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Functional Splice Variants of the Type II G Protein–Coupled Receptor (VPAC2) for Vasoactive Intestinal Peptide in Mouse and Human Lymphocytes

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Abstract

A PCR-based search for splice variants of the VPAC2 G protein–coupled receptor for vasoactive intestinal peptide (VIP) revealed: (a) a short-deletion variant in mouse lymphocytes termed *VPAC2de367–380*, that lacks 14 amino acids in the seventh transmembrane domain, and (b) a long-deletion variant in human lymphocytes termed *VPAC2de325–438(i325–334)*, that lacks 114 amino acids beginning with the carboxyl-terminal end of the third cytoplasmic loop and has 10 new carboxy-terminal amino acids. *VPAC2de367–380* binds VIP normally, but shows reduced VIP-evoked signaling and effects on immune functions, whereas *VPAC2de325–438(i325–334)* shows reduced binding affinity for VIP and a complex pattern of functional differences. These splice variants may modify the immunoregulatory contributions of the VIP–VPAC2 axis.

Keywords

neuropeptide; immunity; T cell; cytokine

Most of the vasoactive intestinal peptide (VIP) at sites of immune cell traffic and functional responses is generated and secreted by neurons and type 2 helper T lymphocytes, termed *Th2 cells*.¹ Many aspects of the specific effects of VIP on immune cells, including T cells and antigen-presenting cells, such as macrophages and dendritic cells, have been elucidated in several laboratories over the past two decades.² Expression of the types I (VPAC1) and II (VPAC2) G protein–coupled receptors for VIP by different immune cells has been documented and some mechanisms controlling their respective expression are clear determinants of the nature of immune responses. For example, stimulation of T cells through their antigen receptor (TCR) downregulates VPAC1 but upregulates VPAC2. This shift in predominance of VPAC1 to VPAC2 on activated T cells permits VIP to control the balance between immediate-type or allergic inflammatory immune responses mediated by Th2 cells and delayed-type cellular protective or hypersensitivity responses mediated by Th1 cells. Further support for this role of the VIP–VPAC2 axis came from the results of immunological studies of VPAC2-knockout (KO) mice and VPAC2-transgenic (TG) mice with a constitutively high level of expression of VPAC2 selectively on T cells. VPAC2-TG mice have blood eosinophilia, elevated serum concentrations of IgE, and greater than normal IgE antibody responses and cutaneous immediate-type reactions to allergy-producing antigens, that all are typical of the allergic state.³ VPAC2-TG mice also exhibit reduced cutaneous delayed-type reactions to antigens that is indicative of deviation from Th1- to Th2-mediated immunity. In contrast, VPAC2-KO mice

manifest greater than normal cutaneous delayed-type reactions to antigens and reduced IgE antibody responses and cutaneous immediate-type reactions to allergy-producing antigens.⁴ This immune deviation from Th1-mediated delayed-type cellular responses to Th2-mediated immediate-type allergic inflammatory responses is a function of the differential effects of the VIP-VPAC2 axis on T cell generation of cytokines that determine the relative numbers and activities of Th1 and Th2 cells.

The VIP-VPAC2 axis in Th cells signals expansion of the Th2 population and contraction of the Th1 set. As a result, stimulation of mixed Th cells from VPAC2-TG mice results in increased generation of interleukin-4 (IL-4) and, to a lesser extent, IL-5 and decreased generation of gamma-interferon. In contrast, similarly stimulated Th cells from VPAC2-KO mice generate less IL-4 and more gamma-interferon than Th cells from wild-type mice. VPAC2 signals these alterations in cytokine-secreting Th cell subsets through c-Maf and JunB, without the more usual involvement of GATA-3 and Tbet.⁵ Other effects of VIP on T cells are mediated by both VPAC1 and VPAC2.² VIP is a chemotactic stimulus for T cells, in part through its capacity to enhance cell surface expression of the matrix metalloproteinase, MMP-9, that facilitates T cell movement across basement membranes and other tissues. The suppression of T cell proliferative responses by VIP is attributable largely to inhibition of generation of IL-2 and its actions as a T cell growth factor. These effects of VIP also reduce Th cell help to B cells that presumably underlies VIP suppression of T cell-dependent production of IgG antibodies. The coreceptor interactions necessary for effective antigen presentation to T cells and the binding of cell surface Fas/Fas ligand proteins that initiate apoptosis also are influenced by VIP-induced changes in expression of one or more such proteins.

The least well-understood aspect of VIP in immunity is the basis for immune regulation of expression of VPAC1 and VPAC2 and their signaling pathways in immune cells. It is generally assumed that some combination of cytokines transcriptionally regulates the constitutive levels of VPAC1 and VPAC2 and that changes in VIP concentration and the composition of the controlling cytokines is responsible for altered expression of VPAC1 and VPAC2 in immune responses. No investigations have addressed independent immune-specific alterations in signaling pathways. No one has considered the possible involvement of structural variants of VPAC receptors, hetero-oligomers of such variants with native wild-type VPAC receptors, or decoy or other signaling-impaired variants in immune-specific regulation of responsiveness of T cells to VIP. We therefore began a search for variants of VPAC2 in immune cells and tissues, using multiple sets of primers in a polymerase chain reaction (PCR)-based approach. Two deletion splice variants have been identified, one in mouse thymocytes and splenic T cells and the other in cultured lines of human malignant T cells and activated T cells of normal human blood. The mouse immune splice variant of VPAC2 is a simple deletion of the 42 base pairs of exon 12 and the resultant loss of 14 amino acids from 367–380 at the carboxyl-terminal end of the seventh transmembrane (TM) domain (Table 1).⁶ In the human immune splice variant of VPAC2, the deletion of exon 11 results in loss of 114 amino acids from 325–438 by disruption of the codon sequence that creates a frame shift allowing transcription of mRNA encoding 10 different amino acids (i325–334) and introduces a premature stop codon with loss of sequence beyond amino acid 334 (Table 1). It is predicted that mouse VPAC2de367–380 will be a six TM domain protein and human VPAC2de325–438(i325–334) will be a five TM domain protein.

Similar levels of expression of mouse VPAC2de367–380 and mouse wild-type VPAC2 were established in human VPAC2 low Jurkat human T cell transfectants, which permitted comparative studies of their properties.⁶ VPAC2de367–380 and wild-type VPAC2 in Jurkat T cells bound VIP with the same affinity, but only wild-type VPAC2-transduced VIP mediation of increases in [cAMP]_i, chemotaxis, and decreases in IL-2 generation in the Jurkat T cells (Fig. 1). Similarly, only mouse wild-type VPAC2 and not VPAC2de 367–380 in mouse Th2

cell transfectants signaled. The results of preliminary studies of human VPAC2de325–438 (i325–334) in contrast with human wild-type VPAC2 suggest lower-affinity binding of VIP, decreased and less sustained VIP-induced downregulation, and diminished transduction of functional signals to T cells (Fig. 1).

Future goals include completion of analyses of the structures of mouse VPAC2de367–380 and human VPAC2de325–438(i325–334), elucidation of their distinctive mechanisms for regulation of expression and for cellular signaling, and delineation of functionally relevant interactions between these splice variants and their respective wild-type VPAC2 receptors.

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FIGURE 1.

Distinctive characteristics of the carboxyl-terminal deletion splice variants of VPAC2. The mouse VPAC2de367–380 is shown as a six TM domain G protein–coupled receptor and human VPAC2de325–438(i325–334) is depicted as a five TM domain GPCR.

TABLE 1

Natural carboxyl-terminal splice variants of the VPAC2

A. Mouse VPAC2	
WT mVPAC2	³⁵⁰ ISSTYQILFELCVGSFQGLVVAVLYCFLNSEVQCELR ³⁹⁰
mVPAC2de367–380	³⁵⁰ ISSTYQILFELCVGSFQ—VQCELR ³⁹⁰
B. Human VPAC2	
WT hVPAC2	³²¹ SQYKRLAKSTLLIPLFGVHYMVFAVFPISISSKYQILFELCLGS ³⁶⁵
hVPAC2de325–438	³²¹ SQYKA <i>WWPSSTVS</i> —
WT hVPAC2	³⁶⁶ FQGLVVAVLYCFLNSEVQCELRK ⁴⁰⁷
hVPAC2de325–438	—
WT hVPAC2	⁴⁰⁸ SSFSRNGSEGALQFHRGSRAQSFLQTETSVI ⁴³⁸
hVPAC2de325–438	—

The sequence underlined in mVPAC2de367–380 is the seventh transmembrane domain and those underlined in hVPAC2de325–438 (i325–334) are the sixth and seventh transmembrane domains. Ten new amino acids (i325–334) at the carboxyl-terminus of hVPAC2de325–438 (i325–334) are shown in italics. WT = wild type.