



The Potential of eCD4-Ig, Delivered by Adeno-Associated Virus (AAV) Vector as a Novel Vaccine for HIV/AIDS Infection

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ABSTRACT

Background: HIV/AIDS has already become one of the world's major health issues taking its toll on millions of lives each year. Developing an HIV vaccine with excellent efficacy has become a global urgency that must be addressed immediately. Recently, researchers have successfully developed a more self-like molecule which is a fusion protein between human CD4 domains and immunoglobulin G (IgG) Fc with a CCR5-mimetic sulfopeptide in the carboxy terminus. This molecule, eCD4-Ig, targets only the conserved regions of HIV Env and thus demonstrated the most remarkable potency and breadth so far. By using adeno-associated virus (AAV) vector, eCD4-Ig's long-term expression *in vivo* can be achieved. **Objectives:** Evaluate the efficacy of AAV-eCD4-Ig as both preventive and therapeutic vaccine for HIV/AIDS infection. **Methods:** A systematic literature study was conducted with the database in PubMed, ScienceDirect, and Proquest. No time and language restriction were applied. **Discussion:** This review shows that eCD4-Ig eliminates HIV-infected cells through neutralization and antibody-dependent cell-mediated cytotoxicity (ADCC). Moreover, eCD4-Ig is also capable of preventing HIV infection *in vivo*. Delivered with AAV, eCD4-Ig is maintained stably at both protective and therapeutic levels, as well as gives robust protection for rhesus macaques for almost a year long through a single injection. **Conclusion:** This study offers evidences that AAV-eCD4-Ig appears to have the potential to be an effective vaccine to prevent HIV infection.

Keywords: AAV, AIDS, eCD4-Ig, HIV, vaccine

ABSTRAK

Latar Belakang: Pengembangan vaksin HIV yang efektif menjadi sangat penting mengingat tingginya angka kematian yang ditimbulkan oleh HIV/AIDS. Beberapa tahun terakhir, peneliti berhasil menemukan sebuah molekul yang tersusun atas domain CD4 manusia, immunoglobulin G (IgG) Fc, dan sulfopeptida yang menyerupai CCR5. Molekul yang dinamakan eCD4-Ig ini menargetkan area konservatif dari HIV Env sehingga berpotensi untuk menjadi vaksin HIV yang efektif. Ekspresi eCD4-Ig akan dipertahankan menggunakan Adeno-associated Virus Vector (AAV). **Tujuan:** Evaluasi efektivitas AAV-eCD4-Ig sebagai vaksin untuk HIV/AIDS. **Metode:** Penelitian dilakukan dengan melakukan tinjauan pustaka dari beberapa database jurnal, yakni PubMed, ScienceDirect, dan Proquest tanpa ada batasan waktu dan bahasa. **Pembahasan:** eCD4-Ig membunuh sel-sel yang terinfeksi HIV melalui proses netralisasi dan antibody-dependent cell-mediated cytotoxicity (ADCC). eCD4-Ig juga memberikan perlindungan terhadap infeksi HIV. Ekspresi AAV-eCD4-Ig sangat stabil untuk dosis protektif dan terapeutik, sekaligus melindungi rhesus macaques dari infeksi HIV selama hampir 1 tahun lamanya hanya dengan sekali injeksi. **Kesimpulan:** AAV-eCD4-Ig memiliki potensi yang menjanjikan untuk menjadi vaksin HIV yang efektif bagi seluruh penderita HIV/AIDS di seluruh dunia.

Kata Kunci: AAV, AIDS, eCD4-Ig, HIV, vaksin

Received [30 Apr 2020] | Revised [17 Nov 2020] | Accepted [18 Nov 2020]

INTRODUCTION

Human immunodeficiency virus (HIV) is a virus that attacks humans' immune system. This highly contagious

virus specifically targets the CD4⁺ T cells and later destroys the entire cells colonies. If left untreated, the number of CD4⁺ T cells will continue to decline rendering the immune system incapable of fighting off

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infection – a condition known as acquired immunodeficiency syndrome (AIDS).^[1,2]

HIV/AIDS has become a worldwide major health issue. According to World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS), there were approximately 37.9 million people worldwide living with HIV/AIDS in 2018.^[2,3] Since the beginning of the AIDS epidemic, HIV has claimed more than 32 million of lives globally.^[2]

For the last few decades, the medicine used for treating HIV/AIDS is called antiretroviral therapy (ART). It does not cure HIV infection, but only suppress viral load temporarily.^[4-7] In 2018, WHO reported that 23.3 million people (62%) living with HIV/AIDS are currently receiving ART.^[2] ART as the current HIV therapy is necessary, but not competent enough to end the global AIDS epidemic.

In the early years of the AIDS epidemic, immunotherapy against HIV-1 was developed. Human monoclonal antibodies (mAb) appear to shed light on HIV/AIDS therapy. Treatment with mAb showed a slight improvement than the previously used therapies. However, mAbs had limited breadth against multiple HIV-1 strains.^[8,9]

Several years after the discovery of mAb, researchers have tried to transfer antibodies from the sera of HIV-infected subjects to AIDS patients. Unfortunately, these antibodies were ineffective against the diverse and highly adaptive virus.^[5] However, studies demonstrated that 20–50% of chronically HIV-infected individuals could produce antibodies that target shared epitopes across various clades of HIV – later known as broadly neutralizing antibodies (bNAbs).^[4,5,9,10]

Since then, multiple bNAbs targeting different HIV envelope glycoprotein (Env) had been identified. The best ones ever found, N6 and 10E8, manage to neutralize 98% of HIV strains with 50% inhibitory concentration (IC₅₀) up to 50 µg/mL.^[5,6,8,9,11] Although seems

promising, however, developing a potent and broad bNAb requires excessive mutation of antibody genes.^[7] Thus, the newly-formed bNAbs are frequently subjected to the host's immune system – inducing the formation of anti-drug antibodies (ADA) – that further decrease their efficacy even more.^[7,12-15]

In order to ensure neutralization of HIV, excellent potency and breadth is a necessity for developing an effective HIV vaccine. At the same time, it should maintain a low immunogenicity profile when administered to humans. Considering this, researchers have begun to explore the possibility of molecules resembling antibodies that could recognize HIV conserved epitopes. Recently, researchers have successfully developed a fusion protein which functions like an antibody. This more self-like molecule is a fusion between human CD4 domains and immunoglobulin G (IgG) Fc with a CCR5-mimetic sulfopeptide in the carboxy terminus.^[11,12,16,17] This molecule, eCD4-Ig, targets only the conserved regions of HIV Env and thus demonstrated the most remarkable potency and breadth so far.

Besides being potent and broad, eCD4-Ig's long-term expression *in vivo* must be maintained at protective and therapeutic levels. This response can be achieved by using adeno-associated virus (AAV) vector. AAV-expressed eCD4-Ig is maintained stably for almost a year-long through a single injection.^[11,12,16]

Therefore, this study is conducted to highlight the efficacy of eCD4-Ig, delivered with AAV vector, as both preventive and therapeutic vaccine for HIV/AIDS infection.

METHOD

Data Sources and Search

A systematic literature study was conducted with the database in PubMed, ScienceDirect, and Proquest. No time and language restriction were applied. The keywords used are described as follow:

“eCD4-Ig” AND “HIV” OR “human immunodeficiency virus” OR “AIDS” OR “acquired immunodeficiency syndrome”. The results from the literature search were first reviewed by reading the titles and abstracts. The relevant studies were further checked by reading the full texts and assessed for inclusion. Search in electronic database yielded 323 studies. Screening through titles and abstracts found 32 articles, 9 of which met the eligibility criteria of current study. Search in electronic database yielded 323 studies. Screening through titles and abstracts found 32 articles, 9 of which met the eligibility criteria of current study.

Eligibility Criteria

The following criteria are considered for studies’ eligibility: type of studies, problem, intervention, comparison, outcome (PICO).

Type of Studies. Randomised-controlled trials are included for this study. Identified articles included humans and non-humans’ studies. Cross-sectional, case control, cohort, case report, case series, conference abstracts, expert opinion, and review papers are excluded. Articles with unavailable full-text, languages other than English, and irrelevant topics are also omitted.

Population. All subjects (humans or non-humans) challenged with infectious doses of HIV are included for this study.

Intervention. Studies evaluating the efficacy of eCD4-Ig to neutralize HIV-infected subjects are included. In addition, included studies also evaluate AAV-eCD4-Ig to protect subjects from HIV infection.

Comparison. Included studies compared HIV-infected subjects inoculated with and without eCD4-Ig to evaluate the efficacy at both therapeutic and preventive level.

Outcome: The key findings of included studies are the number of subjects fully protected from HIV infection.

DISCUSSION

eCD4-Ig as a Promising Broad and Potent Neutralizing Agent against HIV Infection

Current trend in HIV/AIDS treatment is only limited to the suppression of viral load temporarily by using ART. The therapy developed for the last few decades focuses on targeting HIV Env by using bNAbs. Despite the promising potency and breadth, bNAbs still have a major drawback regarding of immunogenicity. Considering this, researchers have shifted their focus on using a more self-like molecule to target the conserved regions of HIV Env.^[8,11]

Recently, researchers have successfully engineered eCD4-Ig, a Fc-fusion protein between domains 1 and 2 of human CD4 and IgG Fc with CCR5-mimetic sulfopeptide attached in the carboxy terminus (Figure 1a). eCD4-Ig cooperatively binds to the CD4- and coreceptor-binding sites (Figure 1b) on all HIV subtypes hence its markedly superior potency and breadth.^[12,16,17]

eCD4-Ig eliminates HIV-infected cells through 2 main mechanisms: neutralization and antibody-dependent cell-mediated cytotoxicity (ADCC).^[12,16,18] eCD4-Ig engages the Env and potently

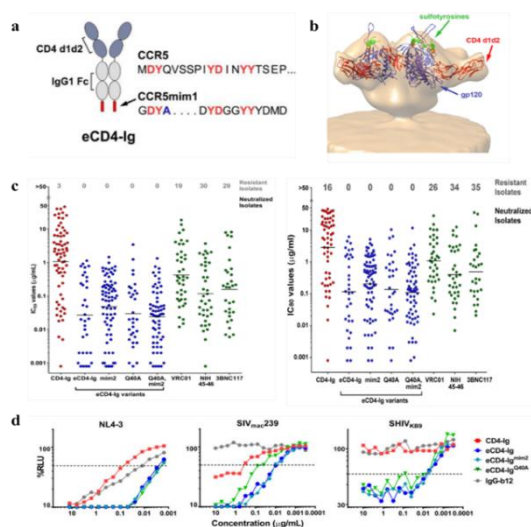


Figure 1. Structure (a,b) and Capabilities of eCD4-Ig in Neutralizing HIV Strains (c) and Enhancing ADCC (d)^[16]

neutralizes the highly diverse HIV isolates. According to an *in vitro* study by Gardner *et al.*, eCD4-Ig neutralized 100% of HIV-1, HIV-2, and SIV strains with IC₅₀ and IC₈₀ less than 1.5 µg/mL and 10 µg/mL, respectively (Figure 1c).^[16] This finding is supported by another study done by Fetzer *et al.* which has shown the excellent potency of eCD4-Ig and its variants in neutralizing diverse HIV-1 isolates.^[17]

In addition of enhancing neutralization, CCR5-mimetic sulfopeptides ensure no viral escape and HIV-infection occurring in CD4-negative cells.^[11,16,19,20] According to the study by Fellingner *et al.*, eCD4-Ig was capable of altering HIV-1 Envs, hence making the viruses less infectious and more sensitive to antibodies present in the serum of HIV-infected people.^[20] Other studies done by Davis-Gardner *et al.* and Fetzer *et al.* also supported this finding. eCD4-Ig has the ability to expose the V3-loop of HIV Env hence its ability to enhance the potency of V3-loop antibodies, which are abundant but non-neutralizing in HIV-infected individuals.^[18,21]

The remarkable potency of eCD4-Ig also comes from its ability in mediating ADCC. An *in vitro* study by Davis-Gardner *et al.* revealed that eCD4-Ig mediated killing of HIV-infected cells up to 10 times more efficiently by natural killer (NK) cells.^[18] This finding is supported by another study by Gardner *et al.* It is revealed that eCD4-Ig enhanced ADCC activity by 30-40 times higher when tested against diverse HIV tropisms (Figure 1d).^[16] Moreover, eCD4-Ig could alter the conformation of HIV Env so that pre-existing non-neutralizing antibodies in patients' sera could bind to HIV Env and promote ADCC activity by 100-fold.^[18]

Besides having a remarkable therapeutic effect, eCD4-Ig is also proven to be capable of preventing HIV infection. An *in vivo* study by Gardner *et al.* revealed that eCD4-Ig fully protected humanized NOD/SCID/γc (NSG) mice infected with 5x10⁴ infectious units of HIV_{NL4-3}.

Complete protection was achieved at the concentration of 2–4 µg/mL and maintained up to 5 weeks after inoculation.^[16] Moreover, the more self-like structure of eCD4-Ig makes it less likely subjected to endogenous antibodies that can impair the efficacy. Even the additional CCR5-mimetic sulfopeptides do not elicit significant antibody responses.^[12,16,19] These findings strongly suggested eCD4-Ig as a promising candidate for HIV vaccine development.

eCD4-Ig Delivered by Adeno-associated Virus Vector (AAV-eCD4-Ig) as a Promising Novel Vaccine for HIV Infection

Another important issue in developing an effective vaccine for HIV/AIDS is the use of an appropriate packaging system to ensure eCD4-Ig's expression at protective and therapeutic levels. Previously, antibodies or antibody-like inhibitors were passively administered. However, this method prompted the need of repeated infusion which could lower patient's compliance.^[22,23] This approach is deemed inefficient and thus an alternative is needed to express biologically active molecules in a prolonged manner with a single inoculation. In this case, AAV vector appears to be a major breakthrough of this problem.

Adeno-associated virus (AAV) is a small (20 nm), non-enveloped virus. It belongs to genus *Dependoparvovirus*, which in turn belonging to family *Parvoviridae*.^[22] AAV vector-mediated gene transfer has gained a lot of attention as it is able to express desired-protein for the lifetime of the cell as long as the product is considered as self by one's immune system.^[23] AAV particles also show high resistance to high temperature and widely-range pH, as well as stability after being stored for a long time.^[22,23] Therefore, this paper strongly suggest the use of AAV to efficiently deliver eCD4-Ig (AAV-eCD4-Ig) both as a prevention and treatment for HIV infection.

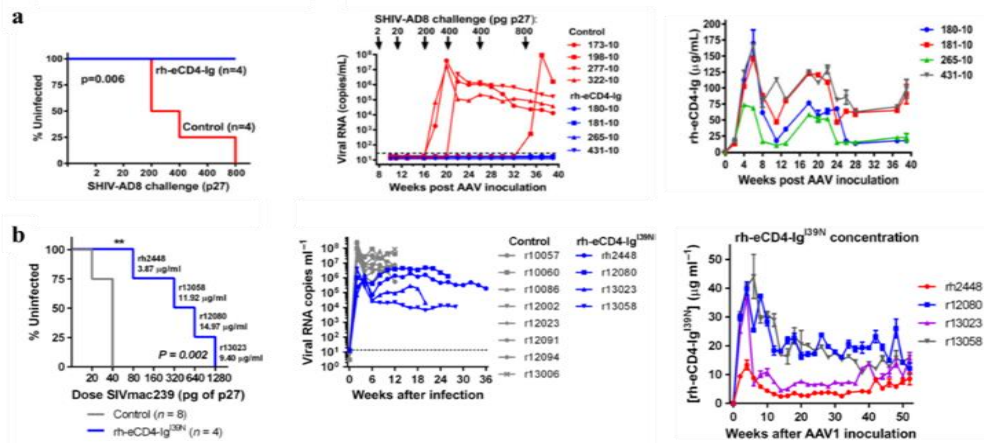


Figure 2. AAV-eCD4-Ig Promotes Full Protection of Rhesus Macaques from SHIV-AD8 (a) and SIVmac239 Infection^[12,16]

AAV-eCD4-Ig can give full protection from HIV infection in non-human studies. According to a study by Gardner *et al.* in 2015, AAV-eCD4-Ig completely protected four of four rhesus macaque from six escalating doses of simian-human immunodeficiency virus (SHIV-AD8) infection. A single administration of 2.5×10^{13} genomic copies (GCs) of AAV-eCD4-Ig was capable of maintaining stable eCD4-Ig expression between 17–77 $\mu\text{g}/\text{mL}$ and giving robust protection for inoculated macaques up to 40-week long (Figure 2a).^[16] Another study by Gardner *et al.* in 2019 revealed that AAV-eCD4-Ig was capable of preventing SIVmac239 infection in four of four inoculated macaques with serum concentration as low as 3–18 $\mu\text{g}/\text{mL}$ by the end of week-50 (Figure 2b).^[12] These studies highlight the potential of AAV to achieve a long-term and stable transgene delivery in humans' bodies, particularly in HIV-infected individuals. The delivery of eCD4-Ig is boosted to a point where its effectivity reaches beyond average.

CONCLUSION

The extensive genetic diversity in HIV has been a challenge to develop an effective HIV vaccine. Recently, eCD4-Ig has been developed and proven to be the most potent and broad therapy at present. At a low concentration, eCD4-Ig is able to neutralize 100% of HIV-1, HIV-2, and

SIV strains. It also mediates efficient killing by NK cells. In addition, the self-like eCD4-Ig is less likely targeted by pre-existing antibodies. This review revealed that one-time administration AAV-eCD4-Ig fully protected test subjects from HIV infection for at least a year. Thus, AAV-eCD4-Ig appears to have the potential to be an effective vaccine to prevent HIV infection.

RECOMMENDATIONS

Further comprehensive studies should be done to assess the efficacy of AAV-eCD4-Ig in vitro and in vivo, as it would serve as a foundation for future management of HIV/AIDS infection. Moreover, it is recommended that clinical trials are initiated to investigate the ability of AAV-eCD4-Ig in giving protection and treatment for HIV-infected patients.

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