Vasoactive Intestinal Peptide Receptor-1 (VPAC-1) Is a Novel Gene Target of the Hemolymphopoietic Transcription Factor Ikaros*

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Glenn Dorsam‡ and Edward J. Goetzl
From the Departments of Medicine and Microbiology-Immunology, University of California Medical Center, San Francisco, California 94143-0711

Vasoactive intestinal peptide and its G-protein-coupled receptors, VPAC-1 and VPAC-2, are highly expressed in the immune system and modulate diverse T cell functions. The human VPAC-1 5’-flanking region (1.4 kb) contains four high affinity Ikaros (IK) consensus sequences. Ikaros native protein from T cell nuclear extracts and IK-1 and IK-2 recombinant proteins recognized an IK high affinity binding motif in the VPAC-1 promoter in electrophoretic mobility shift assays by a sequence-specific mechanism, and anti-IK antibodies supershifted this complex. Stable NIH-3T3 clones overexpressing IK-1 or IK-2 isoforms were generated to investigate Ikaros regulation of endogenous VPAC-1 expression as assessed by quantifying VPAC-1 mRNA and protein. By traditional and fluorometric-based kinetic reverse transcription-PCR and 125I-labeled vasoactive intestinal peptide binding, both IK-1 and IK-2 suppressed endogenous VPAC-1 expression in NIH-3T3 clones by a range of 50–93%. When a series of nested deletions of the VPAC-1 luciferase reporter construct were transiently transfected into IK-2 clones there was up to a 41% decrease in transcriptional activity compared with vector control. Two major IK-2 binding domains also were identified at −1076 to −623 bp and at −222 to −35 bp, respectively. As both Ikaros and its novel target VPAC-1 are highly expressed in T cells, this system may be a dominant determinant of the VPAC-1 expression in immune responses.

Vasoactive intestinal peptide (VIP)† is a 28-amino acid peptide expressed and secreted by neurons innervating primary and secondary immune organs such as the thymus and lymph nodes (1, 2). VIP is a potent neurotrophic factor (3), vasodilator (4), and regulator of the hypothalamo-pituitary-adrenal axis (5). VIP also modulates several T lymphocyte activities including motility, cytokine production, proliferation, and apoptosis (6–9). VIP exerts its biological activity by binding (Kd, 1–10 μM) to two closely related class II G-protein-coupled receptors designated VPAC-1 and VPAC-2 with structural similarities to receptors for glucagon, secretin, parathyroid hormone, and calcitonin (2, 10). VPAC-1 and VPAC-2 are structurally similar, share 49% amino acid sequence identity, and illicit intracellular cyclic AMP protein kinase A and phospholipase C-calcium signals (11).

VPAC-1 is widely expressed throughout the body in several species studied including the central nervous system, peripheral nervous system, liver, lung, and intestines (10). In addition, immune cells such as peripheral blood mononuclear cells and T lymphocytes constitutively express VPAC-1 (2, 6, 7, 12). Naïve human CD4+ T cells express VPAC-1 at levels 10-fold higher than CD8+ T cells and down-regulate this receptor by greater than 70% within 10 h of TCR-mediated activation. Thus, VPAC-1 levels may be indicative of the activation status and thus represent a naïve CD4+ T cell surface marker (13).

We have identified four high affinity Ikaros binding elements located within 550 bp of the transcriptional start site of the human VPAC-1 promoter. Ikaros is a hemolymphopoietic restricted zinc finger transcription factor and is absolutely necessary for the proper ontogeny of lymphocytes (for review see Refs.14 and 15). The Ikaros gene is highly conserved between rodent and man and generates at least eight isoforms through alternative splicing (14, 15). All Ikaros isoforms have a common C terminus containing a bipartite transcriptional activation domain and two zinc fingers that facilitate dimerization with other Ikaros isoforms. All eight isoforms, however, differ in their N-terminal domain, which consists of four zinc fingers, three of which are necessary to bind DNA with high affinity. Thus, only IK-1, IK-2, and IK-3 demonstrate high affinity DNA binding, while IK-4 through IK-8 have little to no DNA binding (16, 17). Ikaros homo- or heterodimers recognize the sequence TGGGA(A/T), where the core GGGGA sequence is more important than flanking nucleotides. Non-DNA-binding isoforms (IK-4–8) generating heterodimers with DNA-binding isoforms (IK-1–3) do not bind DNA and are transcriptionally inactive. Consequently, non-DNA-binding Ikaros isoforms act in a dominant negative fashion (18).

This study began by an analysis of 1400 bp of the 5’-flanking region of human VPAC-1 that revealed four high affinity Ikaros binding motifs (TGGGA(A/T)) and 20 additional Ikaros core binding elements (GGGA). Electrophoretic mobility shift assays using Jurkat T cell nuclear extracts or recombinant Ikaros protein generated a sequence-specific retardation signal, which was further shifted by an anti-Ikaros antibody. Therefore, we hypothesized that the predominant DNA-binding Ikaros isoforms expressed in CD4+ T lymphocytes, IK-1 and IK-2, may regulate endogenous levels of VPAC-1 receptor. To this end, stable Ikaros NIH-3T3 mouse fibroblast clones overexpressing IK-1 and IK-2 isoforms were generated, and endogenous levels of VPAC-1 expression were quantified by traditional and fluorometric-based kinetic RT-PCR and 125I-labeled VIP binding.
Endogenous VPAC-1 mRNA and protein levels were significantly reduced (50–93%) compared with vector control. Luciferase reporter assays utilizing nested VPAC-1-deleted constructs further confirmed transcriptional down-regulation of VPAC-1 expression and identified two IK-2 regulatory domains of 453 and 187 bp, respectively. These data strongly suggest that VPAC-1 is a novel gene target for Ikaros and may mediate VPAC-1 regulation in the immune compartment.

MATERIALS AND METHODS

Reagents—DMEM-H21 media, 1× PBS (without Ca2+ and Mg2+), 0.05% trypsin, WI-38 cells, pyrogen-free water, and Opti-Mem were purchased from the Tissue Culture facility at the University of California. Fetal bovine serum, Tq polymerase, Pfu polymerase, Glutamax II, T4 ligase, TRizol®, First Strand cDNA reverse transcription kit, Genetin (G418), penicillin/streptomycin, and LipofectAMINE Plus were purchased from Invitrogen. Falcon tissue culture flasks were purchased from BD PharMingen. Stop and Grow luciferase, Renilla luciferase substrate assay kit, and the Renilla luciferase control vector were purchased from Promega. Invitrogen supplied the mammalian expression vector pCMV-tag2B, luciferase-pCMV-Tag2B, TOPO cloning vectors, and Top10 competent cells. Bradford reagent was purchased from Bio-Rad. Radioactive [γ-32P]ATP (6000 Ci/mmol, 10 μCi/ml) was purchased from Amersham Biosciences. Restriction enzymes BamHI, HindIII, and EcoRI were obtained from New England Biolabs. DNA oligonucleotides were synthesized by the University of California San Francisco DNA core facility, and fluorometric-based kinetic RT-PCR primers and probes were purchased from Integrated DNA Technologies. AmpliFrog Gold polymerase, PCR buffer, MgCl2, plates, and caps for fluorometric-based kinetic RT-PCR were purchased from Applied Biosystems. RNase inhibitor, Moloney murine leukemia virus reverse transcriptase, and deoxynucleotides were received from Roche Molecular Biochemicals. M2 anti-FLAG antibody, normal rabbit serum, dibutyl phthalate, and all other reagents used for buffers were purchased from Sigma.

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as described previously by Dorsam et al. (19). Briefly, cells were lysed with 100 μl of lysis buffer (20 mM Tris, pH 7.4, 140 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 0.5% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride), and nuclei were washed twice with 1 ml of lysis buffer lacking Nonidet P-40. Nuclear extraction buffer (250 mM Tris, pH 7.8, 60 mM KC1, 1 mM EDTA, 1 mM EGTA, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) was used to resuspend nuclei, and after centrifuging at 13,000 × g for 15 min, supernatants (nuclear extracts) were collected.

A 28-bp double-stranded DNA probe known to recognize all Ikaros DNA-binding isoforms and containing two inverted Ikaros binding sites (IK-oligo; 5′-TCACTTCCTCTGCGACCTTCCCTGTCA-3′) was used as a 24-bp DNA probe for determining sequence specificity of the probe. The 24-bp DNA element found in the human VPAC-1 promoter (VPAC-1 probe; 5′-CTTCGACAGCTGGAGAAGATGGTGCG-3′) was labeled for 30 min along with IK-oligo with T4 polynucleotide kinase using [γ-32P]ATP, then phenol/chloroform was extracted, and ethanol was precipitated. The phenol-DNA complexes were then resolved on a 5% PAGE gel in 0.5× Tris borate-EDTA buffer with 40 V for 2 h. Gels were dried for 45 min under vacuum and exposed to x-ray film.

Cell Culture—WI-38 cells were grown in 85% (v/v) DMEM-21, Jurkat T cells were grown in 80% RPMI, and both media were supplemented with 10% fetal bovine serum, 1× penicillin/streptomycin, 1× Glutamax II and incubated at 37 °C, 5% CO2. Stable Ikaros NIH-3T3 clones were cultured in WI-38 media supplemented with 500 μg/ml Genetin (G418).

Subcloning of Ikaros cDNA Isoforms—Ikaros cDNAs encoding for isoforms 1 and 2 (a kind gift from Dr. Katia Georgopoulos) were amplified by PCR using specific primers containing BamHI and HindIII overhangs. Each reaction was catalyzed by the high fidelity Pfu Taq polymerase. Amplified PCR products were ligated into the TOPO blunt cloning vector. Positive clones were isolated and sequenced bidirectionally with T7/SphI primers. Only Ikaros cDNAs that matched the published murine Ikaros cDNA sequences were used. Both Ikaros cDNAs were subsequently subcloned into the mammalian expression vector pCMV-Tag2B (IK-pCMV-Tag2B) and the bacterial expression vector pGXT-2TK (IK-GXT-2TK) by digesting all DNA species with BamHI/HindIII and ligating with T4 ligase at a 15:1 insert/vector ratio. Isolated subcloned vectors were sequenced to verify polymerase fidelity, and microgram quantities were amplified and isolated. IK-pCMV-Tag2B was used to generate NIH-3T3 stable clones, and IK-GXT-2TK was used to generate recombinant Ikaros-1 and Ikaros-2.

Stable Transfections—Subconfluent (50%) NIH-3T3 cells in 6-well plates were transfected with 0.7 μg of IK-pCMV-Tag2B (IK-1 or IK-2), luciferase-pCMV-Tag2B, or empty pCMV-Tag2B vector by the LipofectAMINE Plus technique as described by the manufacturer. Addition of the antibiotic Genetin (G418) was added to the growth media at 500 μg/ml 24 h after transfection. The cells were cloned by cell sorting and placed into 96-well plates. Clones were analyzed by traditional RT-PCR, fluorescence-activated cell sorter (data not shown), and Western blot analysis (data not shown) to verify expression of FLAG-tagged Ikaros recombinant expression. Positive clones were expanded, and all experiments were performed between passages 4 and 10 after expansion.

Recombinant Ikaros Expression—Competent Top10 bacteria were transformed with the IK-GXT-2TK expression vector and induced by 100 mg/ml isopropyl-1-thio-β-D-galactopyranoside overnight at room temperature. Bacterial cells were pelleted and lysed by sonication in 8 mM urea, 100 mM Tris, 150 mM NaCl buffer. Lysates were centrifuged at 10,000 × g in a JA-17 rotor for 15 min at 4 °C. Supernatants containing the recombinant Ikaros-2 protein were purified using 1 × PBS (4 liters) for 10 min and precipitated with 10% PEG 8000/4000 and 2 M NaCl. Precipitated proteins were resuspended in 1 × PBS (4 liters) and boiled for 10 min. Purified recombinant proteins were used in subsequent EMSA experiments.

Transient Transfections and Reporter Assays—VPAC-1 luciferase constructs with deleted 5′ or internal sequences were generated from human VPAC-1 5′-flanking sequence isolated from a human placenta genomic library (20). Restriction endonuclease digestion liberated either 5′ or internally deleted nested VPAC-1 5′-flanking sequences. These deleted sequences were subcloned into the pG2-2 basic luciferase vector as described by the manufacturer. Nested deleted VPAC-1 luciferase constructs or pG2-2 basic vector were transfection into subconfluent (50%) NIH-3T3 stable Ikaros-2 or vector clones by the LipofectAMINE Plus or LipofectAMINE 2000 procedure as described by the manufacturer. Briefly, cells were transfected in Opti-Mem for 5 h in the absence of serum. Cells were washed once with 1× PBS, and normal growth media were added. Cells were washed 24 h later with 1× PBS and lysed with 1× passive lysis buffer, and luciferase activity was measured by a 96-well Microlumat Plus luminometer for 15 s/well. Firefly luciferase activity was normalized with Renilla luciferase and represented as -fold activation over pG2-2 luciferase control vector.

Fluorometric-based Kinetic RT-PCR—Confined NIH-3T3 wild type and stable clones were trypanosed from tissue culture plates, centrifuged, washed with 1× PBS, and lysed with 1 ml of TRizol®. Total RNA was isolated as described by the manufacturer and quantitated by optical density, and 0.5–5.0 μg of mRNA-treated total RNA was used to generate first strand cDNA using reverse transcriptase. Ikaros message was amplified using primers specific to exons 5′-5′ (forward) and 3′-3′ (reverse), 5′-GATCCACCAACGCTGCGACAGCTGGAGAAGATGGTGCG-3′ and 5′-GATCCACCAACGCTGCGACAGCTGGAGAAGATGGTGCG-3′, respectively. Expected sizes for the Ikaros PCR products were 891 bp for IFK-1 and 629 bp for IFK-2. PCR products were separated on a 2% agarose gel and visualized by ethidium bromide fluorescence. The PCR reaction conditions were: 1 min at 94 °C, 1 min at 63 °C, and 1 min at 72 °C for 35–40 cycles.

Fluorometric-based Kinetic RT-PCR—Reactions contained 10 μl of DNase-treated total RNA template or RNAfree H2O with 15 μl of a 1.67× master mix (final concentrations: 4% glycerol, 0.01% Tween 20, 0.01% gelatin, 1× AmpliTaq Gold PCR buffer, 75 mM 5′-FAM-CTTCCTCTGCGACCTTCCCTGTCA-3′ internal probe, 2.5 mM MgCl2, 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 200 μM dGTP, 300 μM forward (VPMAC-1, 5′-AACCTTTAGGCCACAGTGAAT-3′; RGAPDH, 5′-TGCACCACTGCGACGCTGCGACAGCTGGAGAAGATGGTGCG-3′; and reverse primers (VPMAC-1, 5′-CTTCGACAGCTGGAGAAGATGGTGCG-3′; RGAPDH, 5′-GATCCACCAACGCTGCGACAGCTGGAGAAGATGGTGCG-3′, 5′-GATCCACCAACGCTGCGACAGCTGGAGAAGATGGTGCG-3′, and 5′-GATCCACCAACGCTGCGACAGCTGGAGAAGATGGTGCG-3′, 0.5 units/μl AmpliTaq gold polymerase, 0.5 units/μl Moloney murine leukemia virus reverse transcriptase) for a final volume of 25 μl. Reactions for both amplicons were run in the absence of reverse transcriptase to ensure no genomic DNA contamination. The reaction was conducted by the following procedure: one cycle of 30 min at 48 °C (first strand cDNA synthesis), one cycle for 5 min at 99 °C (inactivate reverse transcriptase and activate AmpliTaq gold polymerase), one cycle for 2 min at 95 °C, and 35 cycles.
and 40 cycles of denaturation for 15 s at 94 °C and annealing/extension for 1 min at 60 °C. Standard curves for both MVPAC-1 and RGAPDH were performed with every experiment, and coefficient of variance between runs for 5 μg of RNA template was 3.7 and 1.8%, respectively.

Confluent clones were detached and lysed with 1 ml of TRIzol®, and total RNA was isolated as described by the manufacturer. Total RNA was DNase-treated for 30 min at room temperature, and 5 ng of template RNA was used for subsequent measurement of MVPAC-1 and RGAPDH. Reactions were read by a 7700 sequence detector thermocycler linked to a Macintosh G4 computer using the sequence detector software. All cycle threshold values were obtained in the linear range of both amplicons.

Statistics—Data was analyzed by the Student’s t test for independent samples. Coefficient of variance was determined by running independent experiments on three different days, and the precision of each standard curve was compared for each amplicon.

RESULTS

Search for Putative Transcriptional Binding Motifs in the Human and Rat VPAC-1 5′-Flanking Regions—Analysis of the proximal 1.4 kb of human and rat VPAC-1 5′-flanking sequences (promoter) revealed 24 and 32 GGGA Ikaros core binding elements, respectively. This frequency is 4.5- and 6-fold greater than expected based on a random nucleotide distribution. Aligning the transcriptional start sites of both species further showed that 22 of these Ikaros core binding elements spatially matched. The human VPAC-1 promoter also contains four high affinity Ikaros binding sites (Fig. 1). Consequently, we hypothesized that Ikaros binds to and transcriptionally regulates VPAC-1.

In Vitro Binding of Ikaros to the Human VPAC-1 Promoter—EMSA demonstrated that protein from Jurkat T cells bound a 24-bp probe (VPAC-1 probe) containing a high affinity Ikaros binding site (TGAGGAT) found in the human VPAC-1 promoter. As a positive control, a 28-bp probe (IK-oligo) containing two inverted, high affinity Ikaros binding sites generated a similar retardation signal as the VPAC-1 probe (Fig. 1). Nuclear protein from human WI-38 fibroblast cells, which do not express Ikaros, were used as a negative control and did not generate a detectable retardation signal with either Ikaros probe. The Ikaros retardation signal generated by nuclear protein from Jurkat T cells could be competed away by excess unlabeled probe indicating a sequence-specific interaction (Fig. 2A). A highly specific Ikaros polyclonal antibody that recognizes the C terminus common to all Ikaros isoforms supershifted both the VPAC-1 and IK-oligo probes, whereas normal serum did not (Fig. 2B). Furthermore, EMSA analysis showed that GST re-
Regulation of Vasoactive Intestinal Peptide Receptor-1 by Ikaros

The functional consequences of Ikaros binding to the promoter of VPAC-1 were investigated by measuring changes in endogenous VPAC-1 receptor levels in Ikaros NIH-3T3 fibroblast clones stably expressing IK-1 and IK-2 protein. Cloned transfected were analyzed for VPAC-1 expression by traditional RT-PCR (Fig. 3C), fluorometric-based kinetic RT-PCR, and Western blot analysis (data not shown). By fluorometric-based kinetic RT-PCR, VPAC-1 mRNA expression was significantly lowered in stable clones overexpressing IK-1 (93%) and IK-2 (83%) isoforms compared with controls (Fig. 3A). Likewise, protein levels of VPAC-1 were significantly repressed (range = 54–60%, n = 3) as assessed by 125I-labeled VIP binding (Fig. 3B). This decrease in VIP binding is a direct reflection of VPAC-1 protein because NIH-3T3 cells do not express detectable levels of VPAC-2 by traditional RT-PCR (Fig. 3C). To confirm that this modulation of VPAC-1 expression was at the transcriptional level, we transiently transfected several VPAC-1 luciferase reporter constructs with defined deletions into IK-2 stable clones or vector control. The luciferase activity was repressed with several of the VPAC-1 deleted constructs compared with vector control. The Ikaros-mediated decreases were up to 41% in this reporter system (Fig. 4). In addition, two major IK-2 regulatory domains were identified in the VPAC-1 promoter at regions distal (−1076 to −623 bp) and proximal (−222 to −35 bp) to the transcriptional start site. Reporter assays were performed in IK-2 clones, which are similar to IK-1 clones in DNA binding activity and function, but were more stable in culture.

DISCUSSION

The capacity of the hemolymphopoietic transcription factors IK-1 and IK-2 to down-regulate VPAC-1, a G-protein-coupled receptor for VIP, is attributed to the presence of functional, high affinity Ikaros binding elements in the VPAC-1 promoter (Fig. 1). Because mouse NIH-3T3 fibroblasts do not express Ikaros, the isolated introduction of IK-1 or IK-2 by transfection assumes association with their DNA binding elements found in promoters of Ikaros target genes. Specific down-regulation of endogenous VPAC-1 receptors in Ikaros stable NIH-3T3 clones was demonstrated by several quantitative measurements at both the mRNA and protein levels. To our knowledge this is the first demonstration of a link between a master regulator of the development and maintenance of T and B cells and the transcriptional regulation of a neuropeptide receptor constitutively expressed in the immune system.

The observation that human and rat VPAC-1 promoters contain a tetrad of high affinity Ikaros binding elements introduces VPAC-1 into the cluster of T cell genes also containing high affinity Ikaros binding motifs, such as CD2, CD3, IL-2Ra, NF-xB, and terminal deoxynucleotidyl transferase (18). The high frequency of additional Ikaros core binding elements conserved between rodent and man further strengthens the importance of Ikaros transcriptional regulation of VPAC-1. By luciferase reporter analysis using 5’ or internal VPAC-1 nested deleted constructs, two IK-2 regulatory domains spanning 453 bp (proximal region; −1076 to −623 bp) and 187 bp (proximal region; −222 to −35 bp) to the transcriptional start site. Reporter assays were performed in IK-2 clones, which are similar to IK-1 clones in DNA binding activity and function, but were more stable in culture.
region; -222 to -35 bp) were identified. The distal domain contains 10 Ikaros core binding elements, whereas the proximal region contains one high affinity and two Ikaros core binding elements (Figs. 1 and 4). Both domains repress luciferase activity, with the distal domain showing greater repression (41% versus 30%). Interestingly, these two IK-2 regulatory domains correlate with only one of the four high affinity Ikaros binding elements. This binding element is within the VPAC-1 minimal promoter and overlaps a crucial Sp-1 binding site (21, 22). Therefore, IK-2, and presumably IK-1, may compete for this SP-1 binding site and cause transcriptional repression when overexpressed in NIH-3T3 fibroblasts. It has been demonstrated that Ikaros can compete for other binding sites and displace transcription factors such as ETS-1 (23). A major future goal is to identify which of these Ikaros binding elements are functionally active.

Ikaros has been shown to both activate and suppress transcription in transient reporter assays designed to detect specific recognition of synthesized tandem repeats of high affinity Ikaros DNA binding elements (24). The presence of Ikaros binding sites in T cell-specific genes suggests a classical trans-activating role for Ikaros, which has been demonstrated for VPAC-1 (25). However, Ikaros can also interact with centromeric heterochromatin and co-localize with transcriptionally silenced genes, suggesting that Ikaros can physically interact with certain gene promoters to silence their expression by promoting translocation to transcriptionally refractory nuclear compartments (26). Ikaros may repress transcription by at least two other mechanisms, which include recruitment of histone deacetylase (HDAC) enzymes and the engagement of the co-repressor CtBP (20, 27). With respect to the first possibility, it is noteworthy that the transcriptional rate of VPAC-1 is up-regulated by the HDAC inhibitor trichostatin A (data not shown) in numerous cell types including NIH-3T3 and Jurkat T cells. This may suggest that the extent of acetylation of critical nuclear proteins, such as histones, may be an important mechanism for the regulation of VPAC-1. Therefore, we propose that Ikaros may recruit additional HDAC enzymes to the promoter of VPAC-1 when overexpressed in NIH-3T3 cells and shift the equilibrium toward a hypoacetylated state, which thus silences transcription. Whether this down-regulation of VPAC-1 by Ikaros is also associated with its localization in heterochromatin will be the focus of future research.

VPAC-1 is constitutively expressed on human, naïve CD4 T cells of immune tissues (2, 12, 13). VIP represses TCR-mediated activation of human CD4 T cells and IL-2 generation initially through VPAC-1 receptor signaling (28). VPAC-1 is also down-regulated by more than 70% by TCR-mediated activation. These observations by our laboratory and others suggest that optimal activation of human CD4 T cells may require the down-regulation of VPAC-1 receptors. Indeed, VPAC-1 has been suggested to play a role in suppressing bystander T cell activation (13).

Ikaros, which sets the threshold for TCR activation (29), is redistributed to heterochromatin during TCR-mediated activation of T cells (14, 15). Several Ikaros isoforms and other family members such as Aiolos and Helios, aggregate into a higher order, 2 MDa toroidal complex with chromatin remodeling nu-

![Luciferase Activity Graph](image-url)
cleosome remodelling and deacetylase proteins and HDAC-1 and HDAC-2 enzymes (14, 30). We surmise that the redistribution of Ikaros, bound to the VPAC-1 promoter, to regions of heterochromatin after TCR-induced activation of CD4+ T cells may mediate the observed specificity of VPAC-1 down-regulation. The high frequency of Ikaros core binding elements in the VPAC-1 expression and enabling full CD4+ T cell activation when recruited to heterochromatin.

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