Received: 16 November 2022 | Revised: 5 June 2023 | Accepted: 7 June 2023

DOI: 10.1002/jca.22069

RESEARCH ARTICLE

Journal of Clinical Apheresis ASFA WILEY

Efficient removal of antibodies to adeno-associated viruses by immunoadsorption

Simone Boedecker-Lips[1] | **Andreas Judel**[2] | **Stefan Holtz**[1] | **Magnus Mayer**[1] | **Pascal Klimpke**[1] | **Daniel Kraus**[1] | **Thomas Schreiner**[3] | **Bernhard Gerstmayer**[3] | **Klaus Eulitz**[2] | **Magnus Christopher Mayer**[2] | **Julia Weinmann-Menke**[1,4]

[1]Division of Nephrology, I. Department of Medicine, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

[2]Miltenyi Biotec B.V. & Co. KG, Teterow, Germany

[3]Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

[4]Research Center for Immunotherapy (FZI), University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

Correspondence
Julia Weinmann-Menke, Department of Nephrology, Medical Center of the Johannes-Gutenberg University, Langenbeckstrasse 1, 55131 Mainz, Germany.
Email: julia.weinmann-menke@unimedizin-mainz.de

Abstract

Background: Gene therapies based on adeno-associated viruses (AAV) are a therapeutic option to successfully treat monogenetic diseases. However, the influence of pre-existing immunity to AAV can compromise the application of AAV gene therapy, most notably by the presence of neutralizing antibodies (NAb) to AAV.

Methods: In the following study, we investigated to what extent the treatment by immunoadsorption (IA) would reduce the levels of human anti-AAV antibodies to AAV2 and AAV5. To that end, we screened blood sera from 40 patients receiving IA treatment because of underlying autoimmune disease or transplant rejection, with detectable AAV-antibodies in 23 patients (22 by NAb detection, and 1 additionally by anti-AAV5 ELISA analysis).

Results: Our results show that IA efficiently depleted anti-AAV2 NAb with a mean reduction of 3.92 ± 1.09 log2 titer steps (93.4%) after three to five single IA treatments, 45% of seropositive subjects had an anti-AAV2 titer below the threshold titer of 1:5 after the IA treatment series. Anti-AAV5 NAb were reduced to below the threshold titer of 1:5 in all but one of five seropositive subjects. Analysis of total anti-AAV5 antibodies by ELISA demonstrated an anti-AAV5 antibody reduction over the IA treatment series of 2.67 ± 1.16 log2 titer steps (84.3%).

Conclusion: In summary, IA may represent a safe strategy to precondition patients with pre-existing anti-AAV antibodies to make this population eligible for an effective AAV-based gene therapy.

Abbreviations: AAV, adeno-associated viruses; Ab, antibodies; ABMR, antibody-mediated rejection; IA, immunoadsorption; NAb, neutralizing antibodies.

KEYWORDS
adeno-associated viral vector-based gene therapies, adeno-associated virus, antibody, immunoadsorption, immunoglobulin, monogenetic diseases, plasmapheresis

1 | INTRODUCTION

Adeno-associated viral (AAV) vector-based gene therapies have proven to be successful for the treatment of a variety of human diseases.[1-4] The low toxicological profile of AAV vectors and their ability to target specific tissues/organs in vivo in a serotype-specific manner allows systemic administration to patients. Yet, pre-existing immunity against the AAV capsid limits the application of AAV vectors substantially for a significant proportion of potential patients. Depending on the AAV serotype, prevalence of anti-AAV antibodies can be higher than 50% in the population.[5,6] Since anti-AAV antibodies have been found to preclude successful AAV gene therapy application,[7,8] presence of anti-AAV antibodies in patients' blood before AAV gene therapy serves as an exclusion criterion in many AAV-based gene therapy trials.[9]

Several strategies have been tested in animal models to overcome pre-existing humoral immunity to AAV. This included the design of less seroprevalent AAV capsids,[10] systemic immune suppression, and the use of IgG-degrading enzymes.[11-14] However, all of these strategies have limitations, as they may require adjustment of the drug formulation, increase the risk of side effects, or allow therapy only for patients with low anti-AAV neutralizing Ab (NAb) titers.

A completely different strategy that could help to overcome these limitations is the removal of AAV antibodies from patients' blood plasma via plasmapheresis techniques. Non-selective therapeutic plasma exchange (TPE) has been shown to successfully deplete anti-AAV antibodies in humans.[15] However, the non-selective removal of plasma proteins prevents application of daily intensive therapeutic apheresis required for efficient removal.[16-18] Alternatively, for daily treatment, replacement with fresh frozen plasma is possible to substitute for example, coagulation factors, but this may introduce donor anti-AAV antibodies.

Immunoadsorption (IA) is a selective therapeutic apheresis technique by which immunoglobulins are selectively removed from patients' plasma. It is frequently used for the treatment of antibody-mediated autoimmune diseases, for example, anti-neutrophil cytoplasmatic antibody-associated vasculitis, systemic lupus erythematosus, and multiple sclerosis, or to enable AB0-incompatible solid organ transplantation.[19-22] Removal of anti-AAV antibodies by IA and subsequent successful re-administration of AAV5 gene therapy has already been demonstrated in non-human primates.[23] Furthermore, an AAV-specific IA column has been developed based on immobilized capsids, which is highly efficient in removing anti-AAV antibodies without affecting total IgG levels.[24]

Here, we applied an IA system with regenerative broadband IA columns that has been shown to efficiently deplete IgG, IgA, and IgM antibodies based on two recombinant antibody fragments with specificity to the constant region of human kappa and lambda light chains.[25] We report real-world data from patients who underwent IA treatments because of autoimmune diseases or transplant rejection. Using blood sera taken before and after IA treatments, we investigated the effectiveness of IA to reduce the levels of antibodies to AAV2 and AAV5.

2 | MATERIALS AND METHODS

This prospective, explorative, observational trial was performed in accordance with the Declaration of Helsinki of the World Medical Association. The ethics committee of the University Medical Centre Mainz, Germany and the Medical Association of the State of Rhineland-Palatinate approved this study (Approval no. 2018-13039). Written informed consent was obtained from all participating patients at the beginning of the study.

2.1 | Patients

Sera from 40 patients undergoing IA treatment because of underlying autoimmune conditions or transplant rejection were collected. Blood samples were taken immediately before and after each treatment. Subsequently, the samples were centrifuged and aliquoted.

2.2 | IA therapy

IA treatments were performed on the TheraSorb LIFE 21 apheresis unit (Miltenyi Biotec, Bergisch Gladbach, Germany), with TheraSorb—Ig omni 5 adsorbers (330-000-965, Miltenyi Biotec), a multiple use (five times) adsorber. The principle of the adsorber is based on

antigen–antibody binding and always a double column system was used during treatment, so that one of the adsorbers could be regenerated while the other adsorber was used for antibody depletion from plasma. During each IA treatment, two patient plasma volumes were processed. Intravenous immunoglobulins (Octagam, Octapharma GmbH, Langenfeld, Germany) were applied to patient 22 after the third IA treatment and thus the fourth and fifth treatment were excluded from statistical analyses.

The treatments were done according to the clinic's internal standard, so usually the first three IA treatments were performed daily and the fourth and fifth treatments were done with a day's treatment break in between. However, treatment schedules were adjusted to fit individual patient's needs resulting in a reduced number of IA applications and/or differing treatment schedules compared to the standard schedule in some patients (Table 1). Additionally, immunosuppressive therapy was applied in the majority of patients as indicated in Table 1.

2.3 | AAV vectors

AAV2/2 and AAV2/5 viral vectors were provided by the University of Iowa Viral Vector Core (http://www.medicine.uiowa.edu/vectorcore).

2.4 | Immunoglobulin measurement

IgG analysis of the sera was performed on the AU480 Clinical Chemistry system (Beckman Coulter) with reanalysis on the Immage 800 analysis system (Beckman Coulter) if the IgG concentration was below 0.75 g/L. The determination of the IgG subclasses was performed by the central laboratory according to clinical routine.

2.5 | NAb assay

Anti-AAV NAb analysis was performed with a protocol similar to the one described by Meliani et al.[26] On the first day, 2×10^4 human embryonic kidney (HEK293T) cells/well were seeded in a 96-well plate. On day 2, AAV2/2-luc and AAV2/5-luc vectors were diluted in serum-free DMEM (12-604F, Lonza) according to the required multiplicity of infection (MOI). Subsequently, vectors were incubated with heat-inactivated serum samples that were prepared as 2-fold serial dilutions in heat-inactivated FBS (SH30071.03IR, Cytiva) from a starting dilution of 1:5 for 1 h at 37°C. Cells were transduced with the AAV-serum mixture with an MOI of 200 (AAV2) or 1500 (AAV5), respectively. After 23 ± 1 h, Bright Glo reagent (E2620, Promega) was applied in a 1:1 mixture to the wells for cell lysis and luciferase reaction. Luciferase activity was determined on a luminescence reader (Infinite M200, Tecan or Synergy H1M, BioTek) as relative light units per second. These results were then related to the signal of a 100% transduction control. NAb titer was determined as the highest serum dilution resulting in an inhibition of AAV vector transduction by 50% or more.

2.6 | ELISA analysis

For anti-AAV ELISA analysis, 96-well ELISA plates (Nunc MaxiSorp Catalog #44-2404-21, ThermoFisher) were coated with 25 ng AAV5 capsid per well over night at 4°C. Plates were blocked by 1% casein/PBS solution for 1 h and then incubated for 1 h with serum samples that were prepared as 2-fold serial dilutions in blocking buffer from a starting dilution of 1:20. Antibody detection was performed with HRP-coupled anti-human secondary Ig antibody (STAR106P, Biorad); colorimetric detection was performed with tetramethylbenzidine (37068.01, Serva). For determination of AAV-antibody-negative samples, sera were pre-incubated for 2 h with AAV capsid before transfer to the ELISA plate. Seronegative samples were then used to establish an absorbance cutoff value with 95% confidence level.[27] ELISA titer of a sample was determined as the highest serum dilution with an OD value above cutoff.

2.7 | Statistical analyses

Values are depicted as means ± SD if not indicated otherwise. Average titer depletion and increase between treatment days were calculated from measurements with titer values that were equal to or above the threshold titer of the NAb and ELISA assay of 1:5 and 1:20, respectively. Average IgG reductions were calculated from measurements that were equal to or above the quantification limit of 0.33 g/L. All statistical analyses were performed with GraphPad Prism version 9.3.1 (www.graphpad.com).

3 | RESULTS

In total, 43 patients suffering from neurological or rheumatic autoimmune diseases or organ transplant rejection were included in the study and treated with IA using regenerative broadband IA columns. Samples from three patients were excluded from further analysis because of

TABLE 1 Treatment days and additional immunosuppressive treatment.

Patient	Day 1 (IA start)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Immunosuppression
1	×	×	×		×		×			None
2	×	×	×	×	×					Steroid pulse (250 mg for 3 d, then 100 mg/d)
3	×	×	×			×				None
4	×		×	×	×		×			Steroid pulse (250 mg for 3 d, then 60 mg/d), cyclophosphamide (500 mg) after the fourth IA
5	×	×	×	×	×					None
6	×		×	×	×					None
7	×	×	×		×	×				Steroid pulse (250 mg for 3 d, then 80 mg/d), 2× rituximab (375 mg/m^2 body surface)
8	×	×	×		×		×			Steroid pulse (250 mg for 3 d, then 80 mg/d)
9	×	×	×	×	×					Steroid pulse (250 mg for 3 d, then 70 mg/d)
10	×	×	×	×	×					Steroid pulse (250 mg for 3 d, then 55 mg/d)
11	×	×	×		×	×				Immunoglobulins before IA, 5× TPE 2 weeks before
12	×	×	×		×	×				Prednisolone 2.5 mg/d
13	×	×	×	×						5× IA, 4 weeks before
14	×	×	×		×		×			Steroid pulse (1000 mg for 5 d)
15	×	×	×	×		×				None
16	×	×		×	×	×				None
17	×	×	×		×	×				Steroid pulse (1000 mg for 5 d)
18	×	×	×			×		×		Steroid pulse (250 mg for 3 d, then 90 mg/d), 1× rituximab (375 mg/m^2 body surface)
19	×	×	×	×	×					Steroid pulse (250 mg for 3 d, then 60 mg/d), 1× rituximab (375 mg/m^2 body surface)
20	×					×	×	×	×	Prednisolone 5 mg/d plus one time rituximab before IA start (375 mg/m^2 body surface)
21	×	×	×		×		×			Prednisolone 40 mg/d, mycophenolate mofetil 2000 mg/d
22	×	×	×			×	×			Mycophenolate mofetil, tacrolimus, prednisolone 5 mg/d, thymoglobulins for 5 d, immunoglobulins after third IA treatment
23	×	×	×		×					None
24	×	×	×		×		×			Steroid pulse (1000 mg for 3 d)
25	×	×	×	×		×				Mycophenolate mofetil 2000 mg/d, prednisolone 70 mg/d

TABLE 1 (Continued)

Patient	Day 1 (IA start)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Immunosuppression
26	×	×	×	×			×			Steroid pulse (250 mg for 3 d, then 80 mg/d), 1× rituximab (375 mg/m^2 body surface)
27	×	×	×		×		×			Steroid pulse (1000 mg for 5 d), 1× rituximab before IA (1000 mg)
28	×	×	×		×		×			2× rituximab (1000 mg) before IA
29	×	×	×		×		×			Ciclosporin, prednisolone 5 mg/d
30	×	×	×			×	×			Prednisolone 40 mg/d, 1× rituximab before IA (375 mg/m^2 body surface), 5× IA 1 week before, mycophenolate mofetil 2000 mg/d, tacrolimus
31	×	×	×		×		×			Steroid pulse (1000 mg for 5 d)
32	×	×	×		×		×			Steroid pulse (250 mg for 3 d, then 80 mg/d), cyclophosphamide (500 mg) after the fourth IA
33	×	×	×							Prednisolone 80 mg/d, immunoglobulins before IA, 3× rituximab (375 mg/m^2 body surface)
34	×	×	×		×		×			Prednisolone 20 mg/d, ciclosporin 250 mg/d
35	×	×	×		×		×			Belimumab 1×/s.c.
36	×	×	×							Steroid pulse (1000 mg for 5 d)
37	×	×	×	×		×				Steroid pulse (250 mg for 3 d, then 100 mg/d), cyclophosphamide (500 mg) after the second IA
38	×	×	×	×	×					Steroid pulse (250 mg for 3 d, then 50 mg/d)
39	×	×	×							None
40	×	×	×		×		×			Steroid pulse (1000 mg for 5 d)

Abbreviations: IA, immunoadsorption; TPE, therapeutic plasma exchange.

very low total pre-apheresis IgG levels before the first IA (<0.75 g/L). Two of these three patients received TPE, whereas the third patient received corticosteroid pulse therapy and interferon beta prior to IA. Baseline characteristics of the 40 screened patients are summarized in Supplementary Table 1.

Thirty-two of the included patients already received additional immunosuppressive drug therapy prior to initiation of therapy by IA and also during therapy. Out of these 32 patients, 19 patients received steroid pulse therapy (250-1000 mg for 3-5 days depending on the disease), out of which 12 patients were then treated with continuous steroid therapy. Other immunosuppressive therapies included continuous cortisone therapy, mycophenolate mofetil, calcineurin inhibitors, and rituximab. The individual immunosuppressive therapy of each patient can be found in Table 1.

3.1 | Measurement of IgG concentration during treatment

In all blood samples of the 40 treated patients, IgG levels were determined to prove the general effectiveness of the treatment (Figure 1A, Supplementary Table 2). In addition, the effectiveness of the adsorber column was

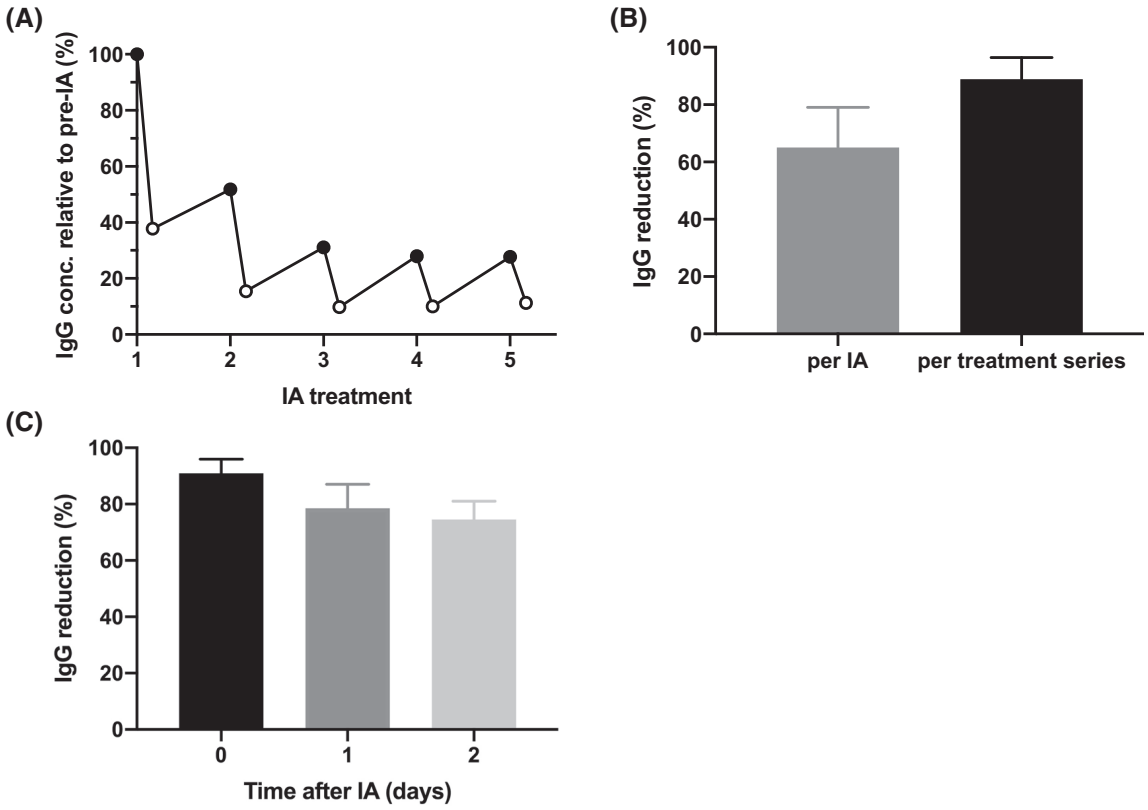

FIGURE 1 Serum IgG reduction during immunoadsorption treatment. (A) Normalized averaged IgG concentration decrease over the treatment period. Full circle: before IA treatment; blank circle: after IA treatment. (B) IgG percentage decrease per IA (black) and from the first to the last IA (grey). (C) Mean IgG reduction compared to pre-IA levels immediately after the IA treatment and before the next IA treatment 1 or 2 days later. Only third and fourth IA treatments of a treatment series are included. IA, immunoadsorption; IgG, immunoglobulin G; conc., concentration. (n = 40).

demonstrated by the study of IgG subclasses (see Supplementary Figure 1). All IgG subclasses were found to be efficiently reduced by IA.

For the majority of patients, the most prominent IgG decrease was measured within the first three treatment days with treatments being applied daily (mean IgG concentration decrease of 90.2 ± 3.9%). A single IA treatment resulted in a mean IgG reduction of 65.0 ± 14.0% (Figure 1B). Lower-than-average reduction levels were observed for patients with very high pre-IA IgG levels (Supplementary Table 2). Between treatment days, serum IgG concentration increased due an IgG-reproduction and by redistribution of IgG from all tissues where IgG deposits can be found into the circulation.[28] This renewed rise in IgG is a typical observation in TPE/IA treatments.[25] According to our clinic's internal standard, the last two treatments within a series of five are for many patients done every other day to improve IgG depletion from tissue. To analyze the kinetics of IgG redistribution in our patient collective, we analyzed the IgG concentration immediately after the third and fourth IA treatment and compared this to the IgG concentration before the next IA treatment in relation to the time between IA sessions (Figure 1C). We found that IgG concentration in the blood is constitutively replenished after IA. Two days after IA, IgG levels were, however, still reduced by 74.5 ± 6.6% compared to pre-IA levels. Stronger redistribution of IgG from the tissue into the circulation with longer time intervals between IA treatments putatively explains why there was no further IgG reduction in the blood after treatment 3.

3.2 | Anti-AAV2- and anti-AAV5-NAb detection

All pre-IA serum samples were analyzed for the presence of NAbs against serotypes AAV2 and AAV5. Of the 40 screened patients, neutralizing AAV-antibodies were detected in a total of 22 patients. Twenty-two out of 40 patients were positive for anti-AAV2 NAbs, and 5 out of 40 patients had anti-AAV5 NAbs (Figure 2).

Each of these patients underwent a series of three to five IA treatments. Anti-AAV2 and anti-AAV5 NAb titers were

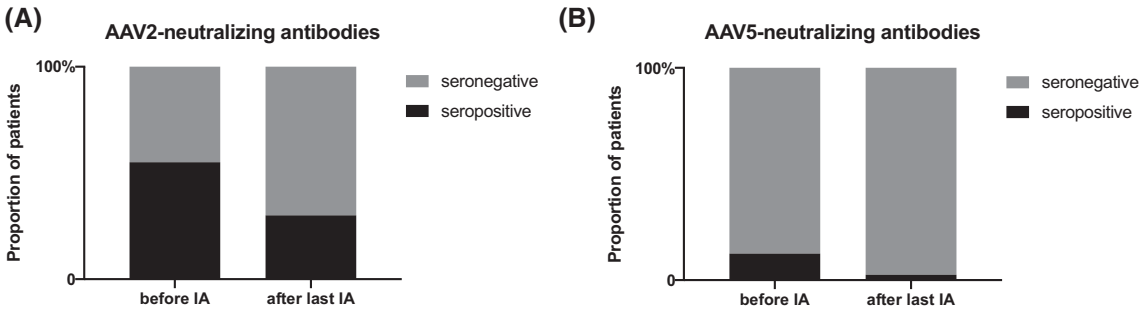

FIGURE 2 Seropositivity before and after immunoadsorption. Proportion of patients with AAV2-neutralizing antibodies (A) and AAV5-neutralizing antibodies (B) before and after IA treatment. Antibody levels were determined by a neutralizing antibody assay. AAV, adeno-associated virus; IA, immunoadsorption. (n = 40).

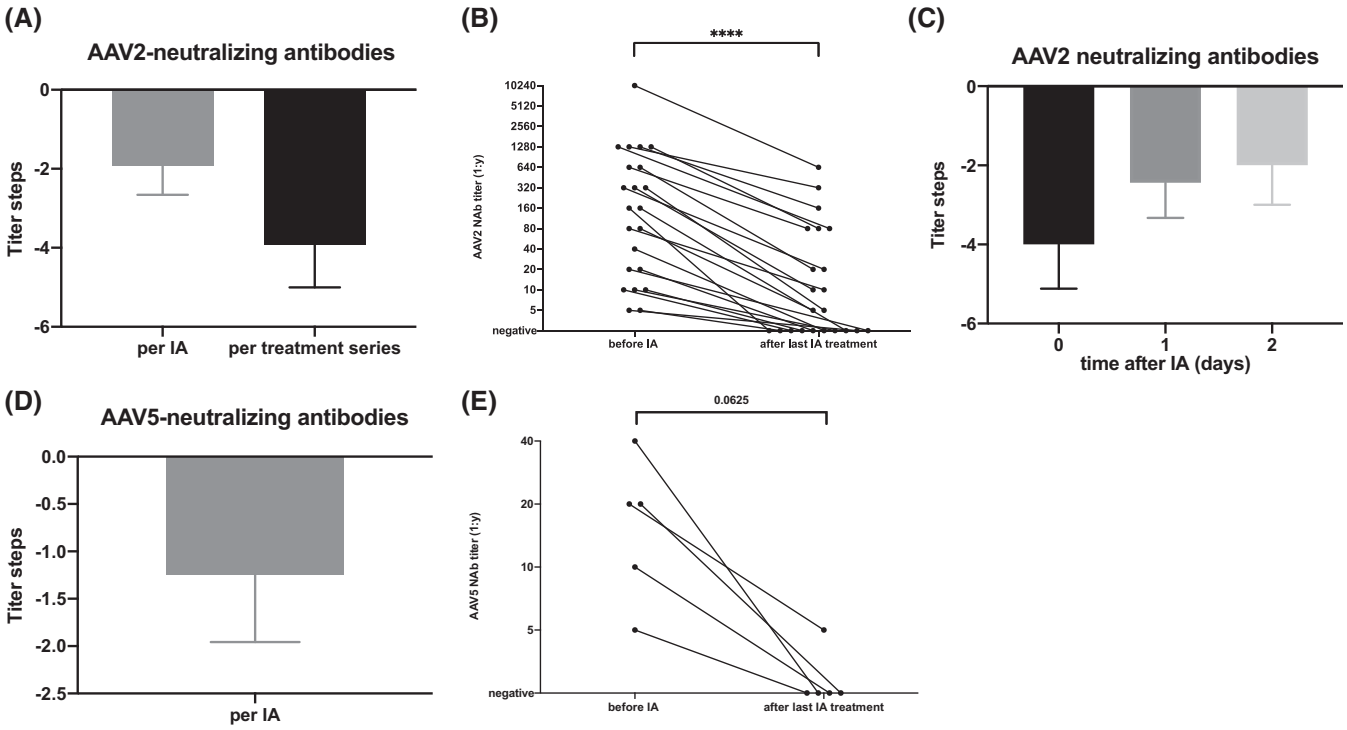

FIGURE 3 Reduction of anti-AAV2 and anti-AAV5 neutralizing antibodies by immunoadsorption. (A) Mean anti-AAV2 NAb titer step reduction per IA and over the IA treatment series. (B) Visualization of the individual anti-AAV2 NAb titers from before the first IA to after the last IA. (C) Mean AAV2 NAb titer reduction compared to pre-IA levels immediately after the IA treatment and before the next IA treatment one or two days later. Only 3rd and 4th IA treatments of a treatment series are included. (D) Mean anti-AAV5 NAb titer step reduction per IA. (E) Visualization of the individual anti-AAV5 NAb titers from before the first IA to after the last IA. Statistical analyses were performed by two-tailed Wilcoxon matched-pairs signed-rank tests (b,e). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. AAV, adeno-associated virus; IA, immunoadsorption; NAb, neutralizing antibody. (AAV2 NAbs n = 22; AAV5 NAbs n = 5).

analyzed before and after each treatment (Supplementary Figures 2 and 3, Figure 3).

Anti-AAV2 titers were reduced by 1.92 ± 0.74 $\log_2$ titer steps per treatment, which corresponds to 73.5% reduction (see Figure 4A,B).

Over the whole series of three to five IA treatments, anti-AAV2 NAbs were reduced by 3.92 ± 1.09 titer steps (93.4%). Out of the 22 patients who had anti-AAV2 NAbs before the start of IA, 10 had no detectable anti-AAV2 NAbs (Figure 2A) after their last treatment, and in all patients, anti-AAV2 NAb titers were reduced (Figure 4B, Supplementary Figure 2).

Anti-AAV2 NAb titers in the patient's sera increased after IA in the following days (Figure 4C). After 2 days, an average reduction of 2.0 titer steps (75%) compared to pre-IA levels was still observed.

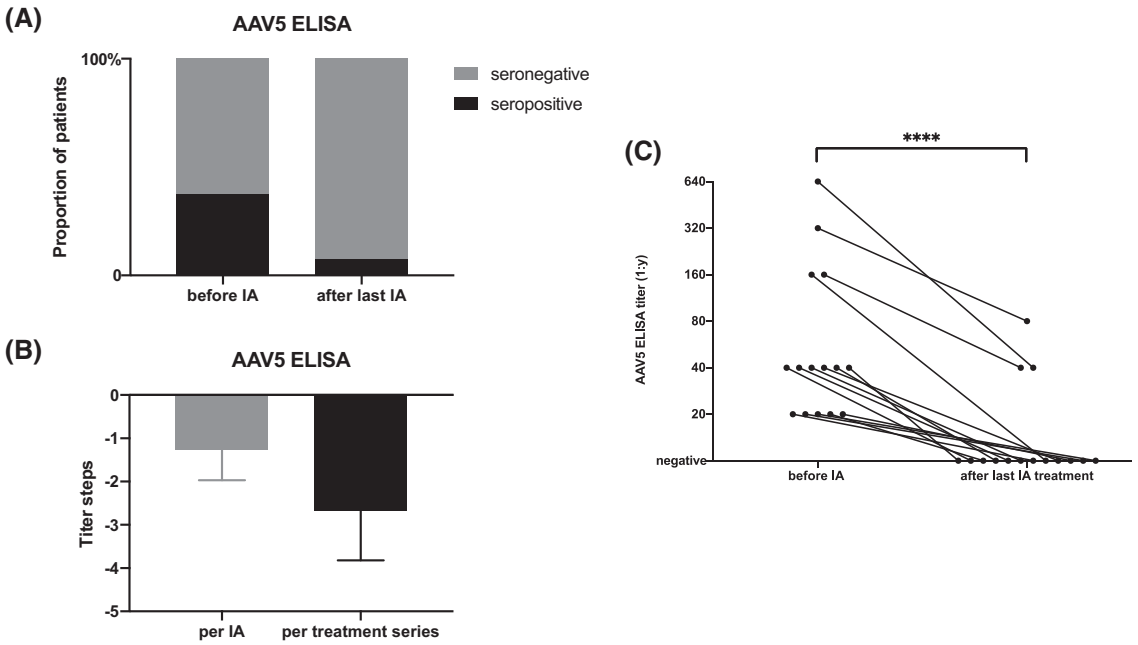

FIGURE 4 ELISA analysis of AAV5 antibodies: seropositivity before and after immunoadsorption and anti-AAV5 antibody reduction by immunoadsorption. (A) Proportion of patients with anti-AAV5 antibodies before and after IA treatment. Antibody levels were determined by ELISA. (B) Mean anti-AAV5 ELISA titer step reduction per IA. (C) Visualization of the individual anti-AAV5 ELISA titers from before the first IA to after the last IA. Statistical analysis was performed by two-tailed Wilcoxon matched-pairs signed-rank test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. AAV, adeno-associated virus; IA, immunoadsorption. (n = 15).

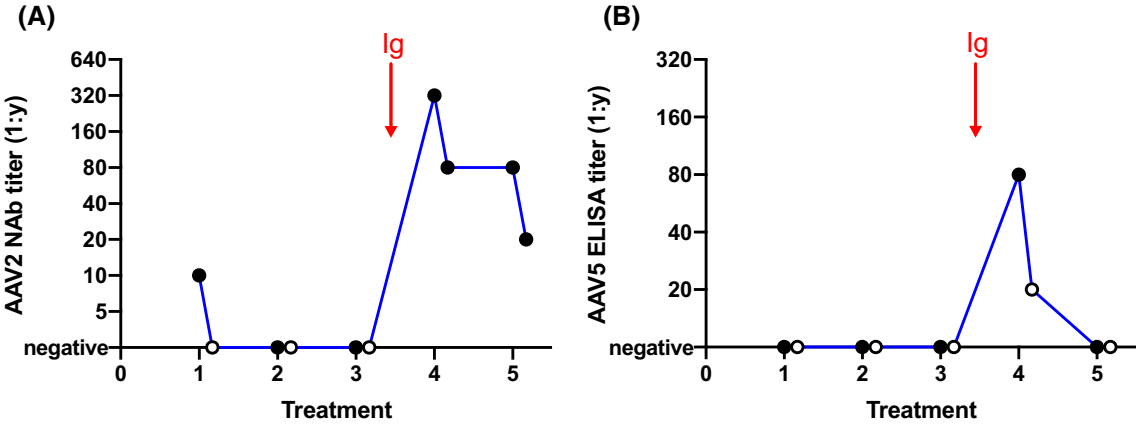

FIGURE 5 Time course of antibody titers with application of immunoglobulins after the third immunoadsorption treatment in one patient with humoral rejection after kidney transplantation. (A) Time course of anti-AAV2 neutralizing antibody titer from before the first IA to after the last IA. (B) Time course of AAV5 ELISA titers. Anti-AAV titers before (full circle) and after (blank circle) the IA treatment are displayed for each treatment day. AAV, adeno-associated virus; IA, immunoadsorption; Ig, immunoglobulin; NAb, neutralizing antibody.

From the five patients with pre-existing anti-AAV5 NAbs, four had no detectable anti-AAV5 NAbs after their last IA treatment (Figure 2B), and anti-AAV5 NAbs were reduced in all patients (Figure 4E, Supplementary Figure 4) with a mean reduction of 1.25 ± 0.71 titer steps (58.0%) per treatment (Figure 4D).

3.3 | Anti-AAV5 ELISA analysis

Analysis of NAb to AAV can be affected by different transduction efficiencies of AAV vectors in cell culture. While AAV2 has good transduction efficiencies for many different cell types, AAV5 results in poor transduction of

cultured cells.[29] Therefore, higher AAV5 doses were applied in this study (MOI AAV2: 200; MOI AAV5: 1500), which, however, potentially masked detection of low amounts of NAb to AAV5. To that end, we analyzed patient serum samples by a direct anti-AAV5 ELISA, which was assumed to provide higher sensitivity compared to the AAV5 NAb assay.

Anti-AAV5-antibodies were detected in 15 out of 40 patients before IA. Twelve out of these 15 patients had no detectable anti-AAV5 antibodies after the last IA treatment (Figure 5A). Anti-AAV5 ELISA titer was reduced in all seropositive patients with a reduction of 1.27 ± 0.71 titer steps per single treatment, which corresponds to 58.4% (Figure 5B,C, Supplementary Figure 5). Overall anti-AAV5 titer reduction over the whole treatment period of three to five IA treatments was 2.67 ± 1.16 (84.3%), respectively. In conclusion, in the first performed NAb assay 5 out of 40 patients were positive for AAV5 antibodies and second, we also performed an AAV5 ELISA with an increased sensitivity, in which Ab were detectable in 15 of the 40 patients. This explains the two different results by assay type.

3.4 | Correlation of IgG concentration and AAV titer

In the real-world setting the patient should ideally be dosed with an AAV gene therapy immediately after IA when antibody levels are the lowest (Figures 1C and 4C). However, analysis of anti-AAV antibody titers is lengthy and can often not be conducted at the clinical center, hereby delaying time to dosing. In contrast to that, standard total IgG assays are widely available and can be completed within a few hours. Therefore, total IgG concentration has been proposed as a surrogate for anti-AAV titer[23] and led us to perform a correlation analysis of IgG levels and anti-AAV2 NAb titers. The analysis did not indicate an association between pre-IA IgG concentrations and pre-IA anti-AAV2 NAb titers (Spearman correlation coefficient $r_s = 0.1728$, $P = .2864$). However, IgG reduction and AAV2 NAb titer reduction highly correlated (Spearman correlation coefficient $r_s = 0.9167$, $P = .0013$) suggesting potential use of IgG reduction measured by a standard IgG assay as anti-AAV antibody reduction surrogate (Supplementary Figure 3).

3.5 | Factors influencing IA therapy

In the context of humoral rejection after kidney transplantation, one patient (patient 22) received immunoglobulins once after the third IA treatment because of the substantial immunosuppressive therapy (Supplementary Table 1).

As expected, an IgG increase was seen at the beginning of the fourth IA. Interestingly, there was also a strong increase in anti-AAV2-NAb and anti-AAV5-ELISA titers in this patient (Figure 3), putatively as a result of anti-AAV antibodies contained in the immunoglobulin preparation. During the fourth and fifth IA treatment, the antibody levels were successfully reduced. However, an AAV2-NAb titer of 1:20 persisted after the fifth IA (starting from 1:320 before the fourth IA).

4 | DISCUSSION

AAV vector-based gene therapies face the problem that a substantial fraction of patients are not eligible for therapy because of the presence of antibodies to AAV in their blood. This is particularly important for therapies based on intravenously applied AAVs.[30] Both neutralizing and non-neutralizing antibodies play a role in pre-existing humoral immunity. While NAbs directly prevent vector transduction, non-neutralizing antibodies have the potential for complement activation and consequently increasing capsid immune responses.[31] Therefore, in clinical practice both neutralizing and non-neutralizing antibodies should be reduced before a possible gene therapy. The aim of this study was to understand the efficiency and timing of anti-AAV antibody removal by IA with regenerative broadband IA columns.

We found that anti-AAV2 NAb titers were decreased by a mean of approximately two $\log_2$ titer steps per treatment day and approximately four titer steps after a treatment series of three to five IA treatments. IgG reduction and anti-AAV2 NAb titer decrease were found to be highly correlated. An increase (rebound) of anti-AAV antibodies after the treatment was observed diminishing the anti-AAV antibody reduction 2 days after IA to two titer steps compared to pre-IA level. Ten out of 22 patients with anti-AAV2 NAbs (all <1:320) reached a titer below the typical cutoff of AAV gene therapy studies of 1:5 after the IA treatment series. Anti-AAV5 NAbs were reduced to a titer of <1:5 in four out of five patients. Levels of anti-AAV5 antibodies were decreased to below the detection limit in 12 out of 15 patients for AAV5, as determined by ELISA. The magnitude of antibody titer reduction was similar throughout all assays, suggesting that there are no quantitative differences in anti-AAV antibody removal between different AAV serotypes and detection methods. This indicates that IA treatment with broadband IA columns is an effective option to reduce anti-AAV antibodies. In addition, the steady decrease of all antibodies even by the fifth IA suggests that continuing the therapy by applying further

IA treatments could lead to an even greater reduction of anti-AAV antibodies. A similar scenario is present in the application of IA in the context of AB0-incompatible transplantation, where the number of IA treatments is adjusted to the individual antibody titer of the patients.[22]

Thus far, the threshold titer, that is, the presence or lack of NAb to AAV gene therapy vectors, cannot be directly correlated to a clinical outcome, because no AAV gene therapy has been performed after IA. Comparing protocols for NAb assays and ELISAs between different groups proved to be difficult due to differences in assay parameters.[32] Still the constant decrease of anti-AAV antibody titers throughout an IA treatment period demonstrated the effect of IA treatment and anti-AAV antibody decrease.

Considering the increased anti-AAV antibody rebound between IA treatments not performed on consecutive days, it becomes clear that daily IA treatment is significantly more effective in removing antibodies against AAV2 and AAV5 from the circulation than treatment on every other day. Accordingly, when planning the treatment, it is of great importance to take into account to what extent and from which body compartment antibodies shall be removed. In autoimmune diseases, a shift of autoantibodies from the tissue (ie, non-vascular compartments) into the vascular system is intended to support immunosuppressive therapy. Therefore, treatment-free days were indicated for the patients studied here. However, if IA is used to prepare patients for AAV-vector-based gene therapies where a minimal antibody concentration in the circulation (regardless of the antibody concentration in other tissues) is intended, daily IA seems to be a more appropriate regime.

Another topic to be considered is the re-occurrence of anti-AAV antibodies within the first few days after AAV gene therapy. From our experience with the preparation of patients for ABO-incompatible living-donor kidney transplantation, we know that the measurement of isoagglutinins is indispensable during the first 14 days after transplantation. In case of an increase of isoagglutinins to 1:8 during these 14 days, further treatment by IA is necessary to avoid an increased risk of antibody-mediated rejection (ABMR).[33] IA treatment after AAV dosing during AAV-based gene therapy could be similarly useful to maintain a low level of anti-AAV antibody to suppress an immune reaction for the duration of AAV vector shedding. This warrants further studies, for example, it needs to be excluded if non-specific AAV adsorption to the IA column occurs, which would be detrimental to the effectiveness of AAV gene therapy in a situation where not all AAV vectors have already bound to their target cells.

With regard to the effective removal of antibodies by IA, it should also be noted that immunoglobulin substitution carries the risk of introducing unwanted antibodies, for example, against AAV, which must be removed during therapy. This was observed in one of the patients we treated. Therefore, administration of immunoglobulins under IA should be avoided.

As a potential alternative to IA, there is also the possibility of apheresis therapy using TPE. Here, Monteilhet et al. among others demonstrated that AAV-1, -2, -6, and -8 antibodies can be effectively reduced by TPE. TPE was performed by substitution with 5% albumin and the treatment intervals were between 1 and 5 days. Patients treated with immunoglobulins or fresh frozen plasma were excluded from the study. Our study results clearly show that daily treatment with IA produces a more effective reduction in AAV antibodies. This means that, according to our study results, a treatment regimen of 4–5 daily treatments prior to possible AAV vector-based gene therapy is recommendable. However, this is only possible to a limited extent by using non-selective TPE, due to the furthermore prevailing removal of important plasma proteins such as coagulation factors or cytokines. Alternatively to the exchange with human albumin, an exchange with fresh frozen plasma is also available in order to perform daily treatments with plasma exchange. This is an effective therapy option, for example, in the treatment of autoimmune diseases. However, prior to prospective treatment using AAV vector-based gene therapy, exchange with fresh frozen plasma could bend risks. On the one hand, there even seems to be an increase in proinflammatory cytokines during plasma exchange therapy with fresh frozen plasma, which indicates an activation of the cellular immunity.[34] On the other hand, substitution of FFPs brings the possibility of introduction of AAV antibodies to the patient. This should be investigated with further studies.

Another interesting therapeutic apheresis concept was recently suggested by which not all immunoglobulins, but only AAV-specific antibodies, were removed through binding to full capsids coupled to an adsorber matrix.[24,35] While in vitro analyses and studies with passive immunization models look promising, efficacy and safety need to be confirmed in further preclinical and clinical studies.

In addition, as already described, there are other approaches that circumvent pre-existing anti-AAV antibodies to make AAV vector-based gene therapy possible for a larger number of patients. These treatments and IA should not be regarded as mutually exclusive. On the contrary, a combination of different therapy strategies may result in a more efficient anti-AAV antibody removal and thus in a further increase in the number of patients amenable to AAV-based gene therapy compared to the application of a single treatment type.

5 | CONCLUSION

In summary, our results indicate that IA with regenerative broadband IA columns represents a potentially safe and effective strategy to increase the patient population amenable to AAV-based gene therapy by lowering pre-existing anti-AAV antibodies to below threshold levels that are prerequisite for AAV gene therapy application.

AUTHOR CONTRIBUTIONS

Julia Weinmann-Menke, Thomas Schreiner, Bernhard Gerstmayer, and Magnus Mayer designed the study. Simone Boedecker-Lips, Pascal Klimpke, and Stefan Holtz carried out the study procedure. Julia Weinmann-Menke oversaw conduct of the study. Andreas Judel, Klaus Eulitz, and Magnus Mayer developed and performed the anti-AAV antibody assays. MEM analyzed the IgG subclasses. Simone Boedecker-Lips, Andreas Judel, Klaus Eulitz, and Magnus Mayer analyzed the data. Simone Boedecker-Lips and Magnus Mayer compiled the first version of the manuscript. All authors contributed to the interpretation of results and provided critical input during drafting of the article.

ACKNOWLEDGMENTS

We wish to thank the team of the dialysis ward of the University Hospital Mainz for excellent technical assistance and for the support and Ralph Schaloske from Miltenyi Biotec for final proofreading of the manuscript. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

No external funding.

CONFLICT OF INTEREST STATEMENT

Simone Boedecker-Lips, Stefan Holtz, Magnus Mayer, Pascal Klimpke, Daniel Kraus, and Julia Weinmann-Menke declare no conflict of interest. Thomas Schreiner, Bernhard Gerstmayer, Klaus Eulitz, Andreas Judel, and Magnus Christopher Mayer are current employees of Miltenyi Biotec.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Boedecker-Lips S, Judel A, Holtz S, et al. Efficient removal of antibodies to adeno-associated viruses by immunoadsorption. *J Clin Apher*. 2023;1-12. doi:10.1002/jca.22069