

EDTA and heparinized plasma, as well as serum from the donor, induced comparable, high-level degranulation of basophils from healthy individuals (Figure 1B). Reactivity of donor's serum from 2020 was 5% higher compared to 2022, showing 87% *versus* 82% of CD63-positive basophils. Surprisingly, dilution of crude serum showed that even a 0.06% concentration induced degranulation of basophils (25%) (Figure 1C). To determine the serological trigger of the response, donor's serum was depleted of IgG and tested on basophils from healthy individuals. The isolated IgG fractions induced responses comparable to crude serum, while the IgG-depleted fractions had no effect, indicating IgG antibodies as the causative agent of AATR (Figure 1D). Randomized analysis of archival plasma samples from the donor including samples with evident clinical AATR (N=3) and from before onset of reactivity in 2018 (N=18) revealed positive degranulation in only the post-2018 samples (Figure 1E).

Since the basophil response to the AATR-inducing serum was comparable to anti-IgE activation, we assumed that the FCεRIα pathway was targeted by the IgG antibodies in the donor's serum. After preincubation of serum with either FcεRIα-expressing LAD2 cells, KU812 cells, or IgE-positive microbeads, only sera from the LAD2 cells showed reduced capacity to activate basophils, indicating that FcεRIα, rather than IgE, was the IgG-antibody target (Figure 2A). Following immunoprecipitation of antibody/antigen complexes after incubation of the donor's serum with LAD2 cells, mass spectrometry analysis could identify antibodies directed against both α- and β-subunits of FcεRI (FCER1A, MS4A2) with manifold increased detection compared to a healthy control (Figure 2B).

In conclusion, multiple consecutive severe AATR had occurred in recipients who received donations from a single blood donor, that had remained undetected by standard clinical measures for AATR detection for several years. The chance finding of two transfusion reactions to blood components from the same donor led to a large scale look-back revealing seven additional cases. Despite research suggesting BAT as a screening tool, no international recommendations exist regarding prevention of AATR or donor screening.

Milder AATR were observed in patients receiving RBC components, which contain less plasma than platelets or FFP also implying the causative mechanism as plasma associated. Here, we suspect a FcεRI-specific IgG autoantibody in the AATR-inducing donor's plasma/serum as the causative mechanism. The presence of anti-FcεRI autoantibodies is well known in patients with chronic spontaneous urticaria (CSU).[11] One possible etiology of the AATR-inducing mechanism could be subclinical CSU in the AATR-inducing donor, resulting in the extreme potency of the donor's serum/plasma in inducing basophilic degranulation. Another mechanism could be an undetected/occult infection leading to immune activation triggering the change in reactivity, possible mediated by somatic hypermutation and affinity maturation.[12] Population studies have found that IgG directed against FcεRI are prevalent in both patients with CSU and healthy controls,[13] which could complicate large-scale blood donor screening for AATR-inducing FcεRI antibodies if parallel functional assessment is omitted.

Clinical AATR-signs are difficult to distinguish from allergic reactions to concurrently administered medication, which are more common and should be investigated first. Identifying AATR-inducing donors through clinical reporting and backwards traceability of AATR seems insufficient. The addition of high-throughput functional assays could help to screen donor populations for prevalence of AATR induction. Our results highlight the possibility of identifying causal AATR-mechanisms by going beyond BAT. While we acknowledge this level of workup cannot be performed on all donors, this approach may enable in-depth immunological assessments to identify the few but relevant donors responsible for AATR, with expected benefits for recipient safety. This study illustrates a blood establishment approach to performing clinical assessments of donors involved in AATR. This is an area that would benefit from international consensus guidelines, which we urge the international hemovigilance community to consider.

Authors

Patrick Terrence Brooks,[1*] Christina Mikkelsen,[1*] Ewa Anna Bartko,[2] Vaishnavi Ravikumar,[1] Christoffer Egeberg Hother,[1] Lia Minculescu,[1] Hanne Vibeke Marquart,[1,3] Sisse Rye Ostrowski,[1,3] Morten Bagge Hansen,[1,3] Lars K. Poulsen,[2] Lars H. Blom,[2] Lene H. Garvey[2,3] and Morten Hanefeld Dziegiel[1,3]

[1]Department of Clinical Immunology, Copenhagen University Hospital – Rigshospitalet; [2]Allergy Clinic, Department of Dermatology and Allergy, Copenhagen University Hospital - Herlev and Gentofte and [3]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

**PTB and CM contributed equally as first authors.*

Correspondence:
P.T. BROOKS - patrick.terrence.brooks@regionh.dk

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Contributions

PTB, CM, EAB, and LHG conceived, designed, and supervised the study, collected, analyzed, and interpreted the data, and authored the manuscript. MHD, LHB, and LKP designed and supervised the study, interpreted the data, and authored the manuscript. HM, VR, CEH, and LM collected, analyzed, and interpreted data, and authored the manuscript. SRO, and MBH interpreted data and authored the manuscript.

Data-sharing statement

Data supporting the study findings are available from the corresponding author upon reasonable request.

References

1. Savage WJ, Tobian AAR, Savage JH, Wood RA, Schroeder JT, Ness PM. Scratching the surface of allergic transfusion reactions. Transfusion. 2013;53(6):1361-1371.
2. Hirayama F. Current understanding of allergic transfusion reactions: Incidence, pathogenesis, laboratory tests, prevention and treatment. Br J Haematol. 2013;160(4):434-444.
3. Hirayama F, Yasui K, Matsuyama N, Okamura-Shiki I. Possible utility of the basophil activation test for the analysis of mechanisms involved in allergic transfusion reactions. Transfus Med Rev. 2018;32(1):43-51.
4. Yasui K, Matsuyama N, Kimura T, Fujimura Y, Hirayama F. Immunoglobulin (Ig)G antibodies against IgE identified by basophil activation test as the putative causative agent of a serious allergic transfusion reaction: potential utility of the test as a new safety measure for allergic transfusion reactions. Transfusion. 2018;58(11):2572-2580.
5. Soutar R, McSporran W, Tomlinson T, Booth C, Grey S. Guideline on the investigation and management of acute transfusion reactions. Br J Haematol. 2023;201(5):832-844.
6. Lindsted G, Larsen R, Krøigaard M, et al. Transfusion-associated anaphylaxis during anaesthesia and surgery - a retrospective study. Vox Sang. 2014;107(2):158-165.
7. Ronit A, Berg RMG, Bay JT, et al. Compartmental immunophenotyping in COVID-19 ARDS: a case series. J Allergy Clin Immunol. 2021;147(1):81-91.
8. Santos A, Colaço AR, Nielsen AB, et al. A knowledge graph to interpret clinical proteomics data. Nat Biotechnol. 2022;40(5):692-702.
9. Perez-Riverol Y, Bai J, Bandla C, et al. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. Nucleic Acids Res. 2022;50(D1):D543-D552.
10. Brockow K, Garvey LH, Aberer W, et al. Skin test concentrations for systemically administered drugs - An ENDA/EAACI Drug Allergy Interest Group position paper. Allergy. 2013;68(6):702-712.
11. Bracken SJ, Abraham S, MacLeod AS. Autoimmune theories of chronic spontaneous urticaria. Front Immunol. 2019;10:627.
12. Johnson D, Jiang W. Infectious diseases, autoantibodies, and autoimmunity. J Autoimmun. 2023;137:102962.
13. Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: what we know and what we do not know. J Allergy Clin Immunol. 2017;139(6):1772-1781.e1.