**BACKGROUND**

- Even low levels of anti-AAV2 or anti-AAV8 NABs have been related to a decrease or total impairment of AAV liver transduction in NHPs and humans when delivered intravenously [1, 2].
- We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector [3].
- Those results suggest that differences in the neutralization ability of antibodies might exist between AAV serotypes.

**OBJECTIVES**

The aim of the present study was to assess the binding characteristics of the pre-existing anti-AAV NABs, found in the serum samples of healthy donors, to AAV antigen for AAV2, AAV5 and AAV8 serotypes. Prior to avidity analysis, the prevalence of NABs against AAV serotypes 1, 2, 5 and 8 in those serum samples was established.

**METHODS**

Serum samples from 300 healthy human donors (100 American males, 100 European males and 100 American females) were purchased from BioreclamationIVT (West Sussex, UK). Samples were analyzed for the presence of anti-AAV1, anti-AAV2, anti-AAV5 and anti-AAV8 NABs with the use of anti-AAV NABs luciferase-based bio-assays. Serum samples of donors, that returned anti-AAV NABs titers above 50, were analyzed for anti-AAV IgGs avidity (overall strength of antibody-antigen complexes). The Avidity index (AI) assesses effect of chaotrope agent on the antibody-antigen binding. Avidity index is the serum dilution factor for half-maximal binding after urea exposure divided by the corresponding value in the absence of urea, expressed in percentage (Figure 1).

**RESULTS**

Anti-AAV5 and anti-AAV8 NABs are the least prevalent in healthy donor population

- Serum samples of 300 healthy donors were screened for pre-existing NABs against AAV1, AAV2, 5 and 8. Thirty-nine donors (13%) had no detectable anti-AAV NABs against any of the serotypes analyzed (anti-AAV NABs titers were below 2). The highest prevalence of NABs in healthy donor serum was against AAV2 serotype as 81% (n=242 out of 300) of all donors had titers above 2. Less prevalent were NABs against AAV1 serotype, with 64% (n=192 out of 300) of donors being positive for anti-AAV1 NABs. Furthermore, prevalence of NABs against AAV5 and AAV8 in healthy donor serum samples were 55% (n=164 out of 300) and 50% (n=150 out of 300) respectively (Figure 2).

Anti-AAV5 and anti-AAV8 NABs also presented the lowest seroprevalence (4%) of titers above 1030 among positive donors. On the other hand, NABs against AAV2 had the highest prevalence in a higher titer range (22%) (Figure 3).

**CONCLUSION**

- We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector [3].
- The results obtained demonstrate that the avidity of pre-existing AAV-specific IgG antibodies in healthy human population is significantly different between AAV serotypes.
- Pre-existing anti-AAV5 NABs create the weakest antibody-antigen complexes with the AAV5 antigen when compared to anti-AAV2 or anti-AAV8 NABs. Indeed, pre-existing anti-AAV5 NABs measured in vitro do not interfere with the AAV5-based in vivo transduction as much as pre-existing anti-AAV NABs against other serotypes do.

**REFERENCES**


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