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Humoral and Cellular Capsid-Specific Immune Responses to Adeno-Associated Virus Type 1 in Randomized Healthy Donors

Philippe Veron,* Christian Leborgne,⁎† Virginie Monteilhet,⁎† Sylvie Boutin,⁎† Samia Martin, † Philippe Moulière,⁎‡§ and Carole Masurier* 

A major impediment to the use of adeno-associated virus (AAV)-mediated gene delivery to muscle in clinical applications is the pre-existing immune responses against the vector. Pre-existing humoral response to different AAV serotypes is now well documented. In contrast, cellular responses to AAV capsid have not been analyzed in a systematic manner, despite the risk of T cell reactivation upon gene transfer. AAV1 has been widely used in humans to target muscle. In this study, we analyzed PBMCs and sera of healthy donors for the presence of AAV1 capsid-specific T cell responses and AAV1 neutralizing factors. Approximately 30% of donors presented AAV1 capsid-specific T cells, mainly effector memory CD8+ cells. IFN-γ–producing cells were also observed among effector memory CD4+ cells for two of these donors. Moreover, to our knowledge, this study shows for the first time on a large cohort that there was no correlation between AAV1-specific T cell and humoral responses. Indeed, most donors presenting specific Ig and neutralizing factors were negative for cellular response (and vice versa). These new data raise the question of prescreening patients not only for the humoral response, but also for the cellular response. Clearly, a better understanding of the natural immunology of AAV serotypes will allow us to improve AAV gene therapy and make it an efficient treatment for genetic disease. The Journal of Immunology, 2012, 188: 6418–6424.

aden-associated virus (AAV) has emerged as an attractive treatment strategy for viral vector-mediated gene replacement. The development of such strategy with recombinant AAV is probably due to the lack of pathogenicity of the wild-type virus, the ability to establish long-term transgene expression, the ability to transduce both dividing and nondividing cells over a broad host range (human, simian, murine, canine, and avian cells), the tendency for the viral genome to remain as episomal concatemers, and its relative lack of immunogenicity. Because of its wide tissue tropism, rAAV has been used for in situ viral gene transfer, and serotypes 1 and 2 AAV showed some success in human trials (1–5). However, the host immune response to the viral capsid protein has emerged as a serious obstacle for successful translations of AAV-based strategies to humans. Indeed, we and others have shown that the presence of specific Ig and neutralizing factors (NAF) against wild-type AAV, which commonly circulates in humans, is one of the limitations of in situ transduction efficacy using cognate recombinant vector (6–8). We previously showed that the two highest NAF seroprevalences were in the French population for AAV2 (59%) and AAV1 (50.5%) (8), which was consistent with another report concerning Europe. The lowest NAF seroprevalences were observed for AAV8 (19%) and AAV5 (3.2%). Of note, we observed only one serum sample in which the titer of NAF against AAV1 exceeded that of AAV2, and no serum sample in which the titer of NAF against AAV5, 6, 8, and 9 exceeded that of AAV2 (8). Furthermore, it has been shown that the host immune response to the AAV capsid mediated by circulating Abs may prevent repeated administrations (9). Data indicate that even low levels of neutralizing Abs (1:5 to 1:10) can completely abrogate AAV-mediated transduction (9–11). In clinical trials, a cellular immune response against the AAV1 and AAV2 capsids has been observed after several routes of administration and may have been responsible for the loss of transgene-expressing cells (12–15). Recently, pre-existing memory T cells specific for AAV2 viral capsid protein were observed in naturally infected humans, and it was suggested that this cellular response may also prevent sustained transgene expression (12, 16). Furthermore, it has been shown that AAV2 capsid-primed CD8+ memory T cells in humans cross-react with epitopes from AAV8 and AAV1 capsids and result in expansion of functional CD8+ cytotoxic lymphocytes that are indistinguishable from those elicited by AAV2 capsid epitopes (12).

Despite the fact that AAV2 has been isolated from humans and AAV1 and AAV8 from nonhuman primates (17, 18), pre-existing immune responses have been observed in humans against these different serotypes, which share >80% homology at the nucleotide level, suggesting cross-reactions between them (18). Because of its tropism for skeletal muscle AAV1 has been delivered i.m. in several human clinical trials (15, 19–21).

Our goal in this study was to determine the correspondence between AAV1 capsid-specific T cell responses and the humoral response in healthy donors. We tested PBMCs for the presence of AAV1-specific T cells and sera from the same donors for the prevalence of AAV1-specific Ig and NAF. Our results show that AAV1 capsid-specific T cells were mainly effector memory CD8+ T cells. Furthermore, we showed that there is no correlation between the AAV1-specific T cell response and the Ig or NAF
responses. These data indicate that prescreening of patients for AAV-specific NAF only is not sufficient; prescreening of patients for specific cellular responses should be implemented to increase the safety of gene therapy with AAV. It is now admitted that patients enrolled in a clinical trial using AAV need to be prescreened for AAV-specific humoral responses. We think that it would be useful to determine whether prescreening of AAV-specific cellular responses may also be required.

Materials and Methods

Samples

Serum samples were collected from normal volunteers. A total of 55 blood samples from donors, recruited in the Ile de France community in France, were collected by the French Etablissement Français du Sang according to their procedures.

Production of AAV vectors and contaminant proteins

Pseudotyped AAV vectors were generated by packaging AAV2-based recombinant genomes in AAV1, as previously described (8). Briefly, all the vectors used in the study were produced using the three-plasmid transfection protocol as described elsewhere. Briefly, HEK293 cells were tritiated overnight with adenovirus helper plasmid pX26 (22), a pAA packaging plasmid expressing the rep and cap genes pLTC02 for AAV1 (23) and the relevant pAA2 vector plasmid. Single-stranded AAV vectors were produced with conventional PG22 AAV vector plasmid expressing luciferase (24) under the transcriptional control of the CMV immediate early promoter associated with the SV40 poly(A) signal. The contaminant proteins were obtained using the same transfection protocol (HEK293 cells, but lacking the packaging plasmid. Recombinant vectors and contaminant proteins were purified by double-ceolide chromatography and ultracentrifugation followed by dialysis against sterile PBS. Viral genomes were quantified by real-time PCR, vector titers are expressed as viral genomes per milliliter, and contaminant proteins were quantified by the Bradford protein assay.

Lentiviral vector production and titration

Vesicular stomatitis virus-pseudotyped third-generation lentiviral vectors (LV) were produced by transient four-plasmid cotransfection into 293T cells with the vectors used in the study were produced using the three-plasmid packaging plasmid expressing the rep and cap genes pLTC02 for AAV1 (23) and the relevant pAA2 vector plasmid. Single-stranded AAV vectors were produced with conventional PG22 AAV vector plasmid expressing luciferase (24) under the transcriptional control of the CMV immediate early promoter associated with the SV40 poly(A) signal. The contaminant proteins were obtained using the same transfection protocol (HEK293 cells, but lacking the packaging plasmid. Recombinant vectors and contaminant proteins were purified by double-chloride chromatography and ultracentrifugation followed by dialysis against sterile PBS. Viral genomes were quantified by real-time PCR, vector titers are expressed as viral genomes per milliliter, and contaminant proteins were quantified by the Bradford protein assay.

PMBC preparation and freeze-thawing

PMBCs were obtained after centrifugation in Lymphoprep tubes (Abecys, Paris, France). For freezing, PMBCs were suspended at a concentration of 5–7 × 10^6 cells/mL in 0.5 mL human AB serum maintained at 4°C. An equal volume of freezing medium (RPMI 1640, 20% DMSO), also at 4°C, was added dropwise, while gently mixing by shaking the tube. The resulting cell suspension was transferred in 1.8-mL cryovials (Nunc, Roskilde, Denmark). The tubes were placed into a prechilled (4°C) Nalgene cryogenic controlled freezing container (Perki-nElmer, Waltham, MA) and expressed in ng pg/mL.

To obtain Yac-s described elsewhere (23), HEK293 cells were produced and titrated: a control vector produced with a plasmid vector construct without transgene and a nonspecific signal (OD AA V-OD nonspecific signal). Sera were considered positive for VP1 when the number of IFN-γ–producing cells/10^6 cells was >1.3 times (cut-off based on mean ratio of six negative donors ± 3 SD) the corresponding control with LV-empty. Assays were scored when the number of spots under stimulation was >10 spots per 2 × 10^6 PMBCs. Under the conditions used in the assay, PMA/ionomycin produced >10,000 spots per 10^6 cells, anti-CD3 >3,900 spots per 10^6 cells, and CMV/EBV/influenza pool peptides >100 spots per 10^6 cells.

Flow cytometry analyses for LV-stimulated production of IFN-γ by PMBCs

PMBCs were incubated either with LV-VPI, LV-empty, and polyclonal controls under similar conditions to those described above in a 96-well U-bottom culture plate (Thermo Fisher Scientific, Roskilde, Denmark) for 24 h. A polyclonal stimulation with Con A was also performed. After stimulation, cells were incubated for 6 h at 37°C and 5% CO_2 with 1 μL GolgiStop (Becton Dickinson, Mountain View, CA) for 2 × 10^6 cells and washed once in PBS with 3% FCS. The lymphocyte phenotype was assessed using eight-color immunostaining with FITC-conjugated anti-CD62L (SK11), PE-conjugated anti–IFN-γ (25723.11), allophycocyanin-conjugated anti-CD45RA (SP6), Pacific Blue-conjugated anti-CD8 (RPA-T8), cyanin 7-allophycocyanin-cyamin-conjugated anti-CD3 (SP34-2), cyanin 7-PE-conjugated anti–IL-4 (SD-8), cyanine 5.5-PEC-conjugated anti-CD25 (M-A251), and Alexa Fluor 700-conjugated anti-CD4 (RPA-T4). All mAbs were purchased from Becton Dickinson. Briefly, CD3, CD4, CD8, CD45RA, CD62L, and CD25 Abs were diluted in PBS with 3% FCS at recommended concentrations and were added to the cells for 30 min at 4°C in the dark. After incubation, cells were fixed and permeabilized with BD Fix/Perm solution (Becton Dickinson) for 20 min, washed twice with BD Fix/Perm wash buffer 1×, and then stained for intracellular IFN-γ for 30 min at 4°C in the dark. After two washes, cells were resuspended in 200 μL PBS/3% FCS and analyzed by flow cytometry. Data were acquired using an LSR II flow cytometer (Becton Dickinson), and data analysis was performed using DIVA software (Becton Dickinson). Results are shown as IFN–γ–producing cells/10^6 cells. Samples were considered positive for VP1 when the number of IFN–γ–producing cells was >1.3 times (cut-off based on mean ratio of six negative donors ± 3 SD) the corresponding control with LV-empty.

Ab ELISA

ELISA was performed as previously described (27). Briefly, rAAV particles were diluted in coating buffer (0.1 M carbonate buffer at pH 9.5) to a final concentration of 2 × 10^10 viral genomes/ml. Fifty microtiter was added to each well in a 96-well Nunc MaxiSorp immunoplate (Thermo Fisher Scientific). At the same time, proteins corresponding to contaminants purified during the steps of rAAV production, but in the absence of viral particle formation, were diluted in the same buffer and seeded, in parallel, in different wells in the same Immunoplate at a concentration of 3.4 μg/ml. This amount of protein corresponds to an amount of contaminant protein equivalent to that seeded in the AAV wells, and the signal obtained at the cut-off responds to the nonspecific signal that was removed when not obtained with the same serum in the corresponding AAV wells. Plates were then incubated overnight at 4°C. The next day, plates were washed three times with blocking buffer (6% fat milk buffer in PBS) and then blocked with blocking buffer for 2 h at room temperature. Plates were washed again three times with wash buffer (0.05% Tween 20 in PBS) and then incubated with heat-inactivated serum (at 56°C for 30 min) diluted 1:3 to 1:65,610 for 1 h at 37°C. After three washes, HRP-conjugated Abs specific for Ig purchased from SouthernBiotech (Birmingham, U.K.) were added and incubated 1 h at 37°C. Finally, plates were washed three times with wash buffer and revealed with TMB substrate solution (Becton Dickinson) for 30 min in the dark. The reaction was stopped with H_2SO_4 solution and measurements were made at 450 nm. The results are expressed in arbitrary units (AU) using a colorimetric amplification system based on PAMPA.

The AAV-specific signal was reported as the OD from AAV-coated ELISA after removal of the OD obtained on contaminant protein ELISA, which is a nonspecific signal (OD AAV-OD nonspecific signal). Sera were considered
positive for AAV-specific Ig when the OD signal was ≥0.5 (cut-off based on mean OD of 58 negative healthy donors ± 3 SD) at dilution ≥1:3.

Neutralizing assay
The neutralizing assay was performed as previously described, with some modifications (27). Briefly, 96-well plates were seeded with 4 × 10^5 HeLa cells per well for 3 h. Recombinant AAV1-CMV-luciferase was diluted in DMEM (Invitrogen Life Technology, Auckland, CA) supplemented with 10% FCS (HyClone, Logan, UT) and incubated with 2- to 10-fold serial dilutions (1:2 to 1:12,800) of heat-inactivated serum samples (at 56˚C for 30 min) for 1 h at 37˚C. Subsequently, the serum/vector mixtures corresponding to 5 × 10^3 viral genomes per cell were added to cells plated earlier and incubated in DMEM plus 10% FCS for 48 h at 37˚C and 5% CO2. Each mix was performed in duplicate. Cells were then washed in PBS and lysed for 10 min in 0.2% Triton lysis buffer at 4˚C. The lysate was transferred to 96-well plates and the luciferase activity was read on a luminometer (Victor2; PerkinElmer Life Sciences, Waltham, MA). Transduction efficiency was measured as relative light units per second per well and normalized per amount of protein per well expressed as OD.

The neutralizing titer was reported as the highest serum dilution that inhibited the rAAV transduction by ≥50% compared with the control without serum and correlated with the amount of protein quantified in each well after cell lysis, by the Bradford assay.

Statistical analyses
Results were presented as the mean ± SD. A Student t test for paired data was used to determine significant differences between the two groups. A correlation test was performed employing ANCOVA to determine the correlation coefficient or covariance between two parameters. A covariance close to 0 implies that these parameters are not correlated.

Results
Frequency of AAV1 capsid-specific T cells in human PBMCs
We first analyzed the natural prevalence of AAV1-specific memory T cells from PBMCs in the general human population by testing 55 healthy adults for frequencies of circulating capsid-specific T cells by an ELISPOT assay (Fig. 1), as previously described (26). Briefly, to screen for T cell responses to these Ags, we used ELISPOT assay for IFN-γ secretion after stimulation of donor PBMCs with an LV encoding for VP1, and thus spanning VP2 and VP3 proteins (LV-VP1), at an MOI of 40. LV-VP1–mediated PBMC transduction was confirmed by PCR assay (data not shown), and we used an LV encoding for the enhanced-GFP for quantification. At an MOI of 40, 48 h after vector transduction 23.1 ± 4.7% PBMCs (n = 9 donors) were efficiently transduced (data not shown). Because empty LV itself can generate IFN-γ secretion by PBMCs, we systematically added a condition, used as background control, in which PBMCs were incubated with an LV that did not encode any transgene (LV-empty). The amount of LV-empty was defined as the amount of physical particles (p24) equivalent to those used under the corresponding LV-VP1 conditions. Samples were considered positive for VP1 when the number of spots was >1.8-fold (cut-off based on mean ratio of 32 negative donors ± 3 SD) the corresponding control using LV-empty. The ELISPOT data showed detectable IFN-γ secretion after stimulation with AAV1 capsid protein for 16 of 55 donors (29.1%), as illustrated in Fig. 1. Frequency of specific responding PBMCs ranged from 0.013 to 0.15%. To confirm these results, we performed similar experiments on PBMCs from 15 of the 55 healthy adult donors, but with intracellular cytokine staining for IFN-γ, as readout (Fig. 1). Cells were gated on forward and side scatter and samples were considered positive for VP1 when the number of cells was >1.3-fold (cut-off based on mean ratio of six negative donors ± 3 SD) the corresponding control using LV-empty (Fig. 1A). Cells were also analyzed for the expression of CD3, as illustrated in Fig. 1B. We showed detectable IFN-γ secretion by

![FIGURE 1.](http://www.jimmunol.org/) AAV1 capsid-specific IFN-γ secretion data from PBMCs of healthy donors. (A) Histogram showing the IFN-γ secretion specific for AAV1-VP1 from PBMCs of 16 responding healthy donors and 6 nonresponding donors revealed by ELISPOT or intracellular staining. IFN-γ secretion was revealed either by ELISPOT or by intracellular staining 48 or 24 h, respectively, after transduction of PBMCs by LV-VP1 or LV-empty. Results are expressed as ratio of number of spot-forming units secreting IFN-γ per 10^5 PBMCs after stimulation with LV-VP1 to number of spot-forming units secreting IFN-γ per 10^6 PBMCs after stimulation with LV-empty for each donor. nd, not done. (B) Images illustrating the number of spots observed, in duplicate, in IFN-γ ELISPOT for a responding donor (left columns) or for a nonresponding donor (right columns) after transduction of 2 × 10^5 PBMCs with LV-empty or LV-VP1. Flow cytometry analyses were performed to monitor the expression of CD3 marker and IFN-γ secretion, after gating on forward scatter/side scatter parameters. Dot plots show the results for the same donors as those of ELISPOT illustration. Values indicate the percentage of cells in the corresponding quadrant of dot plots.
CD3+ cells for the nine donors (nine of nine) previously screened positive by ELISPOT, at frequencies ranging from 0.2 to 0.8%. Of note, we also observed detectable IFN-γ secretion by CD3+ cells at frequencies ranging from 0.06 to 0.43%. This profile suggested that these cells were likely NK cells. Furthermore, the six donors (six of six) negative in intracellular staining assays were also negative in ELISPOT assays. Thus, there was a robust correlation between results obtained in both assays using the LV strategy.

Frequency of AAV1 capsid-specific CD4+ or CD8+ cells in PBMCs

Frequency and further characterization of AAV1 capsid-specific T cell subpopulations were assessed for five IFN-γ+ donors and three IFN-γ− donors by multicolor staining with Abs to differentiation and activation markers and for IFN-γ secretion, followed by flow cytometry analyses (Fig. 2). The five analyzed positive donors had detectable AAV1 capsid-specific CD3+ cells in blood. These cells were either CD8+CD4− T cells or double-negative CD8+CD4+ cells, with frequencies of 0.6 ± 0.4 and 1.3 ± 1.1% of the cells in the same subset, respectively. These cells were able to produce IFN-γ and they expressed the activation marker CD25 (Figs. 2B, 3A). Furthermore, we showed that AAV1 capsid-specific CD8+ T cells belong to the CD3+CD8+CD45RA−CD62L+ effector memory cell subpopulation (Figs. 2C, 4A), whereas all subpopulations are able to respond to polyclonal activation (Fig. 4A). A significant AAV1 capsid-specific CD4+ T cell response was observed for two donors (nos. 1001-3 and 1001-4) only (Figs. 2B, 3A). Indeed, for these donors 0.1% of the CD4+ cells produced IFN-γ and expressed the activation marker CD25. Of note, these CD4+ IFN-γ-producing cells are also CD45−CD62L− effector memory cells (Figs. 2C, 4B). As for CD8+ cells, activation and IFN-γ production of the CD4+ cells and CD4+ subsets were observed after a polyclonal stimulation (Figs. 3B, 4B). Also, detectable AAV1 capsid-specific CD3+ cells (Fig. 2), which are likely NK cells, were also observed in two of the donors (nos. 1009-5 and 1003-3) at frequencies of 0.7 and 0.2% in the subset, respectively. Collectively, these data showed IFN-γ-producing cells in different PBMC subsets.

Correlation between specific humoral and cellular responses to AAV1

The seroprevalence of total Ig Abs to AAV of serotype 1 were determined using AAV1-specific ELISA on the serum samples from the 51 adult healthy donors analyzed for AAV1 capsid-specific T cells responses. The prevalence of specific Ig is a relevant indicator of the frequency of individuals who are not naive for one of the AAV serotypes. Sera were considered positive for AAV-specific Ig when an OD signal of ≥0.5 was observed at a dilution of ≥1:5. Ig titer was defined as the highest dilution for which OD signal was ≥0.5. The AAV1 Ig seroprevalence in this population was 64.7%, with titers ranging from 1:3 to 1:65,610 (Fig. 5A). Donors were also analyzed for the prevalence of NAF against AAV of serotype 1 (Fig. 5A). Sera were considered positive for neutralizing capacity when a 1:2 dilution of serum inhibited vector transduction by 50% or more. The magnitude of the neutralizing activity to AAV1 was measured by determining the neutralizing...
Titer was defined as the highest dilution that still inhibited vector transduction by 50% or more. The AAV1 NAF seroprevalence in this population was 58.8%, with titers ranging from 1:2 to 1:3200 (Fig. 5A). As expected, a strong correlation was observed between the Ig and NAF prevalences and between Ig and NAF titers, with a covariance factor of 0.96 (Fig. 4A). These data were confirmed by a Spearman nonparametric correlation coefficient analysis ($r = 0.94$, $p < 0.001$) (data not shown). Similarly, we looked at whether there was a correlation between the humoral and the cellular responses, comparing the cellular response specific to LV-VP1 obtained by ELISPOT and either the AAV1 Ig titers (Fig. 5B) or the AAV1 NAF titers (Fig. 5C). Interestingly, we did not observe a correlation between cellular and Ig or NAF responses (covariance factors are close to 0). Only 27.3 and 23.3% of donors positive for AAV1 Ig and NAF, respectively, were also positive for T cells producing IFN-$\gamma$ upon VP1 stimulation. In contrast, 72.7 and 76.6% of donors positive for AAV1 Ig and NAF, respectively, were negative for T cells producing IFN-$\gamma$ upon VP1 stimulation. Furthermore, 27.8 and 33.3% of donors negative for AAV1 Ig and NAF, respectively,
were positive for T cells producing IFN-γ upon VP1 stimulation. Collectively, these data showed no correlation between titers and ELISPOT responses.

**Discussion**

AAV vectors are increasingly used for human gene therapy clinical trials. A major impediment to the use of AAV-mediated gene delivery to muscle and other target tissues in clinical applications is the pre-existing immune response against the vector. The pre-existing humoral response to different AAV serotypes has now been extensively documented (6–8). Cellular responses to AAV capsid have proven more difficult to apprehend, partly because they have not been analyzed in a systematic fashion. The frequency of circulating capsid-specific T cells was determined by IFN-γ ELISPOT in which cells were stimulated with AAV-specific overlapping peptides (12, 16). IFN-γ ELISPOT is useful as a screening tool because it is sensitive enough to detect low-frequency cells without requiring in vitro expansion of cells. Flow cytometry analyses with multicolor staining coupled with intracellular cytokine detection or pentamers technology were also used to characterize the memory T cell subsets, with sensitivity almost equivalent to that of IFN-γ ELISPOT (12, 16). It was first reported that AAV2-specific memory CD8 T cells may be detected in PBMCs from 2 of 46 adult human subjects (12). More recently, AAV2 capsid CD8+ T cells and CD4+ T cells were found in 8 and 9 of 17 donors, respectively (16). We analyzed the frequency of circulating AAV1 capsid-specific T cells by IFN-γ ELISPOT and flow cytometry with intracellular IFN-γ detection in healthy randomized donors. Nevertheless, instead of stimulating cells with VP1 capsid peptide pools, we chose a strategy in which PBMCs were transduced with an LV vector encoding VP proteins of AAV1. This strategy offers the advantages of a natural processing avoiding peptide excess and thus limiting competition between peptides and MHC class I and II peptide presentation. Furthermore, this strategy is less expensive. We showed that AAV1 capsid-specific T cells were observed in 16 of 55 donors. It is not surprising that the percentage of donors with AAV1 capsid-specific T cells that we observed was lower (~30%) compared with that previously observed for AAV2 (~50%) (16), because AAV1 responses were probably related to cross-reactions with AAV2. Analysis of the different populations involved showed that these CD3+ T cells were mainly effector memory CD8+ T cells. For two of the five positive donors analyzed, IFN-γ–producing cells were also observed among effector memory CD4+ T cells. Li et al. (16) also detected specific AAV2 T cells among CD4+ and CD8+ effector T cells and CD8+ central memory T cells. Therefore, these different profile responses may be related to the different serotypes analyzed. In our study, we also observed AAV1 capsid-specific CD3+ double-negative CD4−CD8− cells, which have not yet been systematically analyzed (28) and are described

**FIGURE 5.** Correlation between humoral and cellular AAV1 capsid-specific responses. Graph shows the correlation between the two parameters indicated on each graph after analysis of samples from the same 51 healthy donors. AAV1-specific Ig and NAF titers were obtained for sera by ELISA and neutralizing assay, respectively. Frequencies of IFN-γ–secreting cells were obtained by ELISPOT after transduction of PBMCs as described in Fig. 1. (A) Correlation between AAV1 Ig titers and NAF titers. (B) Correlation between AAV1 Ig titers and IFN-γ ELISPOT ratio. (C) Correlation between AAV1 NAF titers and IFN-γ ELISPOT ratio. Each diamond represents a donor. n, Number of donors in the corresponding quadrant of graphs.
as having a role in inflammation and autoimmunity. Furthermore, we also observed IFN-γ–producing cells among CD3+ cells, which could be NK cells.

As previously described for AAV2 (6), we recently showed that natural exposure to AAV types 1, 2, 5, 6, 8, and 9 can result in production of Abs from all four IgG subclasses, with a predominant IgG1 response. Furthermore, it was possible to detect neutralizing properties to AAV in most of the sera found to be positive for IgG in the corresponding AAV ELISA, indicating that neutralizing properties of sera were correlated with specific binding Abs (8). Nevertheless, it cannot be excluded, as reported for other viruses (29–32), that in certain cases some other unidentified factors present in the serum can be at least in part responsible for the virus neutralization. In this study, we also showed in this healthy population high Ig and NAF AAV1 seroprevalences equivalent to those previously described (8). Interestingly, the humoral response profiles were not correlated with AAV1 cellular responses. Indeed, 47% of donors presenting specific Ig and NAF against AAV1 capsid were negative for cellular responses, and most importantly 36% of donors presenting a pre-existing cellular response against AAV1 capsid were negative for anti-capsid Ig and NAF.

Our findings indicate that prescreening patients may require assessing not only for humoral response as now commonly performed, but also for a pre-existing cellular response to better understand the clinical consequences of such immune responses. This also emphasizes the importance of understanding the actual in situ AAV capsid metabolism, which in turn depends on multiple factors such as the serotype, dose, and route of administration.

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Disclosures
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