LITERATURE REVIEW

Delivery of Anti-PD-1 Gene with Recombinant Adeno-Associated Virus (RAAV) as Preventive and Curative Therapy of Infectious Diseases in Childhood

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ABSTRACT

Infections in children are common and are the highest cause of hospitalization in children, especially in children aged 0-4 years. The binding of PD-1 (Programmed Cell Death-1) glycoprotein on its ligand in CD4+ and CD8+ T cells activates a pathway that results in T cell dysfunction. Inserting the anti-PD1 gene into rAAV opens opportunities for preventing and treating infections in children. This literature review aims to determine the potential of anti-PD1 gene rAAV as a new modality for preventing and treating pathogen infections in children. The procedure of searching for literature to answer questions was carried out through online searching of journals in the last ten years. The use of anti-PD-1 has shown to increase the immune response against certain viral, bacterial and parasitic infections. Using rAAV as an anti-PD-1 gene vector has great potential to be a preventive or curative therapy for various infections in children. Further research and development are needed to determine the viral model, dose, indications, and contraindications to the use of the rAAV-transmitted anti-PD-1 gene for the treatment of infectious diseases in children.

Keywords: Anti-PD1, recombinant Adeno-Associated Virus (rAAV), Infectious Disease, Pediatric

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INTRODUCTION

Infections in children, whether caused by bacteria, viruses, or parasites, are common (Van den Bruel et al., 2020) and are the highest cause of hospitalization in children (Verbakel et al., 2015). The disease most often occurs in children aged 0-4 years, with an incidence of 1731 infections per 1000 patients per year (Van den Bruel et al., 2020). Some infectious diseases are serious infections that cause morbidities such as hearing loss, neurological disorders, and even mortality (Van den Bruel et al., 2010). Each year, most of the 10 million deaths in children under five years of age are caused by pathogen infection (Bhutta & Saeed, 2008). This is enough to show that infection in children is a severe problem.

In utero, the fetal immune system is tolerant to maternal alloantigens. The mother provides the baby's protection against infection by passive transplacental transfer of IgG antibodies or through breast milk. Nearly all cells at birth provide the glycoprotein CD45RA, a characteristically naive T cell that has never been exposed to a foreign antigen. There were also relatively high Tregs in negative CD45RA cells (Tosato et al., 2015; Simon et al., 2015). After that, the mother's immunity to the baby slowly disappears. The baby is exposed to antigens from the environment, making the baby's body produce immunity quickly, which is suitable for early life (Simon et al., 2015; Ratajczak et al., 2018). During childhood, the number of Treg cells decreases, and the Th1, Th17, and Th2 memory cells gradually increase to equal the number of naive T cells (Tosato et al., 2015; Ratajczak et al., 2018). Children become more susceptible to infection, although better and more mature innate and adaptive immune systems are being formed. With age, memory T and B cells and naive memory cells in children begin to develop by infection, exposure to food antigens, and previous vaccinations. This accumulation of immunological memory is an evolving feature of the adaptive immune response. Of course, with the differences in memory T cells and B cells plus the HLA gene, it is not surprising that the variation of each individual's immune cells is enormous and unique. These memories are preserved into old age but may fade (Simon et al., 2015; Ratajczak et al., 2018; Weng et al., 2012).

PD-1 (Programmed Cell Death-1) is a glycoprotein expressed on CD4+ and CD8+ T cells. PD-1 expression on T cells can be induced by pro-inflammatory cytokines such as IFN-γ. PD-1 plays a role in modulating T cells. The binding of PD-1 with its ligands activates a pathway that results in decreased cell proliferation and an increased risk of apoptosis. In chronic infections or diseases, a persistent increase in PD-1 expression causes T cell dysfunction (Reul et al., 2019). Based on the explanation above, inhibition of PD-1 opens opportunities as a preventive and curative therapy for pathogen infections in children.

AAV (Adeno-Associated Virus) is a virus of the genus

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Parvoviridae. This virus can infect humans without causing disease. This virus can be used as a vector for the desired therapeutic gene by inserting the desired gene into the viral DNA (Wang et al., 2021). Research on the use of AAV as an anti-PD1 gene vector has been carried out and has proven successful (Reul et al., 2019). The ability of AAV combined with the anti-PD1 gene opens opportunities for preventing and treating acute and chronic diseases in children.

Currently, many therapies are used for infectious diseases in children, both preventive and curative therapies, such as vaccines, antivirals, antibiotics, and lifestyle modifications (Drexler, 2010). However, no therapy can prevent and cure various infectious diseases with maximal success and no side effects. Therefore, we propose an anti-PD-1 gene with recombinant carrier Adeno-Associated Virus (rAAV) as a state-of-the-art preventive and curative therapy for bacterial, viral, and parasitic infections in children.

REVIEW

PD-1 (Programmed Cell Death-1) surface monomeric glycoprotein is encoded by the PCDC gene in which an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) are connected to the nucleus (Yokosuka et al., 2012; Boussiotis et al., 2014). Various pathogens utilize the PD-1 co-inhibitory receptor to evade host defenses (Attanasio et al., 2016). During infections, microbial antigens are presented in the Major Histocompatibility Complex (MHC) on Antigen-Presenting Cells (APCs) against naive T cells (Th0). After receiving primary and secondary signals via the MHC-linked Toll-Like Receptor (TCR), effector T cells become memory cells (Jubel et al., 2020). Furthermore, immature T cells will express CD4+, which destroys extracellular pathogens, or CD8+, which works to destroy intracellular pathogens.

Binding of PD-L1 or PD-L2 ligands to PD-1 phosphorylates ITSM and ITIM in the cytoplasm. It recruits protein tyrosine phosphatases, such as SHP2, which then inhibit two essential pathways: first, SHP2 competes with kinases to prevent activation of Phosphoinositide 3-kinase (PI3K), thereby inhibiting the activation of Protein Kinase B or AKT. Inactive AKT reduces T cell proliferation, increases apoptosis, causes T cell exhaustion, and reduces effector functions such as cytokine production and cytolytic function. Second, SHP2 dephosphorylates ZAP-70 and LCK, leading to MEK/ERK1 inactivation. Decreased ERK1 activation minimizes the potential for cell proliferation and differentiation (Jubel et al., 2020). Together, these processes result in reduced immunity and increased risk of bacterial, viral, and parasitic infections.

Therefore, several studies were conducted giving Anti-PD-1 to treat infectious diseases, ranging from viral and bacterial to parasitic infections. A study was conducted in 2016 to prove the effect of Anti-PD-1 on patients infected with Hepatitis B Virus (HBV). Mononuclear cells from peripheral blood were taken from patients with the HLA-A2+ CHB gene, isolated and cultured for ten days, and given anti-PD-1. Cells were harvested, rested, and stained by intracellular IFN-γ staining. The levels of IFN-γ, a macrophage activator, were higher in CD4+ and CD8+ cells treated with anti-PD-1 compared to control cells (Tang et al., 2016; Miller et al., 2019). Furthermore, a study by Holokai et al. developed a co-cultured organoid system/immune cell that was infected with Helicobacter pylori for 72 hours. Then, CTL (Cytotoxic T Cell) was extracted from CD8+ positive cultures and analyzed for cell proliferation by CSFE uptake. It was found that H. pylori infection decreased CTL proliferation, whereas anti-PD-1 administration induced CTL proliferation (Holokai et al., 2019).

After the studies carried out with viral and bacterial infections, a study of the effect of anti-PD-1 against parasites, namely Leishmania donovani, on murine mice was carried out. In addition to the previously described effects, anti-PD-1 improved mice's spleen architecture and significantly reduced the parasite load in infected mice (Habib et al., 2018). Anti-PD-1 has been widely demonstrated to have immunoregulatory functions by increasing T cell proliferation, reducing cell fatigue, reducing viremia, increasing the production of anti-inflammatory cytokines, and decreasing the production of pro-inflammatory cytokines, as well as helping to repair the structure of infected organs and reduce the number of parasites (Jubel et al., 2020; Miller et al., 2019; Holokai et al., 2019; Habib et al., 2018; Li et al., 2014; Seung et al., 2013). In children, it has been proven that anti-PD-1 has minimal side effects and a long-lasting response potential, so it is very well used as a sophisticated preventive and curative therapy for various infections in children (Kabir et al., 2018).

AAV (Adeno-Associated Virus) is a virus that belongs to the genus Parvoviridae. AAV requires the help of other viruses to thrive. An example is an adenovirus (adV). This virus has an icosahedral-shaped capsid measuring 26nm, which consists of 3 types of subunits, namely VP1, VP2, and VP3. AAV genetic material in 4.7 kb ssDNA can be either a positive or negative strand. At both ends of the AAV genome, ITR (Inverted Terminal Repeat) serves as the origin of replication and packaging signal (Wang et al., 2021).

RAAV (recombinant AAV) is an AAV that has the desired therapeutic gene inserted. rAAV has the same capsid protein as wild-type AAV, but the genes encoding the AAV proteins have been replaced with the selected therapeutic genes. Eliminating genes encoding viral proteins is also beneficial for reducing the cytotoxicity and immunogenicity of AAV. Only the ITR portion is not discarded because this section is required for genome replication and packaging during the vector production process. Intravenous rAAV will circulate and insert the desired gene into the body's cells. Body cells can then express the gene (Wang et al., 2021). The ability of rAAV to carry desirable therapeutic genes, including genes encoding anti-PD-1 antibodies, can be an effective form of general preventive and curative therapy for bacterial, viral, and parasitic infections in children.

The use of rAAV as an anti-PD-1 vector has been carried out in both in vitro and in vivo experiments (Reul et al., 2019). These experiments aimed to increase the expression of human anti-PD-1 antibodies (nivolumab) in tumor tissue and decrease the expression of anti-PD-1 in the liver and serum to prevent side effects related to the immune system. Both in vitro and in vivo experiments were carried out successfully, inserting the anti-PD-1 gene into tumor cells and redistributing anti-PD-1, normally concentrated in the liver and serum, to become concentrated in tumor tissue (Reul et al., 2019). The experiment used Her2-AAV, rAAV, which can recognize the Her2/Neu antigen on the surface of tumor cells as a cell entry medium. The success of this experiment proves that rAAV, in some way, can be used as an anti-PD-1 gene vector.

Another use of rAAV is as a vector of broadly-

neutralizing HIV antibody (bnAb) for AIDS therapy. The experiment used a natural AAV serotype that can carry the bnAb gene into liver cells or skeletal muscle cells and cause a persistent increase in serum antibody levels (Fuchs et al., 2021). By adopting the above two experiments, it is hoped that an rAAV can be designed that can carry the anti-PD-1 gene and insert it into long-lived cells, such as muscle cells, to obtain persistent increases in serum anti-PD-1 levels to strengthen the immune system and treat acute and chronic infection in children, whether caused by bacteria, virus, or parasites.

Wild-type AAVs do not cause disease in the host (Wang et al., 2021; Büning & Schmidt, 2015). In addition, rAAV is also safe based on 120 clinical trials that have been conducted, with the heaviest side effect in the form of temporary tissue inflammation (Büning & Schmidt, 2015). But rAAV still has some obstacles that can reduce its effectiveness. Among them is the emergence of an immune response to the antibodies produced (Fuchs et al., 2021), the low number of antibodies due to the low ability of cell synthesis (Wang et al., 2021), and the unknown level of antibodies in the serum that is effective for obtaining the desired therapeutic effect (Wang et al., 2021). In addition, the use of anti-PD1 in certain types of infection, namely M. tuberculosis (Mbt) infection, may be harmful. PD-1-deficient mice infected with mbt experienced decreased survival, focal necrosis, uncontrolled proliferation of mbt, decreased numbers of infiltrating T cells and B cells, and increased pro-inflammatory cytokines (TNFα, IL-1, IL-6, and IL- 17A) in serum and lung (Barber et al., 2011; Lázár-Molnár et al., 2010). This might limit the usefulness of this theraphy in preventing many kinds of infections irrespective of the pathogen. However, these shortcomings might be overcome by adjusting the dose, searching for target cells with high antibody production capabilities, and determining the indications and contraindications for therapy use.

CONCLUSION

Using rAAV as an anti-PD-1 gene vector can be a preventive or curative therapy for various infections in children, whether caused by bacteria, viruses, or parasites. Using rAAV as a gene vector has also been safe based on data from 120 clinical trials. The use of anti-PD-1 has also been shown to increase the immune response against certain types of viral, bacterial, and parasitic infections, reduce the number of pathogens in the body, and help restore organ structure. Further research and development are needed to determine the viral model, dose, indications, and contraindications to using the rAAV-transmitted anti-PD-1 gene for treating infectious diseases in children. It is hoped that this scientific essay can become the basis for researchers in developing the use of the anti-PD-1 gene that is delivered as a preventive and curative therapy for infections in children.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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