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REVIEW

Vasoactive intestinal peptide: a potential target for antiviral therapy

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Abstract: Viral infection is clinically common and some viral diseases, such as the ongoing global outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), have high morbidity and mortality. However, most viral infections are currently lacking in specific therapeutic agents and effective prophylactic vaccines, due to inadequate response, increased rate of drug resistance and severe adverse side effects. Therefore, it is urgent to find new specific therapeutic targets for antiviral defense among which "peptide-based therapeutics" is an emerging field. Peptides may be promising antiviral drugs because of their high efficacy and low toxic side effects. Vasoactive intestinal peptide (VIP) is a prospective antiviral peptide. Since its successful isolation in 1970, VIP has been reported to be involved in infections of SARS-CoV-2, human immune deficiency virus (HIV), vesicular stomatitis virus (VSV), respiratory syncytial virus (RSV), Zika virus (ZIKV) and cytomegalovirus (CMV). Additionally, given that viral attacks sometimes cause severe complications due to overaction of inflammatory and immune responses, the potent anti-inflammatory and immunoregulator properties of VIP facilitate it to be a powerful and promising candidate. This review summarizes the role and mechanisms of VIP in all reported viral infections and suggests its clinical potential as an antiviral therapeutic target.

Key words: vasoactive intestinal peptide; viral infection; antiviral therapy

血管活性肠肽：潜在的的抗病毒治疗靶点

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摘 要：病毒感染在临床上十分常见，且部分病毒性疾病有很高的发病率和死亡率，比如正在全球爆发的由严重急性呼吸道综合征冠状病毒-2 (severe acute respiratory syndrome coronavirus-2, SARS-CoV-2)引起的新型冠状病毒肺炎(coronavirus disease 2019, COVID-19)。然而，由于反应不足、耐药率增加和严重的不良副作用等原因，目前大多数病毒感染缺乏特定的治疗药物和有效的预防性疫苗。因此，寻找抗病毒感染新的特定治疗靶点非常紧迫，其中“基于多肽的治疗方法”是一个新兴领域。因其高效力和低毒副作用，肽类可能成为很有前景的抗病毒药物。血管活性肠肽(vasoactive intestinal peptide, VIP)是一种具有前瞻性的抗病毒多肽。自1970年成功分离以来，研究证明VIP参与调控SARS-CoV-2、人类免疫缺陷病毒(human immune deficiency virus, HIV)、水泡口炎病毒(vesicular stomatitis virus, VSV)、呼吸道合胞病毒(respiratory syncytial virus, RSV)、寨卡病毒(Zika virus, ZIKV)和巨细胞病毒(cytomegalovirus, CMV)的感染。此外，鉴于病毒感染可能会因为免疫和炎症过度激活导致严重的并发症，VIP强大的抗炎和免疫调节特性使其成为有前景的一种候选药物。本综述总结了VIP在病毒感染中的作用和机制，并提出其作为抗病毒治疗靶点的临床潜力。

关键词：血管活性肠肽；病毒感染；抗病毒治疗

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1 Introduction

Consisting of 28 amino acid residues, vasoactive intestinal peptide (VIP) is the hinge center, coordinating the nervous, endocrine, and immune systems to maintain the homeostasis of the organism, generating bidirectional communications through shared mediators and receptors [1, 2]. Exogenous administration of VIP exerts therapeutic effects in models of asthma, acute lung injury, Parkinson's disease and autoimmune/inflammatory diseases [3–14]. Due to its well-characterized bronchiectasis, vasodilatory, anti-inflammatory and immune regulation effects [15–18], the role of VIP in viral infections has also been recognized and investigated.

Viruses can be divided into DNA and RNA viruses, based on the molecular nature of their genomes. They not only encode a remarkable variety of mechanisms to subvert the canonical antiviral responses of the mammalian host, but also rapidly mutate to escape immunity, and the RNA viruses are notoriously efficient in this regard. This is exemplified by the respiratory syncytial virus (RSV) nonstructural proteins 1 and 2, which can disrupt antiviral interferon production and signaling [19]. These viral strategies add to the difficulties of finding specific drugs and effective vaccines against viruses. Although some antivirals have been developed, which includes ribavirin, remdesivir and lopinavir/ritonavir, many are prone to drug resistance and have undesirable side effects in clinical trials [20]. The peptide drugs, in contrast, have a wide spectrum of antiviral activity and low toxicity, which may be a better choice for treating viral infections [21].

In this review, we have comprehensively surveyed the mechanisms of VIP in viral infection to find the commonalities; in addition, we have explored the potential of VIP and VIP inhibitors as therapeutic agents for the treatment of viral infections.

2 Biological characteristics of VIP

2.1 VIP discovery

In 1969, Said described, for the first time, the existence of a vasoactive agent in mammal lungs with generalized vasodilator capacity. In collaboration with Mutt, Said partially purified this peptide from pig lungs. Since lung and intestine have a common embryonic origin, they turned to examine the intestine. Finally, using porcine duodenal tissue, they successfully isolated this vasodilator peptide, calling it the VIP [22].

A few years later, VIP was demonstrated in different areas of the central and peripheral nervous system, such as the bodies, axons, and neuronal dendrites, as well as presynaptic endings [23, 24], and has since been recognized as a neurotransmitter or neuromodulator. As a ubiquitous peptide, it has been found in many organs and tissues, including the heart, lung, thyroid gland, kidney, immune system, urinary tract, and genital organs. Regarding its cellular source, expression of VIP has been demonstrated in mast cells, macrophages, T and B lymphocytes [25–29].

2.2 VIP structure

VIP gene structure and processing of prepro-VIP to VIP are summarized in Fig. 1. VIP belongs to the secretin/glucagon family, which includes secretin, the pituitary adenylate cyclase activating peptide (PACAP) 27 and 38, helodermin, peptide histidine-methionine (PHM, in humans) or peptide histidine-isoleucine (PHI, in other mammals), growth hormone-releasing factor (GRF), glucagon and its related peptides GLP1 and GLP2, and the gastric inhibitor peptide (GIP) [30]. The human VIP gene is located on chromosomal region 6q24 and contains seven exons, each encoding a distinct functional domain. It is translated into a ~9 kb precursor molecule (prepro-VIP) that consists of 170 amino acids, formed by a signal peptide, 1 to 3 bioactive peptides and N- and C-terminal peptides. Prepro-VIP is processed by a signal peptidase in the endoplasmic reticulum to generate the 149-amino acid precursor peptide termed pro-VIP, which is then cleaved by prohormone convertases to VIP-GKR (preproVIP125–155) and peptide histidine methionine (PHM)-GKR (preproVIP81–110) [31]. Finally, VIP-GKR and PHM-GKR are further cleaved by carboxypeptidase-B-like enzymes to VIP-G and PHM-G which are metabolized by peptidyl-glycine alpha-amidating monooxygenase (PAM) to VIP and PHM [32]. The secondary structure of VIP consists of a random coil in the N-terminal region and an α-helix structure in the C-terminal region [33], which is similar to that of the other family members, especially PACAP27, with whom it shares 68% sequence homology [34].

2.3 VIP receptors and signaling pathways

VIP receptors, VPAC1 and VPAC2, belong to the B1 subfamily of G-protein-coupled receptors (GPCRs), consisting of seven transmembrane domains (7TM), three extracellular loops (EC1, EC2, and EC3), three intracellular loops (IC1, IC2, and IC3), a long amino terminal extracellular domain, and an intracellular

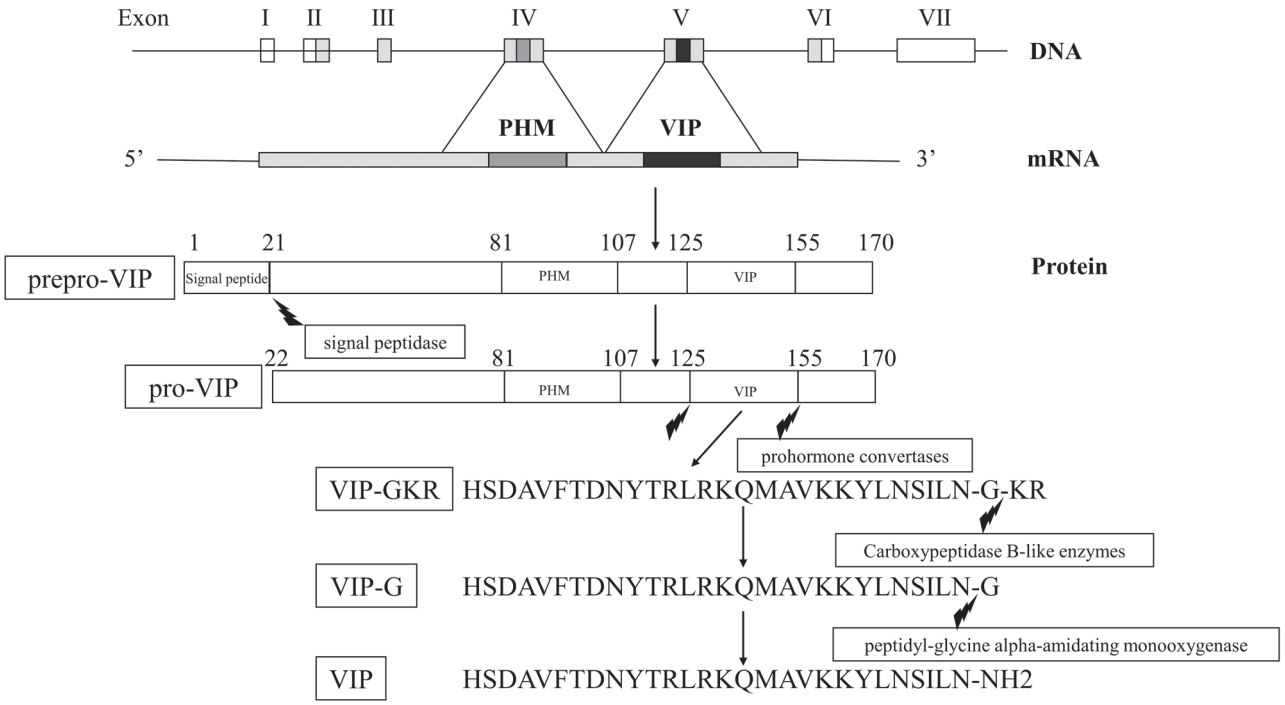

Fig. 1. VIP gene structure and processing of prepro-VIP to VIP. The human VIP gene contains seven exons. It is translated into prepro-VIP that consists of 170 amino acids, and then processed by a signal peptidase to generate pro-VIP. Pro-VIP is then cleaved by prohormone convertases to VIP-GKR. Finally, VIP-GKR is further cleaved by carboxypeptidase-B-like enzymes to VIP-G, which is metabolized by peptidyl-glycine alpha-amidating monooxygenase to VIP. PHM: peptide histidine-methionine.

carboxyl terminus [30]. The interaction of VIP and its receptors follows the "two-site" model, where the central and C-terminal parts of VIP are trapped by the N-terminal of the receptor, which ensures correct ligand positioning and binding of residues 1–6 of VIP to the extracellular loops and transmembrane helices leads to receptor activation [35]. VIP can be recognized by VPAC1 and VPAC2 with equal high affinity [30].

2.3.1 VPAC1 receptor

The VPAC1 receptor was first cloned from rats in 1991 from a cDNA library, and the human VPAC1 was cloned from the HT-29 cell line two years later [36, 37]. The affinity of several peptides for this receptor is as follows: VIP = PACAP > GRF > secretin [38]. VPAC1 is predominantly expressed in the central nervous system (CNS), liver, lung, breast, kidney, prostate, spleen, mucosa of the stomach and small intestine [39]. As for cell types, VPAC1 is expressed constitutively on T cells, monocytes, macrophages and neutrophils [18]. Recently, VPAC1 was found to be expressed on the surface and nuclear membrane of T helper (Th) cells, whereas its expression is limited to the nucleus when these cells are activated [40]. VPAC1 internalization could be attributed to nuclear localization signal sequence in its intracytoplasmic C-terminal. [K^{15},R^{16},L^{27}] VIP(1-7)/GRF(8-27) [41], [$Ala^{11,22,28}$] VIP [42], [Leu^{22}] VIP [43], [R^{16}]PACAP (1-23) [44] are selective agonists for the VPAC1 receptors. The only selective antagonist for the VPAC1 receptor, [Acetyl-His^{1}, D-Phe^{2}, Lys^{15}, Arg^{16}, Leu^{17}] VIP(3-7)/GRF(8-27), also called PG97-269, is currently available [45].

2.3.2 VPAC2 receptor

VPAC2 receptor was first cloned from rats in 1993, with the human and mouse receptors cloned shortly thereafter [46–48]. The order of affinity for human VPAC2 expressed in different cell lines is VIP = PACAP = helodermin > secretin [38]. VPAC2 is mainly localized in the CNS, pancreas, lung, heart, kidney, smooth muscle, adipose tissue, and thyroid follicular cells [49]. As for cell types, VPAC2 is expressed predominantly in human skin mast cells, as well as human mast cell line (HMC-1), and can be induced on T cells and macrophages only after bacterial/or viral infections [18]. VPAC2 sequence does not have a nuclear localization signal sequence in its intracytoplasmic C-terminal, and its intracellular locations has not been depicted. Ro25-1553 [50], Ro25-

1392 [51], BAY 55-9837 [52], Hexanoyl [A[19],K[27,28]]VIP, rRBAYL [52], and LBT-3627 [53] are selective agonists for the VPAC2 receptor. Only PG99-465 [54] and VIpep-3 [55] have been described as VPAC2-selective antagonists.

2.3.3 Signaling pathways

VIP/VPAC receptors' axis signaling pathways are summarized in Fig. 2. VPAC receptors are G protein-coupled receptors (GPCRs), categorized to Gs (stimulatory G proteins), Gi (inhibitory G proteins), Gt (transduction G proteins), or Gq (quiescent G proteins). As heterotrimeric proteins, they are composed of α, β, and γ subunits. When stimulated, the α subunit binds to GTP and dissociates from the βγ dimer. When activated Gα stimulates the enzyme adenylate cyclase (AC) to catalyze cAMP synthesis [38], it leads to the activation of protein kinase A (PKA). The activated PKA further mediates different signaling pathways, depending on the cell type. For instance, the canonical PKA downstream transcription factor is cAMP-response element binding protein (CREB). Another example is the mitogen-activated protein kinase (MAPK). Via a PKA-dependent mechanism, VIP can inhibit AP-1 and IRF activation [56–58]. On the other hand, the βγ dimer interacts

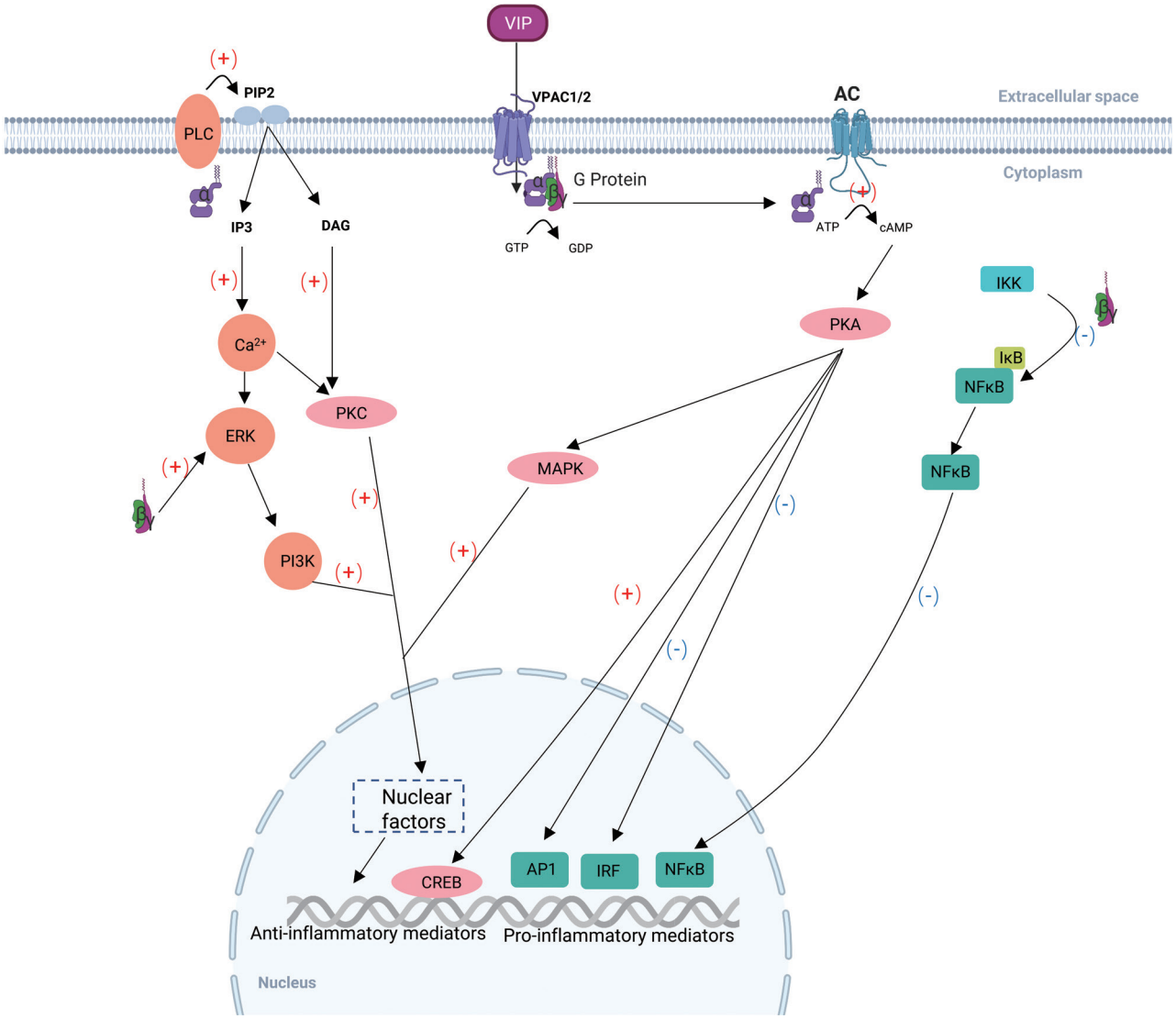

Fig. 2. VIP/VPAC receptors' axis signaling pathways. Activated Gα stimulates the enzyme adenylate cyclase (AC) to catalyze cAMP synthesis, activating PKA, which promotes different cAMP-response element binding protein (CREB) and mitogen-activated protein kinase (MAPK) activation, yet can inhibit AP-1 and IRF activation. The βγ dimer activates phosphoinositide 3-kinase (PI3K) and prevents NF-κB translocation to the nucleus, impeding IKK activation. Additionally, VPAC receptors can also mediate Ca^{2+} release to activate PKC, subsequently activating some nuclear factors.

with several proteins and can activate extracellular regulated kinases (ERK), followed by phosphoinositide 3-kinase (PI3K) activation [59, 60]. Besides, the βγ dimer can prevent NF-κB translocation to the nucleus, impeding IKK activation.

VPAC receptors can also mediate Ca^{2+} release to activate PKC. In this pathway, binding of VIP and VPAC receptors promotes the production of inositol phosphate (IP3), leading to the rise of Ca^{2+} levels, which, together with diacylglycerol (DAG), activates PKC [61]. PKC activation can lead to production of anti-inflammatory factors.

3 Basic functions of VIP

3.1 Gastrointestinal hormone functions of VIP

VIP in the gastrointestinal tract mainly plays a local role in the form of neurotransmitter, including the following ones: (1) Prosecretory action: VIP released from enteric nerves stimulates anion secretion from the enterocytes via Gs-coupled VPAC1 activation [62], such as HCO_3^- and Cl^-, through the activation of PKA and/or cystic fibrosis transmembrane conductance regulator (CFTR) [63, 64]. (2) Vasodilatory action: Vasodilatory effects of VIP are mediated via VPAC1 activation on endothelial cells, followed by release of NO, and via VPAC2 activation on vascular smooth muscle cells in the porcine basilar arteries [65]. (3) Smooth muscle contraction and relaxation: VIP contracts and relaxes gastrointestinal tract smooth muscles depending on the distribution of VPAC1 and VPAC2. Selective VPAC2 agonists, not VPAC1 agonists, relax pre-contracted longitudinal muscles of rat fundic stomach [49]. In contrast, VIP promotes contraction of the longitudinal muscles of guinea pig jejunum via muscarinic receptor and VPAC1 activation [66]. (4) Gastric inhibitory action: VIP inhibits gastric acid secretion via inhibition of gastrin release in dogs [67, 68]. The inhibition by VIP is due to VPAC1 activation on D cells and somatostatin release. (5) VIP effects on epithelial paracellular permeability: VIPergic pathways reduced epithelial paracellular permeability by increasing the expression of the tight junction protein zonula occludens-1 (ZO-1) in human polarized colonic epithelial monolayers [69]. VIP can also prevent the translocation of tight junction proteins ZO-1, occludin, and claudin-3 in a *Citrobacter rodentium*-induced colitis model to meliorate bacterial infection-induced intestinal barrier disruption [70].

In COVID-19, after the lung cells are infected by SARS-CoV-2, effector $CD4^+$ T cells reach the small intestine through the gut-lung axis, causing intestinal immune damage and diarrhea [71]. And early extensive use of antibacterial and antiviral drugs can also lead to diarrhea in the patients. In such cases, the gastrointestinal hormone functions of VIP may provide relief.

3.2 Neuropeptide functions of VIP

VIP has four main neuropeptide functions. (1) It plays a crucial role in regulating mammalian circadian rhythms through VPAC2 [72]. (2) It increases cerebral cortex glycogen metabolism by the cAMP second-messenger pathway, regulates embryonic growth, inhibits neuronal cell death, and has a protective effect on astrocytes [73–75]. (3) It displays neuroprotective and anti-inflammatory properties in models of Parkinson's disease by ameliorating cognitive functions, decreasing the levels of neuroinflammation, and promoting dopaminergic neuronal survival [8]. Lastly, (4) it shapes the CNS development starting with cell generation, but also during cell migration, maturation, synaptogenesis, and myelination as well as during programmed cell death, and modulates hippocampal synaptic plasticity [76, 77].

VIP was reported to pass through the blood-brain barrier (BBB) via transmembrane diffusion, allowing it to reach the CNS to treat neurological symptoms [78]. The acquired immunodeficiency syndrome (AIDS), caused by human immune deficiency virus (HIV), can lead to dementia, which could be reversed by VIP due to its neuroprotective function [79]. Thus, VIP is also worth testing for the resolution of neurological complications caused by other viruses, such as the herpes virus.

3.3 Immune functions of VIP

The immune system clears "foreign" or "non-self" antigens with two temporally separate but physically linked responses, broadly categorized into innate and adaptive immunity. Numerous studies have identified VIP as a potent endogenous anti-inflammatory agent that affects both the innate and adaptive immunity in several ways.

The functions of VIP in innate immunity can be summarized as follows. (1) VIP stimulates macrophage phagocytosis, adherence and migration, or reactive oxygen production via PKC activity, whereas it can change to inhibitory effects through cAMP, depending on the cell differentiation stage and the activation state [18]. (2) VIP inhibits the expression of pro-inflammatory cytokine TNF-α, IL-6, IL-12, and inducible nitric oxide synthase (iNOS) by reducing the binding of the NF-κB transcription factor to the promoter. On the other hand, VIP promotes production of anti-inflammatory cytokine

IL-10 by increasing the binding of the CREB [80]. (3) VIP inhibits the expression of two CXC chemokines (CXCL1/KC and CXCL2/MIP2) and four CC chemokines (CCL2/MCP1, CCL3/MIP1α, CCL4/MIP1β, and CCL5/RANTES) in LPS-stimulated mouse macrophages [81, 82]. In addition, VIP inhibits endotoxin-induced expression of IL-8 (CXCL1) by human peripheral blood monocyte [83]. The inhibitory effect was mediated through VPAC1 with a reduction in NF-κB binding and transactivating activity [81, 84]. (4) VIP can regulate B7 expression in macrophages, which can interact with their counter-receptors on T cells [85]. In resting macrophages, VIP up-regulates B7.2 (CD86) expression, but in LPS/IFN-activated macrophages, VIP down-regulates both B7.1 (CD80) and B7.2 expression [18]. (5) VIP can regain dendritic cell (DC) tolerogenic ability *in vitro* and *in vivo* under different inflammatory situations [86, 87].

The functions of VIP in adaptive immunity are as follows. (1) It affects T cell activation. On the one hand, it down-regulates B7.1/B7.2 expression in LPS/IFNγ-activated macrophages, which correlates with a reduction in the stimulation of antigen-specific T cell proliferation. On the other hand, VIP inhibits IL-2 production and subsequent T cell proliferation via the induction of intracellular cAMP [88], affecting both nuclear factor of activated T cells (NF-AT) and AP-1 binding [89, 90]. (2) VIP affects $CD4^+$ T cell differentiation, in favor of Th2 differentiation, lining in three mechanisms. First, it inhibits IL-12 production from activated macrophages, without which the Th1/Th2 balance will be altered in favor of Th2 [91, 92]. Second, as indicated above, VIP induces B7.2 expression, a Th2 costimulatory molecule, in resting macrophages and immature DCs. Third, VIP treatment of T-cell receptor-transgenic T cells cultured with irradiated antigen presenting cells (APCs) leads to increased levels of IL-4 and decreased levels of IFN, suggesting that VIP promotes a Th2 bias directly by acting on the differentiating $CD4^+$ T cells [93]. In addition, VIP supports the survival and possibly the proliferation of Th2, but not Th1, effectors [94]. (3) VIP was shown to down-regulate CXCL10 and up-regulate CCL22 in spleen cell cultures and bone marrow-derived DC [95]. (4) VIP can also drive the production of IL-22 through a VIP-VPAC2 pathway in group 3 innate lymphoid cells (ILC3s) [96, 97]. (5) It counterbalances the ratio of Th17/Treg, Th1/Treg, or Th2/Th9, leading to reduced pathogenicity and increased tolerance [12, 98, 99].

As implicated earlier, both innate and adaptive immunity are important against virus infections. The immune regulatory role of VIP boosts the prospects of its use in viral infections.

4 VIP in viral infections

4.1 VIP in HIV infection

In 1987, Sacerdote *et al*. found that VIP shared a five-amino-acid (TDNYT) sequence homology with gp120, the attachment sequences of HIV [100]. The shared sequence gives HIV the opportunity to bind to the VIP receptor, disrupting its normal physiological function. Gp120 of HIV was shown to be able to mimic VIP binding, thus interfering with normal VIP-ergic neurotrophic and cerebral blood flow effects and eventually leading to AIDS dementia [101]. Moore *et al*. also proposed that HIV may employ VIP or VIP-like receptors on brain cells and lymphocytes for intracellular access [102]. Similarly, Raymon *et al*. showed that attachment of HIV particles involves VIP receptors in rat brain, at least in part [103]. Lastly, Branch *et al*. discovered that VPAC1 could facilitate HIV-1 infection, and VPAC1 signal blocking antibody inhibited up to 80% of productive infection with HIV-1IIIB in T cells [104]. In contrast, a single study by Nguyen indicated that VIP receptors didn't mediate HIV attachment in three different models (rat intestinal epithelial cell membranes, rat liver plasma membranes and human colonic cells) [105]. It is possible that this apparent discrepancy is caused by differences in cell types.

The therapeutic effect of VIP was first revealed in its ability to completely antagonize neuronal cell death caused by purified gp120 from two diverse HIV isolates and recombinant gp120 [106]. Another study found that VIP antagonist impairs memory for spatially related stimulus control by gp120 [107]. The mechanisms of VIP-mediated prevention of neurotoxicity were shown to relate to VIP promoting MIP-α and RANTES release and counteracting the gp120 inhibition on NK cell function [79, 108]. VIP can also serve as a possible antigen and elicit antibodies that recognize the pC2 peptide, which can block HIV fusion [109] and prevent developmental deficits in infants born to HIV-positive mothers [110]. Temerozo *et al*. expanded the anti-HIV effects of VIP by showing that VIP and the VPAC2 agonists can reduce viral production in HIV-1-infected primary macrophages by promoting β-chemokines and IL-10 production through the production of cAMP and

activation of PKA and PKC [111]. VIP also promoted G-to-A mutations in the HIV-1 provirus, which reduced viral infectivity [111].

Only one clinical trial was conducted by Veljkovic and colleagues. Using HIV-negative blood donors with a significant titer for the anti-VIP/NTM antibody monthly from December 1993 to June 1994 to treat one patient, they found that the CD4 count of the patient increased during the immunotherapy period and remained stable for the following 6 years, documenting the potential therapeutic use of anti-VIP antibodies for the treatment of HIV disease [112]. These findings further strengthened the antiretroviral potential of VIP and pointed to new therapeutic approaches to control the progression of HIV-1 infection. Altogether, these results suggest that VIP can protect the nervous system from HIV injury and reduce viral infectivity. However, clinical trials to test this prospect are currently lacking.

4.2 VIP in vesicular stomatitis virus (VSV) infection

Chelbi-Alix *et al*. first discovered that VIP induced 2'5' oligoadenylate (2'5' A) synthetase activity to inhibit VSV growth in HT-29 cells, which could be abolished by anti-IFN-β/α antibodies [113]. They further showed that in primary glial cultures, VIP also promoted 2'5' A synthetase activity and inhibited VSV multiplication through IFN-α/β synthesis [114]. However, both studies were conducted *in vitro*, and therefore, *in vivo* experiments are needed to further verify the conclusions and validate the molecular mechanism so as to lay the foundation for clinical trials.

4.3 VIP in RSV infection

In 2001, King *et al*. first found that VPAC1 receptor for the anti-inflammatory VIP increased to a much lesser degree compared with NK1 receptor for pro-inflammatory substance P (SP) in RSV-infected intrapulmonary airways of weanling rats [115]. Tan *et al*. established a persistent RSV infection animal model by cyclophosphamide (CYP) pretreatment and found that during persistent infection (day 42 and 60), the SP-positive and calcitonin gene-related peptide-positive fibers increased, but VIP-positive fibers decreased with worse lung function and increased inflammation, suggesting that VIP may play a role in reducing airway inflammation and improving lung function post RSV infection [116]. They obtained the same results by establishing another persistent RSV infection animal model in 3–5-day-old guinea pigs [117]. Our group also had some interesting results in this area. Previously, we demonstrated that degeneration of pulmonary C-fibers (PCFs) increased IFN-α/β induction and STAT1 activation, and reduced virus titers upon RSV infection in mice, which also correlated with alleviated inflammatory responses [118, 119]. Furthermore, we showed that enhanced RSV-induced IFN-α/β antiviral immune responses in PCFs-degenerated mice can be attributed to elevated VIP concentration in bronchoalveolar lavage fluid (BALF) (unpublished). However, there is currently no report of a direct effect of VIP on the airway inflammation and lung function during RSV infection, and therefore, more in-depth mechanistic studies are needed to translate it into clinical use.

4.4 VIP in Zika virus (ZIKV) infection

Vota *et al*. reported that VIP inhibited ZIKV replication in trophoblast cells, which could be correlated with reduced Toll-like receptor-3 and viperin mRNA expression, together with reduced CD56Dim cell trafficking to trophoblast conditioned media [120]. These results point to VIP as a potential candidate molecule to treat ZIKV infection during early pregnancy.

4.5 VIP in SARS-CoV-2 infection

In a seminal recent study, patients with critical COVID-19 showed elevated VIP plasma levels compared with healthy subjects or asymptomatic patients, and the higher serum VIP levels were correlated with further survival rate in patients with severe COVID-19 [121]. In the study, VIP was able to inhibit SARS-CoV-2 RNA synthesis in human monocytes and viral production in lung epithelial cells. Mechanistically, VIP promoted anti-apoptotic CREB activation and reduced the production of pro-inflammatory mediators by suppressing NF-κB activation [121]. Based on the observation, Khodabakhsh *et al*. conclude that the measurment of VIP may be considered as a routine part of COVID-19 patient, and serve as a potential therapeutic target [122].

Currently, synthetic VIP, named Aviptadil in intravenous and inhaled formulation, is under two clinical trials for COVID-19 patients with respiratory failure (clinicaltrials.gov: NCT04311697 and NCT04360096) [122, 123]. All the study results above provided scientific evidence to support that VIP could have the potential as a preventive measure and even therapeutic agent for SARS-CoV-2 infection.

4.6 VIP in cytomegalovirus (CMV) infection

Li *et al*. found that CMV-infected VIP-knockout (VIP-KO) mice had lower weight loss, lower viral loads and faster clearance of virus, leading to better survival,

compared with wild type (WT) mice. Detailed studies revealed that the KO mice indeed had enhanced innate immunity, with increased numbers of IFN-γ^+ NK and NKT cells, and augmented cytolytic activity of NK cells. At the same time, adaptive antiviral cellular immunity was also increased with more Th1/Tc1-polarized T cells, fewer IL-10^+ T cells, and more CMV-M45 epitope peptide MHC class I $tetramer^+$ $CD8^+$ T cells. VIP-KO mice also exhibited a marked upregulation of MHC-II and CD80 costimulatory molecule expression on DC, whereas WT mice had upregulated programmed death-1 and programmed death ligand-1 expression in activated $CD8^+$ T cells and DC following CMV infection. These results established that the absence of VIP in immune cells increased innate and adaptive antiviral immunity following CMV infection [124].

Based on these findings, the same authors conducted another study with one week of daily subcutaneous injections of VIP-receptor antagonist (VIPhyb) to CMV-infected C57BL/6 and BALB/c mice, and found that survival and viral clearance were markedly enhanced, with reduced liver and lung pathology, compared with saline-treated controls [125]. The researchers further demonstrated that even for allogeneic bone marrow

Table 1. Modulatory role of VIP in different virus infections

Virus	Viral genome	Research method	Modulatory effects	Mechanism	References
HIV	*RNA*	*In vivo, in vitro,* clinical trial	VIP protects nervous system from HIV injury, suppresses viral infection and prevents developmental deficits in infants born to HIV-positive mothers	(1) VIP promotes the release of MIP-1α and RANTES in astrocytes (2) Counteracts the inhibitory effect of gp120 on NK cell functions (3) Increases β-chemokines and IL-10 secretion in macrophages via PKA/PKC pathway (4) Promotes G-to-A mutations in the HIV-1 provirus	[79, 106, 108, 110, 111]
VSV	RNA	*In vitro*	VIP inhibits VSV multiplication	IFN-α/β synthesis increases in primary glial cultures	[113,114]
RSV	RNA	*In vivo*	VIP reduces RSV replication and may attenuate airway inflammation and improve lung function post RSV infection	VIP enhances IFN-α/β-STAT1 pathway	[115–119]
ZIKV	RNA	*In vitro*	VIP suppresses ZIKV infection in trophoblast cells	Unknown	[120]
SARS-COV-2	RNA	*In vivo, in vitro,* clinical trial	The higher plasma VIP levels are correlated with survival in severe COVID-19 patients. VIP can inhibit the SARS-CoV-2 virus replication in human lung epithelial cells and human monocytes	VIP prevented the SARS-CoV-2-induced activation of NF-κB and promoted CREB activation	[121]
CMV	DNA	*In vivo*	Both VIP-KO mice model and VIPhyb treatment lead to better survival, and lower virus titers post CMV infection	(1) Lack or suppression of VIP increases numbers of IFN-γ^+ NK and NKT cells, and enhances cytolytic activity of NK cells (2) VIP exhibits a marked upregulation of MHC-II and CD80 costimulatory molecule expression on DC	[124–127]

HIV: human immune deficiency virus; VSV: vesicular stomatitis virus; RSV: respiratory syncytial virus; ZIKV: Zika virus; CMV: cytomegalovirus; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; COVID-19: coronavirus disease 2019; VIPhyb: VIP-receptor antagonist; VIP-KO: VIP knockout.

transplant (allo-BMT) mice, administration of a VIP antagonist is a safe therapeutic approach to enhance anti-CMV immunity [126]. Recently, Forghani *et al*. reported the possibility that VIP could regulate anti-CMV activity of T cells through influencing ability of the immune regulatory cells–monocytic myeloid-derived suppressor cells (MDSC) [127], but the detailed mechanisms remain unknown. Regrettably, like HIV, there is a lack of clinical trials to verify the anti-CMV therapeutic effects of VIP.

5 Prospects for VIP as a target for antiviral therapy

Although significant progress has been made in VIP research, several hurdles still exist in its use in the clinic. (1) VIP is easily degraded by proteases, spontaneous hydrolysis, and catalytic antibodies, which constitute a major obstacle. (2) Cross-interactions is the second limitation for the use of VIP in humans. Its ability to bind different GPCRs also leads to its functional pleiotropism and ubiquity. (3) Lastly, systemic administration of VIP and resultant binding to multiple cell targets with high affinity could cause unwanted effects [128, 129]. Thus, distribution systems directed against specific targets that also protect the peptide against its degradation are desirable options.

Nevertheless, several recent advances should contribute to overcoming these limitations. (1) Metal nanoparticles, modified liposomes and sterically stabilized micelles have successively been used to increase the therapeutic potential of VIP in terms of both targeting and distribution [130–132]. (2) Ongoing exploration of more stable analogues of the VPAC1 and VPAC2 receptors solves the problem of VIP binding to different receptors [53, 133]. (3) Gene therapy with VIP, using lentiviral vectors, has yielded promising results in suppressing diabetes-related inflammation and augmented pancreatic β-cell proliferation [134], and enhancers selective for VIP-expressing interneurons has been discovered recently [135]. However, lack of cellular and tissue specificity is still a problem. Current research attempts to design cell therapy using dendritic cells transduced with a VIP lentiviral vector (LentiVIP-CDs), whose therapeutic effects in sepsis and experimental autoimmune encephalomyelitis (EAE) models have been very positive with a single local administration [136]. Looking toward the future, despite advances in such therapeutic options, research on the design and transfer of stable VIP analogues and specific VPAC1- and VPAC2-receptor drugs to the clinic is still needed.

6 Conclusion

The effects and mechanisms of VIP on reported viruses have been summarized here (Table 1), which shows that VIP has an antiviral role in RNA viruses (HIV, VSV, RSV ZIKV, SARS-CoV-2), while inhibiting VIP defends against a DNA virus (CMV). These viruses are also phylogenetically diverse; while HIV is a retrovirus,

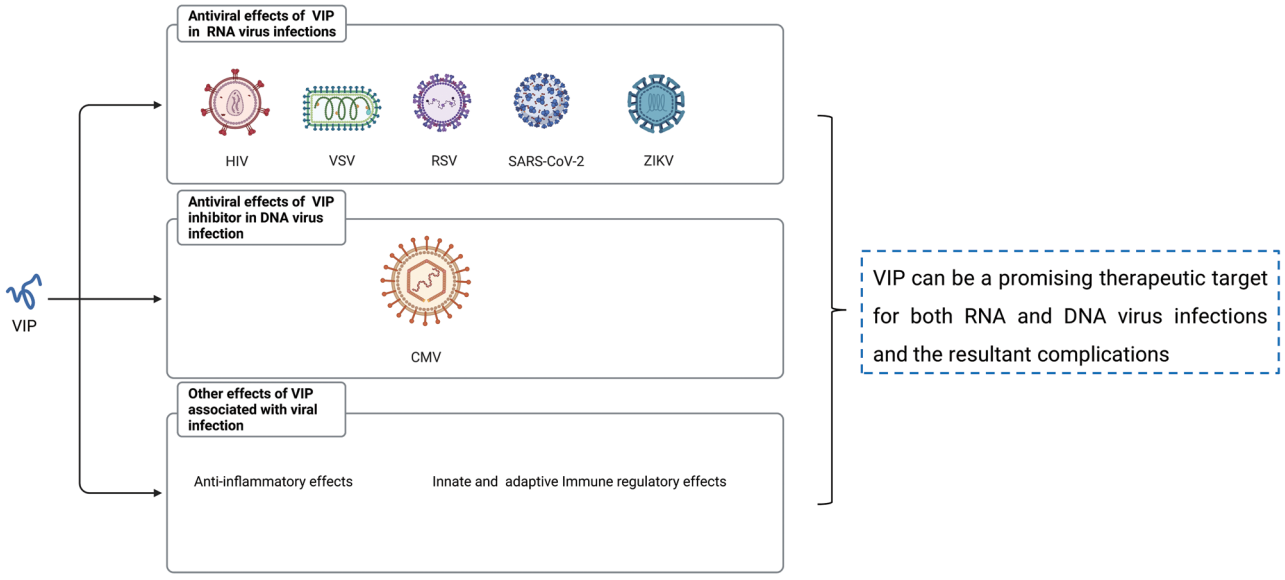

Fig. 3. The summarized prospects of VIP as potential target for antiviral therapy.

VSV and RSV are negative-strand RNA viruses of the *Mononegavirales* order, ZIKV and SARS-CoV-2 are positive-strand RNA virus and CMV is a member of the *Herpesvirales* order. Thus, we speculate that VIP may have effects on a wide spectrum of viral infections and that the underlying pathophysiological mechanism will likely differ between viruses. Additionally, due to overproduction of pro-inflammatory cytokines and the overactivation of immune cells, virus infection often causes complications and some can be severe. So, considering its excellent anti-inflammatory and immunomodulatory properties, VIP may be a promising target for treating viral infections and the resultant complications, generating better outcome and prognosis in viral diseases (Fig. 3).

In summary, VIP is an excellent and potential therapeutic target for viral infections, and its role in HIV, VSV, RSV, SARS-CoV2, ZIKV and CMV as well as other viral infection is worth more detailed mechanistic and clinical studies. Further research on the design of more stable and specific VIP analogues or VPAC1- and VPAC2-receptor drugs is also indispensable for translating the theoretical research into clinical applications.

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