› Anal Biochem. 2020 Feb 15;591:113568. doi: 10.1016/j.ab.2019.113568. Epub 2019 Dec 24.

Semi-quantification of antibody-dependent enhancement (ADE) in the uptake of Adenovirus serotype 5 into THP-1 cells

Lathika Valliyott [1], Ranjeet Dungdung [1], Rajendra Pilankatta [2]

Affiliations + expand

PMID: 31881180 DOI: 10.1016/j.ab.2019.113568

Abstract

Replication defective recombinant Ad5 vectors (rAdV5) are extensively explored for its applications in gene therapy and vaccine delivery. Ad5 enter into monocytes and macrophages through CAR independent route as an immune complex termed as antibody-dependent enhancement (ADE). We developed an effective method for estimating the ADE of rAdV5 encoding GFP (rAdV5-GFP) into THP-1 cells, using fluorimetric semi-quantification of GFP. Initially, twenty numbers of human sera samples were screened in HeLa cells for anti-Ad5 antibody titer using neutralization assay. Uptake of rAdV5-GFP in THP-1 cells was observed only after pre-incubation with the serially diluted human sera which are attributed to ADE. The optimal dilution which showed the maximum GFP expression as per the fluorescence microscopic analysis in THP-1 cells was used for further analysis. Fluorimetric analysis of the THP-1 cell lysate showed a maximum GFP intensity of 17058 RFU, which was equivalent to the 0.397 pmoles of Alexa Fluor 488 under the same experimental condition. Similarly, immunoblot analysis of GFP in THP-1 cell lysate and HeLa cell lysate confirmed the entry of rAdV5-GFP into the cells. The assay can serve as a platform for understanding the molecular events involved in ADE for the uptake of viruses into immune cells.

Keywords: Antibody-dependent enhancement; Coxsackie and adenovirus receptor; Green fluorescent protein; Multiplicity of infection; Replication defective recombinant adenovirus 5 vector encoding GFP.