Efficient removal of antibodies to adeno-associated viruses by immunoadsorption

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Abstract

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 to AAV. 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 analyzed blood sera from patients receiving IA treatment because of underlying autoimmune disease or transplant rejection.

Our results show that IA efficiently reduces pre-existing anti-AAV antibodies with continuous lowering of neutralizing antibodies to below the threshold titer of 1:5 in 45% (anti-AAV2) and 80% (anti-AAV5) of the patients, respectively, after three to five single IA treatments.

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

Introduction

Adeno-associated viral (AAV) vector–based gene therapies have proven to be successful for the treatment of a variety of human diseases in clinical trials, and the first AAV-based treatments have already been granted market authorization (1–3). To date, 5,000–8,000 monogenetic diseases have been found, which affect almost 6% of the world population. These patients are all potential recipients for gene therapies (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 healthy volunteers or patients (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 (9–11).

Several strategies have been tested in animal models to overcome pre-existing humoral immunity to AAV. This includes the design of less seroprevalent AAV capsids (12), systemic immune suppression (13), and the use of IgG-degrading enzymes (14–16). However, all these strategies involve certain drawbacks as they may require an adaptation of the drug product formulation, increase the risk of side effects, or are limited to 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 plasma exchange has been shown to successfully deplete anti-AAV antibodies in humans (17). However, the non-selective removal of plasma proteins prevents application of daily intensive plasmapheresis treatments required for efficient removal (18–20).

Immunoadsorption (IA) is a specific plasmapheresis technique by which immunoglobulins are selectively removed from patients' plasma. It is frequently used in hospitals for the treatment of antibody-mediated autoimmune diseases, e.g., ANCA-associated vasculitis (21), systemic lupus erythematosus (22), and multiple sclerosis (23), or to enable AB0-incompatible solid organ transplantation (24).

Removal of anti-AAV antibodies by IA and subsequent successful re-administration of AAV5 gene therapy has already been demonstrated in non-human primates (NHPs) (25).

Here we applied an IA system, TheraSorb – Ig omni 5 adsorbers, that has been shown to efficiently deplete IgG, IgA, and IgM antibodies (26) based on two recombinant antibody fragments with specificity to the constant region of human kappa and lambda light chains. 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 human anti-AAV antibodies to AAV2 and AAV5.

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).

Donors

Sera from 43 patients undergoing IA treatment because of underlying or suspected autoimmune conditions or transplant rejection were collected. Blood samples were collected immediately before and after each treatment. Subsequently, the samples were centrifuged for serum collection and aliquoted.

Immunoadsorption therapy

IA treatments were performed on the TheraSorb LIFE 21 apheresis unit (Miltenyi Biotec) with TheraSorb – Ig omni 5 adsorbers (330-000-965, Miltenyi Biotec). During each IA treatment, two patient plasma volumes were processed. Intravenous immunoglobulins (Octagam) 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 pauses compared to the standard schedule in some patients (see Suppl. Table 1). Additionally, immunosuppressive therapy was applied in the majority of patients as indicated in Suppl. Table 1.

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).

Immunoglobulin measurement

IgG analysis of the sera was performed on the AU480 Clinical Chemistry system (Beckman Coulter) with re-analysis on the Immage 800 analysis system (Beckman Coulter) if the IgG concentration was below 0.75 g/L.

Neutralizing antibody assay

Anti-AAV NAb analysis was performed with a protocol similar to the one described by Meliani *et al.* (27). On the first day, $2{\cdot}10^4$ 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 (RLU) 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.

ELISA analysis

For anti-AAV ELISA analysis 96-well ELISA plates (Nunc MaxiSorp Catalog #44-2404-21, ThermoFisher) were coated with 100 ng AAV2/2 or 25 ng AAV2/5 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. Samples were determined as seronegative when OD + 3*SD of AAV–pre-incubated samples was lower than OD-3*SD of samples without pre-incubation. Seronegative samples were used to establish an absorbance cutoff value with 95% confidence level (28). ELISA titer of a sample was determined as the highest serum dilution with an OD value above cutoff.

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). The statistical methods applied are specified in the figure legends.

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 TheraSorb – Ig omni 5 adsorbers. Samples from three patients were excluded from further analysis because of very low total pre-apheresis IgG levels before the first IA (< 0.75 g/L). Two of these three patients received therapeutic plasma exchange whereas the third patient received corticosteroid pulse therapy and interferon beta prior to IA. Baseline characteristics of the 40 patients are summarized in 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 (250–1,000 mg for 3–5 days depending on the disease), out of which 12 patients were then treated with continuous steroid therapy. In addition, nine other study patients received continuous cortisone therapy. Other immunosuppressive therapies such as mycophenolate mofetil, calcineurin inhibitors, and rituximab were also given to some study patients. The individual immunosuppressive therapy of each patient can be found in Suppl. Table 1.

Measurement of IgG concentration during treatment

In all blood samples of the 40 patients who were treated with TheraSorb – Ig omni 5 adsorbers, IgG levels were determined in order to prove the general effectiveness of the treatment (Fig. 1A, Supplementary Table 2). 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% (Fig. 1B). Lower-than-average reduction levels were observed for patients with very high pre-IA IgG levels (Suppl. Table 2). Between treatment days serum IgG concentration increased due to redistribution of IgG from the tissue into the circulation which is a typical observation in plasmapheresis/immunoadsorption treatments (26). According to our clinic´s internal standard, the last two treatments within a series of five are usually done every other day to improve IgG depletion from tissue. Stronger redistribution of IgG from the tissue into the circulation with longer time intervals between IA treatments explains why there was no further IgG reduction in the blood after treatment 3.

Anti-AAV2- and anti-AAV5-neutralizing antibody (NAb) detection

All pre-IA serum samples were analyzed for the presence of neutralizing antibodies (NAbs) against serotypes AAV2 and AAV5. Twenty-two out of 40 patients were positive for anti-AAV2 NAbs, and 5 out of 40 patients had anti-AAV5 NAbs (Fig. 2).

Each of these patients underwent a series of three to five TheraSorb – Ig omni 5 treatments. Anti-AAV2 and anti-AAV5 NAb titers were analyzed before and after each treatment (Suppl. Figure 1 + 2, Fig. 6).

In all patients AAV2 titers showed a mean reduction of 1.92 ± 0.74 titer steps, which corresponds to 73.5%, per treatment, respectively (see Fig. 3a-b). Before the next treatment, serum anti-AAV2 NAbs increased by a mean of 1.03 ± 0.83 titer steps when the treatment was performed every day and by a mean of 2.00 ± 1.12 with more than one day between treatments (Fig. 3c).

Over the whole series of three to five TheraSorb – Ig omni 5 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, ten had no detectable anti-AAV2 NAbs (Fig. 2a) after their last treatment, and in all patients anti-AAV2 NAb titers were reduced (Fig. 3b, Suppl. Figure 1).

From the five patients who were seropositive for anti-AAV5 NAbs, four had no detectable anti-AAV5 NAbs after their last IA treatment (Fig. 2b), and anti-AAV5 NAbs were reduced in all patients (Fig. 3e, Suppl. Figure 2). Anti-AAV5 NAbs were reduced by 1.25 ± 0.71 titer steps (58.0%) per treatment (Fig. 3d).

Anti-AAV2 and anti–AAV5 ELISA analysis

Analysis of neutralizing antibodies 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 neutralizing antibodies to AAV5. To that end, we analyzed patient serum samples by a direct anti-AAV5 ELISA.

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 (Fig. 4b). 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% (Fig. 5c, d, Suppl. Figure 4). Overall anti-AAV5 titer reduction over the whole treatment period of three to five IA treatments was 2.67 ± 1.16 (84.3%), respectively.

Additionally, patient sera were analyzed using an anti-AAV2 antibody ELISA. Fourteen out of 40 patients were seropositive, and 8 out of 14 patients had no detectable AAV2 antibodies after the last IA treatment (Fig. 4a). Consistent with the results above, anti-AAV2 antibodies were reduced in all patients over the series of IA treatments with a reduction of 1.49 ± 0.67 titer steps corresponding to 64.3% (Fig. 5a, b, Suppl. Figure 3). Overall titer reduction over the whole IA treatment series was 3.17 ± 0.99 titer steps (88.9%).

Unexpectedly, anti-AAV2 antibody titers measured by ELISA were found to be ~ 2 titer steps lower compared to anti-AAV2 NAb titers throughout the sample set (Suppl. Figure 1, 3). This means that the anti-AAV2 ELISA was less sensitive than the NAb assay, in contrast to the anti-AAV5 ELISA, which was more sensitive than the NAb assay. This could be due to several factors: On the one hand, AAV2 had a very good transduction efficiency enabling design of a very sensitive anti-AAV2 NAb assay. On the other hand, we observed higher background signals in the anti-AAV2 ELISA for the anti-AAV2 negative serum samples compared to the anti-AAV5 ELISA, which led to a higher cutoff value for positive vs. negative discrimination and thus decreased sensitivity of the anti-AAV2 ELISA.

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 (Suppl. 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 as well as anti-AAV2- and anti-AAV5-ELISA titers in this patient (Fig. 6), putatively as a result of anti-AAV antibodies contained in the immunoglobulin preparation. In this context, it should be noted that the anti-AAV2-NAb titer was much higher before the fourth treatment than before the first treatment, and the anti-AAV2 and anti-AAV5 ELISA were positive for the first time (Fig. 6). 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).

Discussion

AAV vector–based gene therapies face the problem that a substantial fraction of patients are not eligible for this type of therapy because of the presence of antibodies to AAV in their blood, putatively as a result of previous encounters to wild-type AAVs. This is particularly important for therapies based on intravenously applied AAVs and to a lesser extent for gene therapies applied to immune privileged sites (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 determined before a possible gene therapy.

Fundamental measures are necessary to apply AAV-based gene therapy successfully despite the presence of anti-AAV antibodies. Thus, the aim of this study was to understand the efficiency and timing of anti-AAV antibody removal by IA with TheraSorb – Ig omni 5 adsorbers.

We found that anti-AAV2 NAb titers were decreased by a mean of approximately two titer steps per treatment day with an increase (rebound) of approx. one titer step over the course of 24 h before the next treatment. Higher rebound rates were observed with longer

intervals between treatments which is in line with stronger IgG redistribution. In all seropositive patients, the anti-AAV pre-IA titer was reduced. Anti-AAV2 NAb titers were found to be as high as 1:10,240. 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 Ig omni 5 treatment series. Anti-AAV5 NAbs were reduced to a titer of < 1:5 in four out of five patients. Levels of AAV2 and AAV5 antibodies were decreased below the detection limit in eight out of 14 patients for AAV2 and in 12 out of 15 patients for AAV5, as determined by ELISA. Differences in the proportion of patients who had detectable anti-AAV antibody titers after the last IA treatment are due to differences in pre-IA anti-AAV titers. The magnitude of antibody titer reduction was similar throughout the study, suggesting that there are no quantitative differences in anti-AAV antibody removal between different AAV serotypes. This indicates that IA treatment with TheraSorb – Ig omni 5 is an effective option to reduce 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. This prolonged treatment may further decrease anti-AAV antibodies to lead patients with higher initial titers of ≥ 1:320 to titers below the threshold titer of 1:5 at the end of an IA treatment session. A similar scenario is 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 (24).

Thus far, the threshold titer, i.e., the presence or lack of neutralizing antibodies 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 neutralizing antibody assays and ELISAs between different groups proved to be difficult due to differences in several parameters such as vector preparations and applied AAV doses or MOI (32). Still the constant decrease of anti-AAV antibody titers throughout an IA treatment period demonstrates the correlation between IA treatment and anti-AAV antibody decrease.

Considering the data showing the titer rebound between IA treatments, it becomes clear that daily IA treatment is significantly more effective in removing antibodies to AAV2 and AAV5 from the circulation than treatment 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 (i.e. non-vascular compartments) into the vascular system is intended to support immunosuppressive therapy. Therefore, pause days between the third and fourth as well as fourth and fifth IA are certainly indicated. 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 an 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 of 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. However, if IA is applied too soon after dosing, AAVs could potentially be removed inadvertently while passing through the adsorber where the residual amounts of anti-AAV antibodies from the blood are captured. In this context it would be of great interest to investigate the timing of a potential IA treatment after AAV vector–based gene therapy.

Regarding the effective removal of antibodies by IA, it is also important to note that the application of immunoglobulins or foreign plasma always carries the risk of introducing undesired antibodies, e.g., against AAV. This can lead to a renewed increase in antibodies that need to be removed during therapy, which was the case in one of our treated patients. Therefore giving immunoglobulins or foreign plasma under IA should be avoided. This also prevents application of plasma exchange treatments with high intensity as a potentially simpler alternative to IA. To enable high intensity plasma exchange treatments use of fresh frozen plasma as substitution solution would be required to replenish physiologically important plasma proteins such as coagulation factors, cytokines, and others, but is not possible due to the likely presence of anti-AAV antibodies. Furthermore there even seems to be an increase in proinflammatory cytokines during plasma exchange therapy with fresh frozen plasma, which could indicate an activation of the cellular immunity by fresh frozen plasma (34).

Another interesting plasmapheresis concept was suggested recently by which not all immunoglobulins, but only AAV-specific antibodies, were removed through binding to full capsids coupled to an adsorber matrix (35, 36). 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 these 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. Combination therapies would be most relevant to the treatment of patients with high titers of anti-AAV antibodies.

In summary, our results indicate that IA with TheraSorb – Ig omni 5 adsorbers 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 threshold levels that are prerequisite for AAV gene therapy application.

Declarations

Data Availability

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

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Author Contributions

Conceptualization: Julia Weinmann-Menke, Thomas Schreiner, Bernhard Gerstmayer and Magnus Mayer; methodology:, Klaus Eulitz and Magnus Mayer; validation: Magnus Mayer; formal analysis: Simone Cosima Boedecker-Lips, Andreas Judel and Magnus Mayer; investigation: Simone Cosima Boedecker-Lips, Pascal Klimpke and Stefan Holtz; data curation: Magnus Mayer; writing—original draft preparation: Simone Cosima Boedecker-Lips and Magnus Mayer; writing—review and editing: Julia Weinmann-Menke, Daniel Kraus, Thomas Schreiner, Bernhard Gerstmayer, Klaus Eulitz and Andreas Judel; supervision: Thomas Schreiner, Klaus Eulitz and Julia Weinmann-Menke; project administration: Magnus Mayer and Julia Weinmann-Menke. All authors have read and agreed to the published version of the manuscript.

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Competing Interests

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

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Tables

Table 1 is available in the Supplementary Files section.

Figures

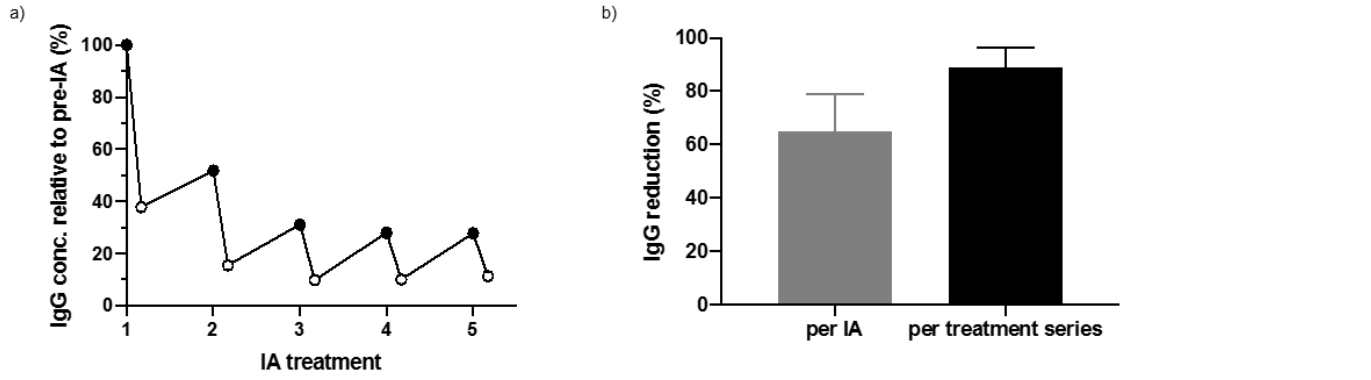

Figure 1

Serum IgG reduction during immunoadsorption (IA) treatment. a) Normalized averaged IgG concentration decrease over the treatment period. b) IgG percentage decrease per IA (black) and from the first to the last IA (grey).

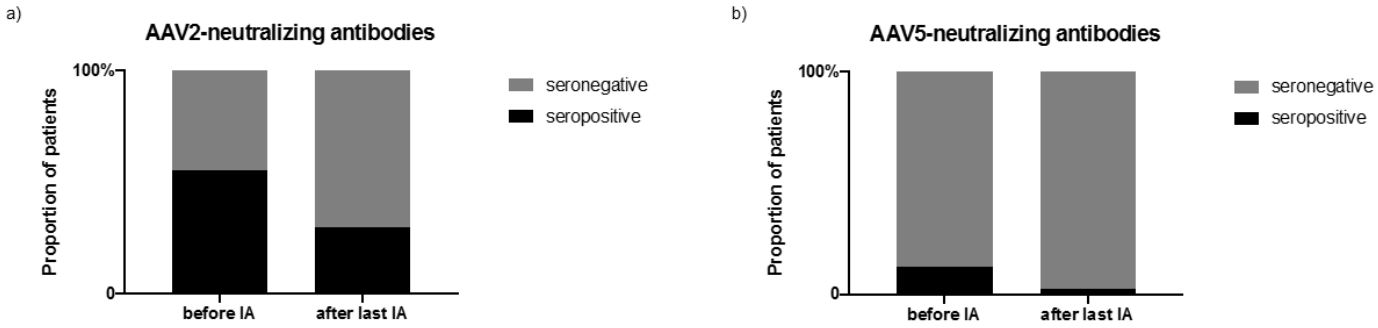

Figure 2

Seropositivity before and after immunoadsorption (IA). 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.

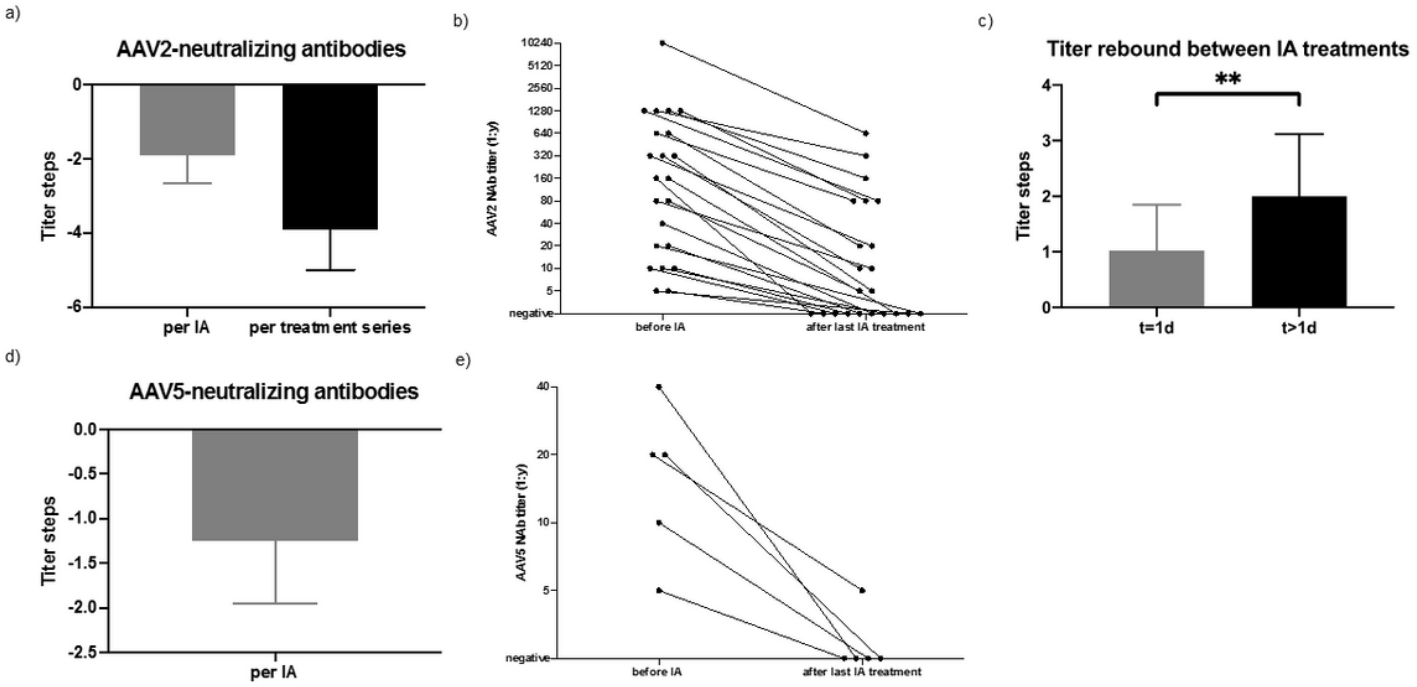

Figure 3

Reduction of anti-AAV2 and anti-AAV5 neutralizing antibodies (NAb) by immunoadsorption (IA). 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 anti-AAV2 NAb titer rebound between treatments performed on successive days (grey) compared to treatments with at least one treatment-free day in between (black). 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 significance was determined by the nonparametric Mann-Whitney test. ** $P < 0.01$.

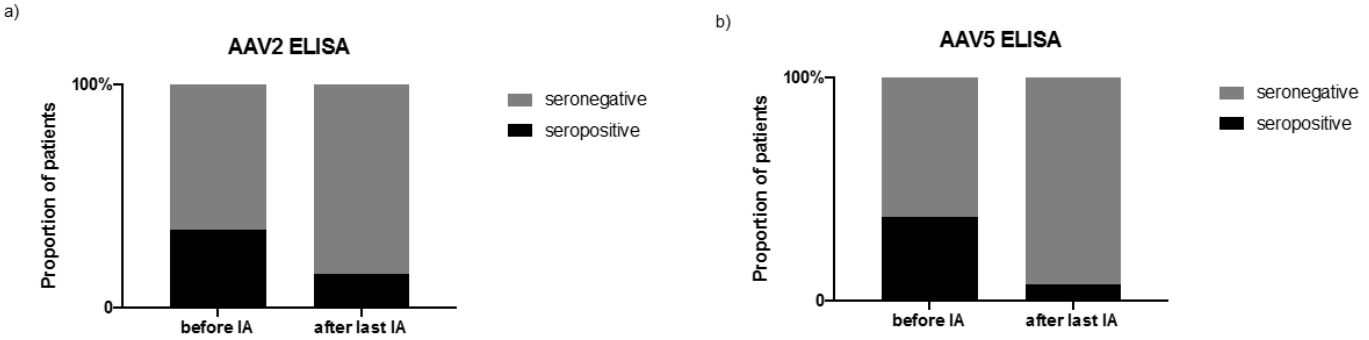

Figure 4

Seropositivity before and after immunoadsorption (IA). Proportion of patients with anti-AAV2 antibodies (a) and anti-AAV5 antibodies (b) before and after IA treatment. Antibody levels were determined by ELISA.

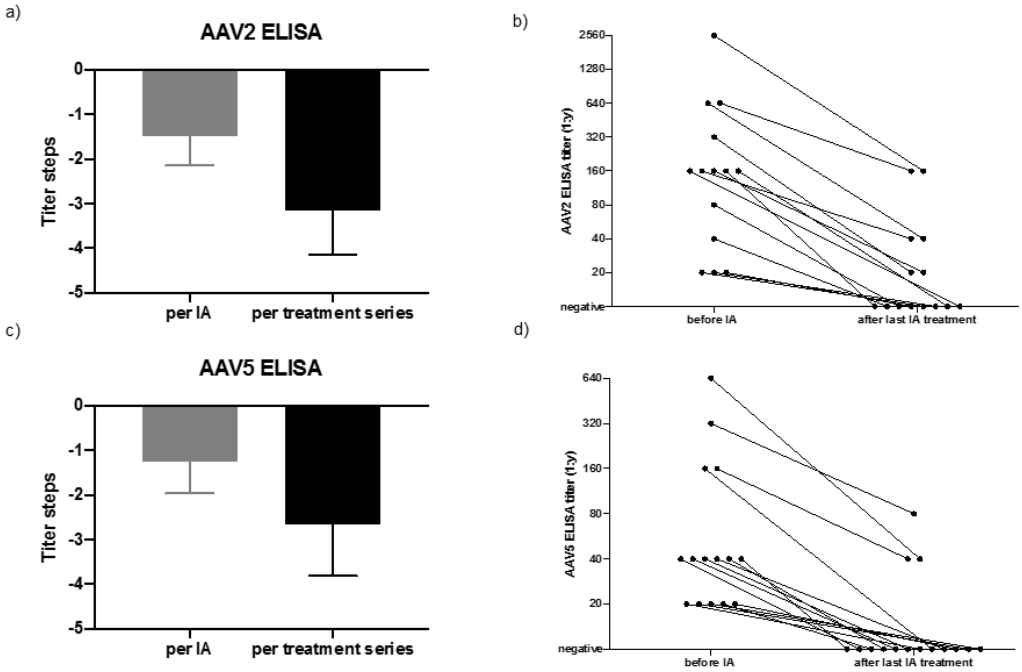

Figure 5

ELISA analysis of anti-AAV2 and anti-AAV5 antibody reduction by immunoadsorption (IA). a) Mean anti-AAV2 ELISA titer step reduction per IA and over the IA treatment series. b) Visualization of the individual Anti-AAV2 ELISA titers from before the first IA to after the last IA. c) Mean anti-AAV5 ELISA titer step reduction per IA. e) Visualization of the individual Anti-AAV5 ELISA titers from before the first IA to after the last IA.

Figure 6

Time course of antibody titers with application of immunoglobulins (Ig) after the third immunoadsorption (IA) treatment in one patient with humoral rejection after kidney transplantation. a) Time course of anti-AAV2 neutralizing antibody (NAb) titer from before the first IA to after the last IA. b) Time course of anti-AAV2 and AAV5 ELISA titers.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.Patientcharacteristics.xlsx
- SupplementaryTable1.Treatmentdaysandadditionalimmunosuppressivetreatment.PEplasmaexchange.IAimmunoadsorption.xlsx
- SupplementaryTable2.IndividualIgGconcentrationsoverthecourseofimmunoadsorptiontreatment.xlsx
- SupplementFigures1.tif
- SupplementFigures2.tif
- SupplementFigures3.tif
- SupplementFigures4.tif