	NYAS	vip073	**Dispatch:** 4-7-2006	**CE:** IV
	Journal	**MSP No.**	**No. of pages:** 6	**PE:** Julia

VIP and Tolerance Induction in Autoimmunity

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ABSTRACT: VIP is a potent anti-inflammatory agent with immunoregulatory properties, skewing the immune response to a Th2 pattern of cytokine production. Here, we studied the effect of treatment with VIP in the development of diabetes in NOD mice, an animal model of type 1 diabetes. Mice treated with VIP from 4 wk of age did not develop diabetes and showed milder insulitis than nontreated mice. The protective mechanism of VIP was associated with a reduction in the circulating levels of Th1 cytokines. In the pancreas of VIP-treated animals, regulatory T cell markers predominate, as indicated by the upregulation of FoxP3 and transforming growth factor (TGF)-β, and the downregulation of the transcription factor T-bet. These findings indicate that VIP restores tolerance to pancreatic islets by promoting the local differentiation and function of regulatory T cells.

KEYWORDS: VIP; autoimmune; FoxP3; TGF-β

INTRODUCTION

The NOD strain of mouse is a useful model of autoimmune type 1 diabetes. The pathological mechanisms that lead to disease development are not known but the disease is associated with loss of immunological tolerance, in which autoreactive T cells play a major role in the pathogenesis. Th1-dependent cellular immune response and its associated cytokine secretion profile are thought to be responsible for the destruction of β cells.[1] *In vivo* systemic or local delivery of Th2 and regulatory cytokines[2] as well as diverting the immune response have been reported to prevent disease development and have been proposed as effective immunotherapeutic strategies.[3]

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Ann. N.Y. Acad. Sci. xxxx: 1–6 (2006). © 2006 New York Academy of Sciences.
doi: 10.1196/annals.1317.073

VIP is widely distributed in the central and peripheral nervous system as well as in the immune system, where it is produced by different cell types and exhibit important immunoregulatory functions,[4] inhibiting Th1 and promoting Th2 immune responses.[5] In this sense, VIP has been proposed as a therapeutic candidate for autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, or autoimmune diabetes.[6] In the present study, we show that treating NOD mice with VIP from 4 wk of age protects them against diabetes development. The *in vivo* effect is correlated with a decrease in Th1/Th2 balance and the induction of markers of T-regulatory function in the pancreas.

RESULTS

VIP Prevents Spontaneous Diabetes and Insulitis

Overt diabetes, as determined by the circulating levels of glucose, was apparent from 18 wk onward in female NOD mice and augmented gradually to reach 70% of diabetic mice at 30 wk of age. None of the NOD mice treated from 4 wk of age with 2.5 nmoles/animal of VIP (i.p.) every other day developed diabetes during the duration of the study (FIG. 1A). Since insulitis is a key feature in the development of type 1 diabetes, we studied the infiltration of the islets by histological methods from 10 wk of age. Infiltration was evident in 80% of the islets in NOD mice from 10 wk of age and its severity augmented progressively to the highest grade of infiltration apparent in 60–80% of the islets from 20 to 30 wk of age (FIG. 1B). Insulitis was not observed in NOD mice treated with VIP at 10 wk of age. Although it was apparent from 15 to 30 wk of age, insulitis in VIP-treated NOD mice appeared in less than 50% of the islets and was mostly restricted to the periphery (FIG. 1B).

VIP Decreases the Th1/Th2 Balance and Enhances the Expression of FoxP3 and TGF-β in the Pancreas

In NOD mice, a generalized intrinsic T cell defect is manifested in a dysregulated cytokine effector function,[1] and a marker of diabetes progression is the increase in the balance of circulating levels of Th1/Th2 cytokines.[7] To find out if the protective effect of VIP against the development of insulitis and diabetes is associated with changes in circulating levels of Th1/Th2 cytokines we have determined the levels of interleukin (IL)-12, IL-4, and IL-10 in serum by enzyme-linked immunosorbent assay (ELISA). As shown in FIGURE 2A, circulating levels of both, IL-4 and IL-12 were readily detected in NOD mice but do not change significantly along the duration of the study. However, the circulating levels of IL-10 were almost undetectable but at 30 wk of age. VIP

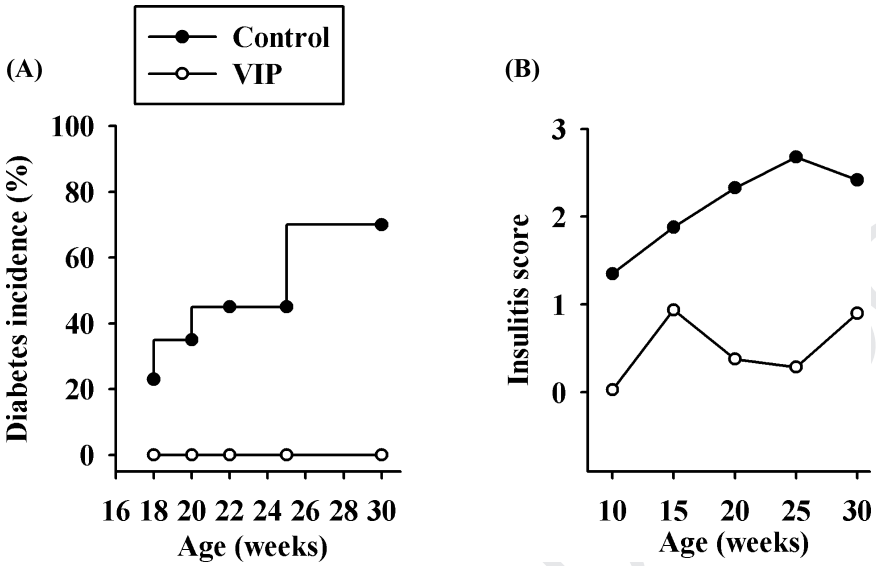

FIGURE 1. Protection against diabetes development and decreased insulitis in VIP-treated NOD mice. Each point represents the mean value ($n = 6$) (**A**) Cumulative incidence of diabetes in vehicle and VIP-treated NOD mice. (**B**) Histological scoring of insulitis was performed on pancreatic sections stained with hematoxilin/eosin. Islets were scored for absence of insulitis (grade 0), peri-insulitis (grade 1), moderate insulitis (grade 2), and severe insulitis (grade 3).

treatment had no significant effects on the circulating levels of IL-4, but reduced the IL-12 serum levels and greatly increased the IL-10 levels. Since a breakdown of immunoregulatory balance in the pancreas has been implicated in the development of diabetes in NOD mice, we paid attention to the Th1/Th2 deviation in the pancreas at 15 wk of age, a time point close to the onset of diabetes, as well as to the presence of regulatory T cells that have been involved in the control of autoimmunity. Markers for the lineage commitment to Th1 and Th2 are the transcription factors T-bet and GATA3, respectively,[8] while the expression of FoxP3 and transforming growth factor (TGF)-β is associated with the commitment to regulatory T cells. The quantitative determination of these markers by reverse-transciption polymerase chain reaction (RT-PCR) in mRNA extracted from the pancreas showed that the transition from the prediabetic stage to diabetes onset was marked by an increase in the Th1 commitment marker T-bet, while markers for Th2 and T-regulatory subsets remained low. VIP treatment significantly reduced the expression of T-bet and increased the expression of the Th2 commitment marker GATA3. Furthermore, the markers for T-regulatory function, FoxP3, and TGF-β, were significantly upregulated in the pancreas of VIP-treated mice.

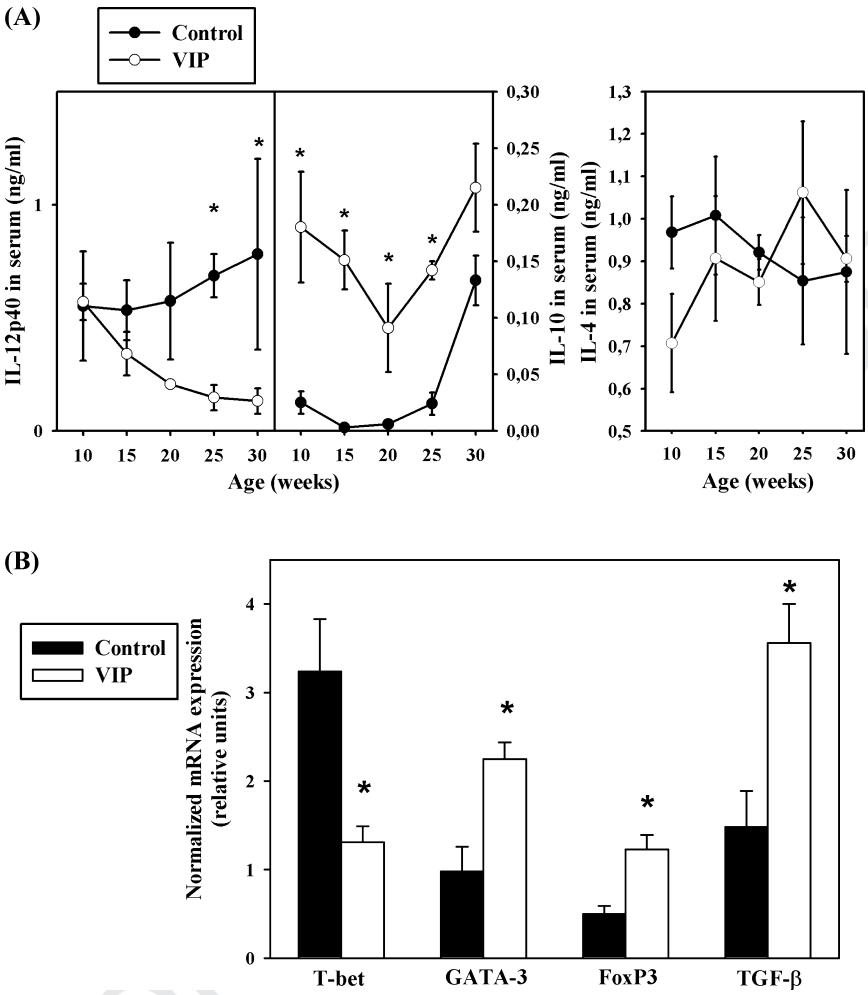

FIGURE 2. (**A**) Circulating cytokine levels. Circulating levels of IL-12, IL-10, and IL-4 were obtained by sandwich ELISA on serum samples. $^{*}P < 0.05$. (**B**) mRNA expression of T-bet, GATA-3, FoxP3, and TGF-β in the pancreas of vehicle and VIP-treated mice at 15 wk of age. Expression of the corresponding mRNA was measured by quantitative RT-PCR and corrected by mRNA expression for β-actin in each sample. $^{*}P < 0.05$.

DISCUSSION

This study shows that treatment of NOD mice with VIP inhibits diabetes development. The data indicate that the protective mechanism may be mediated by the induction of Th2-like immune response and the differentiation of

regulatory T cells counteracting the Th1-dependent cellular immune response in the pancreas.

Central and peripheral tolerance defects are responsible for diabetes in NOD mice. Th1 effectors for autoantigens expressed in the pancreatic islets are associated with disease progression. During its development, the dominance of Th2 cytokines appears at the early stage of nondestructive insulitis that is followed by a predominance of Th1 cytokines during destructive insulitis.[7] Conversely, it appears that deviation to Th2 responses helps protection against diabetes. Elevation of circulating levels of IL-4 and IL-10 by injection or gene therapy has been shown to prevent or ameliorate autoimmune diabetes in NOD mice in a dose-dependent manner, inducing changes in the proportion of T cell subsets in the periphery and the islet lesions, without reduction of islet infiltration.[9] The protective effect of VIP in diabetes development may be due partially to its effect in the Th1/Th2 balance. A shift in this balance was evident in the pancreas after VIP treatment that may prevent islet destruction in spite of a patent infiltration, which nevertheless does not reach the core of the islet. VIP shift toward a Th2-like response locally in the pancreas is indicated by the upregulation of GATA3 and the downregulation of T-bet. In the periphery, however, although VIP decreases IL-12 and increases IL-10 circulating levels, there is no effect in IL-4 levels in serum. However, some authors have questioned the role of the Th1/Th2 balance as a determinant of susceptibility to develop diabetes based on the fact that both IL-12 and interferon (IFN)-γ knockout NOD mice develop diabetes.[10] Some experimental approaches indicate that cytokine shift is an outcome rather than a cause of diabetes protection and may be attributed to the higher resistance of activated Th2 cells to apoptosis in NOD mice.[11] Instead of that, they point to multiple immunoregulatory cell defects.[12] Prominent among the cellular component of this regulatory network are $CD4^+CD25^+$ cells in which expression of the transcription factor FoxP3 is necessary for the development and function of the different subsets of regulatory T cells.[13] Although the mode of action of these cells is not well understood, they express a number of cell surface markers as well as a particular cytokine secretion pattern.[14] In line with these observations, maximal contribution of VIP to prevent diabetes development seems to be mediated by the generation of regulatory T cells. The upregulation of FoxP3 and TGF-β indicate a potentiation of regulatory functions in the pancreas that may lead to tolerance restoration to pancreatic autoantigens. Given the action of VIP in the development of regulatory T cells reported here and the efficacy of these cells to control autoimmune process, this peptide arises as a promising candidate for an effective treatment of type 1 diabetes. Further studies are needed to identify these cells in the islet infiltrates or the pancreatic lymph node and their efficacy to suppress the action of effector diabetogenic cells in transfer experiments.

ACKNOWLEDGMENTS

This work was supported by grants BFI 2002-03489 from Ministerio de Ciencia y Tecnología (Spain) and a postdoctoral fellowship from Fundacion Antorcha (Argentina).

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Queries

Q1 Author: Please spell out VIP.

Q2 Author: Please spell out NOD.