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Neuroimmune Aspects of Sjögren's Syndrome: Role of VIP/VPAC System in Immune and Salivary Gland Epithelial Cell Function

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Abstract: Sjögren's syndrome (SS) is a chronic inflammatory disease characterized by salivary and lacrimal gland dysfunction although extraglandular manifestations are also found. Suitable study models and *in vitro* cell culture designs are used to approach SS pathogenic mechanisms. Cellular and molecular pathways involved in gland homeostasis loss and the autoimmune response are focused in the search of novel drug targets and biomarkers. Vasoactive intestinal peptide (VIP) has trophic, pro-secretory and immunomodulatory effects in several chronic and autoimmune disease models. Here we review evidence pointing to its role as an endogenous modulator of gland homeostasis at early stages of the disease. Particularly, mechanisms involving VIP/VPAC system in the course of salivary function impairment in the non obese diabetic (NOD) mouse model of Sjögren's syndrome are described.

Keywords: Vasoactive intestinal peptide – Sjögren Syndrome – Salivary epithelial cells.

INTRODUCTION

Sjögren's syndrome is a common chronic inflammatory disorder with high economic impact in healthcare. It affects 0.5-1% of adult population being the second most prevalent rheumatic disease after rheumatoid arthritis. The disease affects mostly women in a 9:1 relationship, who are generally mid-aged but women at all ages are diagnosed. SS presents as a primary disease (pSS) or associated to other rheumatic diseases (sSS).

The disease hallmark is the loss of salivary (*xerostomia*) and lacrimal (*keratoconjuntivitis sicca*) gland secretion although multiple organs and systems can be compromised depending on genetic background, sex steroids and environmental triggers [1-5]. Although less frequently, chronic cough can also present associated with high and low respiratory epithelial tract lesions [6]. Symptoms of chronic fatigue raise special attention because they are present in about half of the patients with severe consequences for their work and daily activities [7]. However, the most serious manifestation associated with SS is a Non-Hodgkin B lymphoma of low or intermediate grade and mucosa-associated lymphoid tissue origin that develops in about 5 % of SS patients [7].

Consensus criteria for SS diagnosis are currently updated and treatment mostly relies on local agents to prevent mucosal excessive damage and antimalarials, glucocorticoids and immunosuppressive drugs whereas biologic agents are under study [7-10]. In this regard, some monoclonal antibodies showed acceptable profiles for extraglandular manifestation treatment, with less effect on exocrine dysfunction [10, 11].

Sjögren's syndrome etiopathogenic mechanisms are still unclear; however, evidence on biological pathways involved in exocrine gland dysfunction and the autoimmune response in various disease models of SS have provided relevant information [12, 13]. In fact, the complexity of SS pathogenesis challenges scientists to develop innovative systems analysis [14] as well as to find more specific biomarkers of clinical outcome. Results from animal models and human immunohistochemical studies indicate that the moderate grade of immune infiltrates in the glands does not correlate with the severity of xerostomia. Moreover, it was proposed that a loss of salivary gland homeostasis with a pro-inflammatory role of the epithelium would increase the susceptibility of the gland to an autoimmune response which, in turn, can further impair salivary function [5, 7, 12-17]. Consistently, early functional defects of gland epithelium, namely inappropriate signaling pathways, increased acinar apoptosis and aberrant activation of epithelial cells were described in spontaneous animal models as the *non obese diabetic* (NOD) mouse.

TISSUE HOMEOSTASIS MAINTENANCE

The breakdown of anatomic integrity and tissue homeostasis are among the first events linked to the loss of immune tolerance and, depending on environmental conditions, to autoantigen presentation and the onset of autoimmune responses [18]. Tissue homeostasis maintenance requires a highly controlled cellular response upon stress stimuli that involves key processes such as cell arrest, DNA repair, replicative senescence and apoptosis when damage overwhelms cell repair capacity. Various proteins are induced by stress: among them, the two isoforms of TP53INP1 α and β were described as targets of p53. WhenTP53INP1 is over expressed and cells are induced to apoptosis, it acts as the main mediator of the anti-oxidant function of p53 [19, 20]. These proteins have a short half-life and, along with other stress proteins, they are induced in murine acinar cells upon inflammation [21]. On the other hand, altered subcellular location and impaired function of proteins that participate in the control of secretion like aquaporin-5, muscarinic acetylcholine receptors, type IV collagen and e-cadherin have been associated with the pathogenesis of SS [1]. In line with this, the presence of anti-M3 and anti-M1 muscarinic acetylcholine receptor autoantibodies in the serum of pSS patients has been reported [22, 23] and assessed in other SS patient cohorts and murine models of the disease [24-27]. Likewise, muscarinic receptor variants are currently focused for their potential as SS biomarkers [12, 28].

Macrophages have a central role in tissue homeostasis maintenance. They have been classified according to their classical or alternative activation profiles [29-31]. However, their functional multiplicity is better represented by a spectrum of functional profiles with three mainly defined phenotypes: the inflammatory phenotype that has the characteristics of the classically activated M1

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phenotype; the wound healing phenotype which derives L-arginine to ornithine and polyamine synthesis; and the regulatory phenotype involved in apoptotic cell clearance with suppressant mediators' release [32, 33]. Many stimuli as immunocomplexes, glucocorticoids and vasoactive intestinal peptide (VIP) induce regulatory macrophage phenotype, characterized by the release of high levels of IL-10, TGF-β y PGE2, low levels of IL-12 and high expression of co-stimulatory molecules CD-80/CD-86 [32, 34-36]. Besides, macrophages express serotonin, steroid hormone, VIP and acetylcholine receptors, among other neuroendocrine receptors, that can suppress the expression of pro inflammatory genes through inhibition of the NF-κB pathway [34, 37-39].

VIP IMMUNOMODULATORY AND TROPHIC EFFECTS

VIP is a 28-amino acid peptide structurally related to secretin, pituitary adenylate cyclase activating polypeptide (PACAP), glucagon and growth hormone-releasing factor. It binds to class B members of the G-protein coupled receptor super-family. Two subtypes of VIP receptors named VPAC1 and VPAC2 were described on the basis of sequence, affinity, expression and signaling profiles [40]. They recognize VIP and PACAP with similar affinity whereas other members of class B G-protein coupled receptors like PAC1 bind VIP with lower affinity [41]. Both VPACs are coupled to Gs/AMPc/PKA signaling, they also signal through PLC and MAPK [42, 43]. VIP, first described as a neurotransmitter by Sami Said & Viktor Mutt in 1970, has trophic and potent immunomodulatory effects through its action on VPACs on adult and embryonic tissues. VIP has neurotrophic effects [44] and elicits trophic, prosecretory and vasodilator effects on exocrine gland cells [45-47].

VPACs expressed on immune cells promote anti-inflammatory and tolerogenic responses in human [48] and murine cells [49] and viral disease models [50]. Particularly, VIP reversed salivary gland hypofunction and reduced Th1 cytokines in the NOD model of SS [51] and reduced chronic inflammation in several disease models [52-57]. Due to its low bioavailability, dendritic cells transduced with lentiviral vectors expressing VIP were assayed to locally deliver the peptide in inflammation models [58]. Likewise, VPACs are expressed in synovial cells from arthritis and osteoarthritis patients and *in vitro* VIP treatment modified their inflammatory profile [59-61].

VIP induced regulatory macrophage phenotype [32] with increased IL-10 synthesis and reduced IL-12, TNF-α and inducible nitric oxide (NO) synthase (NOS) activity in human and murine macrophages through VPAC receptors [38, 43]. Dendritic cells are also targeted by VIP to differentiate into a tolerogenic profile that produce high levels of IL-10 and induce antigen specific regulatory T cells [62, 63]. VIP was shown to induce Foxp3+ regulatory T cells [64] and, in the presence of TGF-β, VIP can promote murine CD4+T cell differentiation to a distinct Th17 cell phenotype that generates IL-17 but not IL-6 or IL-21 [56]. It has emerged as a physiological inhibitor of the master regulator of immune responses calcineurin–NFAT pathway [65, 66]. VIP is not synthesized by human or murine macrophages and peptide expression was found in CD4+ T cells after antigen stimulation [67-70]. VPAC1/VPAC2 relative expression on immune cells was proposed as a potential modulatory mechanism of neuropeptide effects [43, 48]. VIP is currently studied as a candidate drug in pulmonary hypertension (https://www.clinicaltrialsregister.eu/ctr-search/search?query= eudract_number:2007-003621-24). Pharmacochemical and biopharmaceutical strategies are also under study to produce VPAC1 and VPAC2 selective agonists and to circumvent its extremely low bioavailability. Based on cumulative evidence on VIP as a prosecretory and anti-inflammatory peptide, its role and potential in Sjögren syndrome has been explored [51, 57, 71-74]. Both the NOD mouse model at the prediabetic stage and genetically modified B6/NOD backcrosses provided valuable information on SS pathogenesis [75-78]. Results from our laboratory working in the NOD strain that will be next summarized point to the role of VIP in gland hypofunction and they also support the concept that the VIP/VPAC system may serve as a marker of gland homeostasis loss and pathology.

Polyamine synthesis by salivary epithelial cells and trophic effects of VIP on rat salivary glands were first described by Ekstrom, Månsson and coworkers [45, 47]. This observation gave support to the hypothesis that endogenous peptidic neurotransmitters could act as long term trophic factors. The concept applied to salivary gland parenchyma is the base of a recently developed device to induce reflex salivary flow in SS patients [79, 80]. Particularly, Konttinen and coworkers described defective innervation of SS patients' salivary glands by VIP-containing nerve fibers compared with normal volunteers and suggested that its depletion could contribute to acinar atrophy [81]. Consistent with this observation, it was recently demonstrated that VIP stimulates neuritogenesis in a neuroblastoma cell line through cAMP/ERK y p38MAPK pathways with increased expression of anti-apoptotic Bcl-2 [82]. This step is required in neuron development and during neuronal regeneration after injury. Interestingly, VIP stimulates neural differentiation of mouse embryo and increases E9 embryo growth [44, 83, 84]. Moreover, the expression of VIP in maternal tissues peaks early during gestation whereas it was detected in peripheral nervous system of embryo at E14,5 [85]. Finally, we have shown a trophic effect of VIP on human trophoblast cells (line Swan 71) which synthesize the polypeptide and express VPACs suggesting possible autocrine and paracrine VIP modulation during pregnancy [86].

VIP/VPAC SYSTEM IN THE NOD MOUSE MODEL OF SJÖGREN SYNDROME

As referred to above, of particular interest is the study of VIP in SS based on its secretory, vasodilator, immune and trophic effects on exocrine gland cells. On the hypothesis of an early functional defect of the glands as a compromising factor to increase gland susceptibility to an autoimmune response, with a role of the local VIP/VPAC system, we analyzed several parameters of gland function and homeostasis in salivary glands of NOD mice at different ages. Results indicate functional impairment of the neural isoform of nitric oxide (NO) synthase expressed in the glands of NOD females of 12 weeks of age, before the appearance of immune infiltrates, with a reduced or altered activity of VIP/NOS/CaMK II-mediated signaling pathways and amylase release [71-73, 87]. On the other hand, a loss of acinar cells through apoptosis mechanisms was described previously by other groups in both NOD and NOD-*scid* mice at 20 weeks of age suggesting a non-immune origin of acinar loss through apoptosis [88, 89]. Consistently, we observed an increased duct-to-acinar cell ratio in submandibular glands of NOD mice at 16 weeks of age and histological images consistent with acinar cell apoptosis appearing at 10 weeks of age in NOD glands [73]. Both signals –reduced number of acinar cells and picnotic nuclei- were concomitant with an increased expression of the stress proteins TP53INP1 in isolated acinar preparations [74]. Together with stress proteins, acinar cells from NOD but not normal mice also exhibited an increased expression of TNF-αR1; and TNF-α was able to further induce TP53INP1 in acinar cells [90]. Acini isolated from normal and NOD mouse submandibular glands treated *in vitro* with VIP showed increased cAMP levels and lower apoptosis levels through functional VPAC receptors coupled to cAMP/PKA mediated pathways [90]. VIP also reduced TNF-α-induced expression of TP53INP1. Moreover, the effect of VIP required the inhibitory phosphorylation of Bad through cAMP and implicated NFkB activation favoring survival/death signals in isolated acinar cells from NOD mice [91]. Figure **1** schematizes these results.

To evaluate the effect of VIP on the immune response, NOD mice were treated every other day with the neuropeptide from week 4 of age. A switch to an immunosuppressant profile was observed

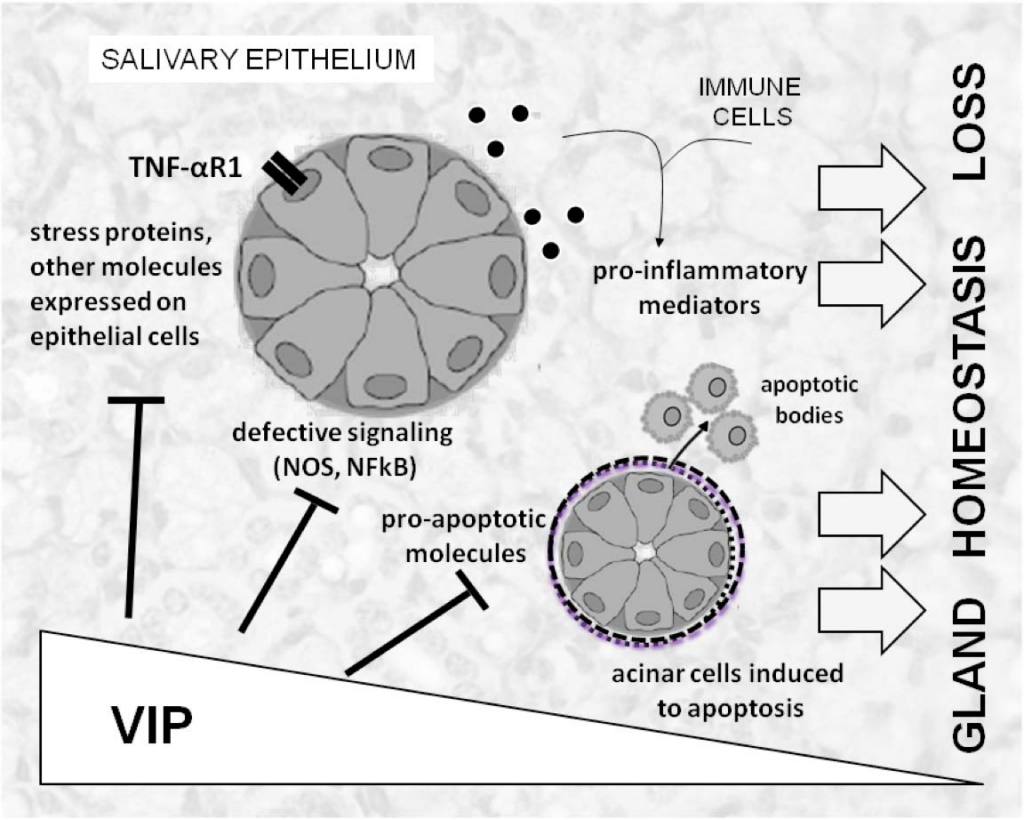

Fig. (1). *Participation of VIP/VPAC system in gland homeostasis loss in the NOD mouse model of SS.* Based on results described in the text, it is proposed that VIP released in the glands inhibits acinar cell apoptosis, aberrant TNF-α receptor 1 and stress protein expression, altered signaling through NFkB and NOS, and the production of inflammatory mediators. The scheme gathers information published on the NOD model that was quoted in the text. NOS: nitric oxide synthase; TNF-αR1: receptor 1 for the tumor necrosis factor α; NFkB: nuclear factor kB.

both in plasma levels of certain cytokines as well as in the expression of immune tolerance markers TGF-β and Foxp3 [57]. A VIP codifying adenoviral construct injected in NOD mice glands prevented SS-like salivary dysfunction and reduced IL-2 levels [51]. In addition, VIP reduced pro-inflammatory cytokines and induced a predominant regulatory profile with IL-10 and PGE2 synthesis in peritoneal macrophages from NOD mice [92]. NOD macrophage inflammatory profile was also modulated to a regulatory phenotype at early gestation and VIP further contributed to NO reduced levels in pregnant NOD mice [93]. Finally, T helper cell phenotypes and Th1/Treg cell ratios were modulated after VIP treatment of NOD mice providing further support to its potential for therapeutic approaches in SS [94, 95].

VIP/VPAC SYSTEM IN THE INTERACTION OF EPITHELIAL CELLS WITH MACROPHAGES

On the hypothesis that VIP released by nerve terminals in the glands can promote long term trophic effects, and that a deficit in this process could underlie acinar cell loss by apoptotic mechanisms, we studied VIP/VPAC system expression and signaling in salivary glands from NOD mice at different ages. A reduction of the neural isoform of nitric oxide synthase along with the progression of the autoimmune response was found [73]. Moreover, there was a reduction of VIP expression in NOD mice glands studied from week 4 to week 20 of age, with no changes in VPACs expression [91]. VIP/VPAC expression ratio in gland parenchyma diminished in parallel with an enhanced susceptibility to apoptosis of acinar cells in the NOD mouse model of SS [89, 90].

Based on these observations and provided the central homeostatic role of macrophages in their 'silent' phagocytosis of apoptotic cells, we analyzed phagocytosis of apoptotic acinar cells by peritoneal macrophages of NOD mice and the potential modulation through the VIP/VPAC system. Reduced levels of pro-inflammatory cytokines and enhanced IL-10 production, favored by VIP, was observed when apoptotic acinar cell were engulfed by NOD mice macrophages [91].

CONCLUDING REMARKS

Few markers fairly reflect pathological disruption of exocrine gland homeostasis early in the course of SS. Mechanisms involved in the high susceptibility of epithelial cells to apoptosis as well as mechanisms of macrophage functional plasticity upon interaction with epithelial salivary gland cells remain to be further explored. These might include the aberrant expression of stress proteins and other factors that could serve in the future as early biomarkers of gland damage. Supporting evidence on the immunomodulatory and trophic effects of VIP in the NOD mouse model may thus contribute to get more insight into SS pathogenesis, particularly its potential role in gland homeostasis maintenance/loss at early stages of the disease.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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REFERENCES

[1] Konttinen YT, Porola P, Konttinen L, Laine M, Poduval P. Immunohistopathology of Sjögren's syndrome. Autoimmunity Rev 2006; 6: 16-20.

[2] García-Carrasco M, Fuentes-Alexandro S, Escárcega RO, Salgado G, Riebeling C, Cervera R. Pathophysiology of Sjögren's syndrome. Arch Med Res 2006; 37: 921-32.

[3] Konttinen YT, Fuellen G, Bing Y, *et al.* Sex steroids in Sjögren's syndrome. J Autoimmun 2012; 39: 49-56.

[4] Meijer JM, Meiners PM, Vissink A, *et al.* Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial. Arthr Rheum 2010; 62: 960-8.

[5] Fox RI. The salivary gland epithelial cell in Sjogren's Syndrome: what are the steps involved in wounding or killing their secretory function? J Rheumatol 2012; 39: 1117-9.

[6] Kassan SS, Moutsopoulos HM. Clinical manifestations and early diagnosis of Sjögren syndrome. Arch Int Med 2004; 164: 1275-84.

[7] Mavragani CP, Moutsopoulos NM, Moutsopoulos HM. The management of Sjögren's syndrome. Nat Clin Pract Rheum 2006; 2: 252-61.

[8] Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002; 61: 554-8.

[9] Shiboski SC, Shiboski CH, Criswell LA, *et al.* American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. Arthr Care Res 2011;64(4):475-87.

[10] Ramos-Casals M B-ZP, Sisó-Almirall A, Bosch X, Tzioufas AG. Topical and systemic medications for the treatment of primary Sjögren's syndrome. Nat Rev Rheumatol 2012; 8: 399-411.

[11] Ng W-f, Bowman SJ. Biological therapies in primary Sögren' s syndrome Expert Opin Biol Ther 2011; 11: 921-36.

[12] Jonsson R, Vogelsang P, Volchenkov R, Espinosa A, Wahren-Herlenius M, Appel S. The complexity of Sjögren's syndrome: Novel aspects on pathogenesis. Immunol Lett 2011;141(1):1-9.

[13] Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjögren's syndrome. Nat Rev Rheumatol 2010; 6: 529-37.

[14] Larsen M, Yamada KM, Musselmann K. Systems analysis of salivary gland development and disease. Wiley Interdisciplinary Rev Systems Biol Med 2010; 2: 670-82.

[15] Manoussakis MN, Kapsogeorgou EK. The role of intrinsic epithelial activation in the pathogenesis of Sjögren's syndrome. J Autoimmun 2010; 35: 219-24.

[16] Mitsias DI, Kapsogeorgou EK, Moutsopoulos HM. Sjögren's syndrome: why autoimmune epithelitis? Oral Dis 2006; 12: 523-32.

[17] Fox RI, Stern M. Sjögren's syndrome: mechanisms of pathogenesis involve interaction of immune and neurosecretory systems. Scand J Rheumatol Supplement 2002; 116: 3-13.

[18] Thorburn A. Apoptosis and autophagy: regulatory connections between two supposedly different processes. Apoptosis 2008; 13: 1-9.

[19] Tomasini R, Samir AA, Carrier A, *et al*. TP53INP1s and homeodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. J Biol Chem 2003; 278: 37722-9.

[20] Cano CE, Gommeaux J, Pietri S, *et al*. Tumor protein 53-induced nuclear protein 1 is a major mediator of p53 antioxidant function. Cancer Res 2009; 69: 219-26.

[21] Tomasini R, Seux M, Nowak J, *et al.* TP53INP1 is a novel p73 target gene that induces cell cycle arrest and cell death by modulating p73 transcriptional activity. Oncogene 2005; 24: 8093-104.

[22] Bacman S, Perez Leiros C, Sterin-Borda L, Hubscher O, Arana R, Borda E. Autoantibodies against lacrimal gland M3 muscarinic acetylcholine receptors in patients with primary Sjögren's syndrome. Invest Ophtalmol Vis Sci 1998; 39: 151-6.

[23] Pérez Leirós C, Sterin-Borda L, Hubscher O, Arana R, Borda ES. Activation of nitric oxide signaling through muscarinic receptors in submandibular glands by primary Sjögren syndrome antibodies. Clin Immunol 1999; 90: 190-5.

[24] Park K, Haberberger RV, Gordon TP, Jackson MW. Antibodies interfering with the type 3 muscarinic receptor pathway inhibit gastrointestinal motility and cholinergic neurotransmission in Sjögren's syndrome. Arthr Rheum 2011; 63: 1426-34.

[25] Nguyen KH, Brayer J, Cha S, *et al.* Evidence for antimuscarinic acetylcholine receptor antibody-mediated secretory dysfunction in nod mice. Arthr Rheum 2000; 43: 2297-306.

[26] Nakamura Y, Wakamatsu E, Matsumoto I, *et al.* High prevalence of autoantibodies to muscarinic-3 acetylcholine receptor in patients with juvenile-onset Sjögren syndrome. Ann Rheum Dis 2008; 67: 136-7.

[27] Delaleu N, Immervoll H, Cornelius J, Jonsson R. Biomarker profiles in serum and saliva of experimental Sjögren's syndrome: associations with specific autoimmune manifestations. Arthr Res Ther 2008; 10: R22-R.

[28] Appel S, Le Hellard S, Bruland O, *et al.* Potential association of muscarinic receptor 3 gene variants with primary Sjogren's syndrome. Ann Rheum Dis 2011; 70: 1327-9.

[29] Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002; 23: 549-55.

[30] Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005; 5: 953-64.

[31] Kurosaka K, Takahashi M, Watanabe N, Kobayashi Y. Silent cleanup of very early apoptotic cells by macrophages. J Immunol 2003; 171: 4672-9.

[32] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8: 958-69.

[33] Brancato SK, Albina JE. Wound Macrophages as Key Regulators of Repair. AJPA 2011; 178: 19-25.

[34] Fairweather D, Cihakova D. Alternatively activated macrophages in infection and autoimmunity. J Autoimmun 2009; 33: 222-30.

[35] Biswas SK, Chittezhath M, Shalova IN, Lim JY. Macrophage polarization and plasticity in health and disease. Immunol Res 2012; 53: 11-24.

[36] Galli SJ BN, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Nat Immunol 2011; 12: 1035-44.

[37] Freire-Garabal M NMJ, Balboa J, López-Delgado P, *et al.* Serotonin upregulates the activity of phagocytosis through 5-HT1A receptors Br J Pharmacol 2003; 139: 457-63.

[38] Delgado M, Munoz-Elias EJ, Martinez C, Gomariz RP, Ganea D. VIP and PACAP38 modulate cytokine and nitric oxide production in peritoneal macrophages and macrophage cell lines. Ann N Y Acad Sci 1999; 897: 401-14.

[39] Su L, Sun Y, Ma F, Lü P, Huang H, Zhou J. Progesterone inhibits Toll-like receptor 4-mediated innate immune response in macrophages by suppressing NF-kappaB activation and enhancing SOCS1 expression. Immunol Lett 2009; 125: 151-5.

[40] Couvineau A, Laburthe M. VPAC receptors: structure, molecular pharmacology and interaction with accessory proteins. Br J Pharmacol 2011; 166: 42-50.

[41] Harmar AJ FJ, Gozes I, Laburthe M, *et al*. Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR Review 1. Br J Pharmacol 2012; 166:4-17

[42] Onoue S, Misaka S, Yamada S. Structure-activity relationship of vasoactive intestinal peptide (VIP): potent agonists and potential clinical applications. Naunyn-Schmiedeberg's Arch Pharmacol 2008; 377: 579-90.

[43] Leceta J, Gomariz RP, Martinez C, Carrión M, Arranz A, Juarranz Y. Vasoactive intestinal peptide regulates Th17 function in autoimmune inflammation. Neuroimmunomodulation 2007; 14: 134-8.

[44] Gressens P MS, Hill JM, Brenneman DE, Gozes I, Fridkin M, Evrard P. Vasoactive intestinal peptide prevents excitotoxic cell death in the murine developing brain. J Clin Invest 1997; 100: 390-7.

[45] Månsson B, Nilsson BO, Ekström J. Effects of repeated infusions of substance P and vasoactive intestinal peptide on the weights of salivary glands subjected to atrophying influences in rats. Br J Pharmacol 1990; 101: 853-8.

[46] Ekström J NB, Rosengren E. Substance P and vasoactive intestinal peptide influence polyamine metabolism in salivary glands of the rat. Acta Physiol Scand 1989; 136: 427-33.

[47] Ekström J MB, Tobin G. Vasoactive intestinal peptide evoked secretion of fluid and protein from rat salivary glands and the development of supersensitivity. Acta Physiol Scand 1983; 119: 169-75.

[48] Lara-Marquez M, O'Dorisio M, O'Dorisio T, Shah M, Karacay B. Selective gene expression and activation-dependent regulation of vasoactive intestinal peptide receptor type 1 and type 2 in human T cells. J Immunol 2001; 166: 2522-30.

[49] Martinez C, Delgado M, Abad C, Gomariz RP, Ganea D, Leceta J. Regulation of VIP production and secretion by murine lymphocytes. J Neuroimmunol 1999; 93: 126-38.

[50] Li JM SL, Hossain MS, Giver CR, *et al*. Absence of vasoactive intestinal peptide expression in hematopoietic cells enhances Th1 polarization and antiviral immunity in mice. J Immunol 2011; 187: 1057-65.

[51] Lodde BM, Mineshiba F, Wang J, *et al*. Effect of human vasoactive intestinal peptide gene transfer in a murine model of Sjogren's syndrome. Ann Rheum Dis 2006; 65: 195-200.

[52] Delgado M, Toscano MG, Benabdellah K, *et al*. *In vivo* delivery of lentiviral vectors expressing vasoactive intestinal peptide complementary DNA as gene therapy for collagen-induced arthritis. Arthr Rheum 2008; 58: 1026-37.

[53] Gonzalez-Rey E, Delgado M. Vasoactive intestinal peptide and regulatory T-cell induction: a new mechanism and therapeutic potential for immune homeostasis. Trends Mol Med 2007; 13: 241-51.

[54] Gomariz RP, Arranz A, Juarranz Y, *et al*. Regulation of TLR expression, a new perspective for the role of VIP in immunity. Peptides 2007; 28: 1825-32.

[55] Delgado M AC, Martinez C, Leceta J, Gomariz RP. Vasoactive intestinal peptide prevents experimental arthritis by downregulating both autoimmune and inflammatory components of the disease. Nat Med 2001; 7: 563-8.

[56] Yadav M, Goetzl EJ. Vasoactive intestinal peptide-mediated Th17 differentiation: an expanding spectrum of vasoactive intestinal peptide effects in immunity and autoimmunity. Ann N Y Acad Sci 2008; 1144: 83-9.

[57] Rosignoli F TM, Juarranz Y, García-Gómez M, Martinez C, Gomariz RP, Pérez-Leirós C, Leceta J. VIP and Tolerance Induction in Autoimmunity. Ann N Y Acad Sci 2006; 1070: 525-30.

[58] Toscano MG DM, Kong W, Martin F, Skarica M, Ganea D. Dendritic cells transduced with lentiviral vectors expressing VIP differentiate into VIP-secreting tolerogenic-like DCs. Mol Ther 2010; 182:1035-45.

[59] Juarranz MG SB, Torroba M, Gutierrez-Cañas I, *et al*. Vasoactive intestinal peptide modulates proinflammatory mediator synthesis in osteoarthritic and rheumatoid synovial cells. Rheumatology (Oxford) 2004; 43: 416-22

[60] Arranz A, Abad C, Juarranz Y, Leceta J, Martinez C, Gomariz RP. Vasoactive intestinal peptide as a healing mediator in Crohn's disease. Neuroimmunomodulation 2008; 15: 46-53.

[61] Juarranz Y, Gutiérrez-Cañas I, Santiago B, Carrión M, Pablos JL, Gomariz RP. Differential expression of vasoactive intestinal peptide and its functional receptors in human osteoarthritic and rheumatoid synovial fibroblasts. Arthr Rheum 2008; 58: 1086-95.

[62] Delgado M G-RE, Ganea D. The neuropeptide vasoactive intestinal peptide generates tolerogenic dendritic cells. J Immunol 2005; 175: 7311-24.

[63] Chorny A G-RE, Fernandez-Martin A, Pozo D, Ganea D, Delgado M. Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. Proc Natl Acad Sci U S A 2005; 102: 13562-7.

[64] Pozo D G-RE, Chorny A, Anderson P, Varela N, Delgado M. Tuning immune tolerance with vasoactive intestinal peptide: a new therapeutic approach for immune disorders. Peptides 2007; 28: 1833-46.

[65] Said SI HS, Dickman KG, Szema AM, *et al*. Moderate pulmonary arterial hypertension in male mice lacking the vasoactive intestinal peptide gene. Circulation 2007; 115: 1260-8.

[66] Said SI HS, Gonzalez Bosc L. Asthma and pulmonary arterial hypertension: do they share a key mechanism of pathogenesis? Eur Respir J 2010; 35: 730-4.

[67] Voice JK GC, Kong Y, Bangale Y, Paul S, Goetzl EJ. Roles of vasoactive intestinal peptide (VIP) in the expression of different immune phenotypes by wild-type mice and T cell-targeted type II VIP receptor transgenic mice. J Immunol 2003; 170: 308-14.

[68] Delgado M PD, Martinez C, Garrido E, Leceta J, Calvo JR, Gomariz RP. Characterization of gene expression of VIP and VIP1-receptor in rat peritoneal lymphocytes and macrophages. Regul Pept 1996; 62: 161-6.

[69] Leceta J MC, Delgado M, Garrido E, Gomariz RP. Expression of vasoactive intestinal peptide in lymphocytes: a possible endogenous role in the regulation of the immune system. Adv Neuroimmunol 1996; 6: 29-36.

[70] Vassiliou E JX, Delgado M, Ganea D. TH2 lymphocytes secrete functional VIP upon antigen stimulation. Arch Physiol Biochem 2001; 109: 365-8.

[71] Rosignoli F, Goren NB, Pe C. Alterations in Nitric Oxide Synthase Activity and Expression in Submandibular Glands of NOD Mice. Clin Immunol 2001; 101: 86-93.

[72] Rosignoli F, Pe C. Nitric oxide synthase I and VIP-activated signaling are affected in salivary glands of NOD mice. J Neuroimmunol 2002; 130: 109-16.

[73] Rosignoli F, Roca V, Meiss R, Leceta J, Gomariz RP, Pérez Leirós C. Defective signalling in salivary glands precedes the autoimmune response in the non-obese diabetic mouse model of sialadenitis. Clin Exp Immunol 2005; 142: 411-8.

[74] Calafat M, Larocca L, Roca V, Pérez Leirós C. NOD mice exocrinopathy: towards a neuroimmune link. Neuroimmunomodulation 2007; 14: 175-81.

[75] Roescher N, Lodde B, Vosters J, *et al*. Temporal changes in salivary glands of non-obese diabetic mice as a model for Sjögren's syndrome. Oral Dis 2011; 18: 96-106.

[76] Jonsson MV, Delaleu N, Brokstad Ka, Berggreen E, Skarstein K. Impaired salivary gland function in NOD mice: association with changes in cytokine profile but not with histopathologic changes in the salivary gland. Arthr Rheum 2006; 54: 2300-5.

[77] Lavoie TN, Lee BH, Nguyen CQ. Current concepts: mouse models of Sjögren's syndrome. J Biomed Biotechnol 2011; 2011: 549107-.

[78] Nguyen CQ, Kim H, Cornelius JG, Peck AB. Development of Sjogren's syndrome in nonobese diabetic-derived autoimmune-prone C57BL/6.NOD-Aec1Aec2 mice is dependent on complement component-3. J Immunol 2007; 179: 2318-29.

[79] Strietzel FP, Lafaurie GI, Mendoza GRB, *et al*. Efficacy and safety of an intraoral electrostimulation device for xerostomia relief: a multicenter, randomized trial. Arthr Rheum 2011; 63: 180-90.

[80] Wolff A, Fox PC, Porter S, Konttinen YT. Established and novel approaches for the management of hyposalivation and xerostomia. Curr Pharm Des 2012; 18: 5515-21.

[81] Konttinen YT HM, Kemppinen P, Segerberg M, *et al*. Peptide-containing nerves in labial salivary glands in Sjögren's syndrome. Arthritis Rheum 1992; 35: 815-20.

[82] Monaghan TK, Mackenzie CJ, Plevin R, Lutz EM. PACAP-38 induces neuronal differentiation of human SH-SY5Y neuroblastoma cells via cAMP-mediated activation of ERK and p38 MAP kinases. J Neurochem 2008; 104: 74-88.

[83] Spong CY, Lee SJ, Cune SKMC, *et al*. Maternal Regulation of Embryonic Growth: The Role of Vasoactive Intestinal Peptide Endocrinology 1999; 140: 917-24.

[84] Lim Ma, Stack CM, Cuasay K, Stone MM, *et al*. Regardless of genotype, offspring of VIP-deficient female mice exhibit developmental delays and deficits in social behavior. Int J Dev Neurosci 2008; 26: 423-34.

[85] Tyrrell S LS. The appearance of NPY and VIP in sympathetic neuroblasts and subsequent alterations in their expression. . J Neurosci 1994; 14: 4529-47

[86] Fraccaroli L, Alfieri J, Larocca L, *et al*. VIP modulates the pro-inflammatory maternal response inducing tolerance to trophoblast cells. Br J Pharmacol 2009; 156: 116-26.

[87] Roca V RF, Calafat M, Perez Leiros C. Lack of nitric oxide-mediated regulation of amylase secretion stimulated by VIP in parotid glands of NOD mice. Int Immunopharmacol 2004; 4: 1837-44.

[88] Kong L RC, Peck AB, Vela-Roch N, *et al*. Inappropriate apoptosis of salivary and lacrimal gland epithelium of immunodeficient NOD-scid mice. Clin Exp Rheumatol 1998;16:675-81.

[89] Robinson CP YS, Alford CE, Cooper C, *et al*. Elevated levels of cysteine protease activity in saliva and salivary glands of the nono-

bese diabetic (NOD) mouse model for Sjögren syndrome. Proc Natl Acad Sci U S A 1997; 94: 5767-71.

[90] Calafat M, Larocca L, Roca V, *et al.* Vasoactive intestinal peptide inhibits TNF-alpha-induced apoptotic events in acinar cells from nonobese diabetic mice submandibular glands. Arthr Res Ther 2009; 11:R53-.

[91] Hauk V, Calafat M, Larocca L, *et al.* Vasoactive intestinal peptide/vasoactive intestinal peptide receptor relative expression in salivary glands as one endogenous modulator of acinar cell apoptosis in a murine model of Sjögren's syndrome. Clin Exp Immunol 2011; 166: 309-16.

[92] Larocca L, Calafat M, Roca V, Franchi AM, Pérez C. VIP limits LPS-induced nitric oxide production through IL-10 in NOD mice macrophages. Int Immunopharmacol 2007; 7: 1343-9.

[93] Larocca L, Hauk V, Calafat M, *et al.* Modulation of macrophage inflammatory profile in pregnant nonobese diabetic (NOD) mice. Mol Cell Endocrinol 2011; 333: 112-8.

[94] Jimeno R, Gomariz RP, Gutiérrez-Cañas I, Martínez C, Juarranz Y, Leceta J. New insights into the role of VIP on the ratio of T-cell subsets during the development of autoimmune diabetes. Immunol Cell Biol 2010; 88: 734-45.

[95] Jimeno R, Leceta J, Martínez C, *et al.* Effect of VIP on the balance between cytokines and master regulators of activated helper T cells. Immunol Cell Biol 2012; 90: 178-86.

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