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Do cytosine guanine dinucleotide (CpG) fragments induce vasoactive neuropeptide mediated fatigue-related autoimmune disorders?

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Summary Autoimmune dysfunction of certain vasoactive neuropeptides (e.g., vasoactive intestinal peptide, pituitary adenylate cyclase activating polypeptide) may be implicated in a range of disorders associated with fatigue-like states (chronic fatigue syndrome, Gulf War syndrome) and even sudden infant death syndrome (SIDS). The important roles of these vasoactive neuropeptides make them a vulnerable target for autoimmune dysfunction. They are known to be associated with heat shock proteins for intracellular functioning with which they may form immunostimulating complexes. Cytosine guanine dinucleotide (CpG) fragments are potentially immunogenic DNA fragments which serve as friend or foe recognition systems between bacterial (hypomethylated) and mammalian (methylated) DNA and are being assessed for suitability for use in human vaccines as adjuvants. Interactions between CpG fragments, heat shock proteins and vasoactive neuropeptides may be associated with fatigue-related autoimmune conditions.

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Do cytosine guanine dinucleotide (CpG) fragments induce vasoactive neuropeptide mediated fatigue-related autoimmune disorders?

Certain vasoactive neuropeptides (VNs), such as pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP)

are widely distributed in mammalian brain and other tissues and have immunoregulatory, neuroregulatory, neurotrophic, neuroprotective, neurotransmitter and hormonal functions with significant influence on carbohydrate and lipid metabolism [1,2]. They are regulators of gaseous (e.g., NO, CO) and noradrenergic and cholinergic transmission [3–5]. These VNs are critical for an extraordinary array of biological processes including promoting muscle mass and contractive force [6], and brain function particularly hippocampal and other major CNS processes [7–9]. PACAP and

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VIP act through only three G-protein coupled receptors (PAC1, VPAC1 and VPAC2) with limited redundancy between them [10].

PACAP and VIP effects are mediated through activation of adenylate cyclase, an essential step in cyclic AMP metabolism. Collocation and co-function of their receptors with gaseous, noradrenergic and cholinergic neurotransmitters suggests a plausible causal association with fatigue-related disorders.

These VNs are implicated in autoimmune disorders. They prevent or ameliorate pathology in experimental autoimmune uveoretinitis and experimental autoimmune encephalomyelitis [11,12] which raises the question of whether other autoimmune conditions may arise through dysfunction of these VNs. Molecular mimicry with bacterial or viral fragments has been postulated as one mechanism triggering autoimmune dysfunction against certain VNs possibly resulting in fatigue-related states [13,14].

Cytosine guanine dinucleotide fragments (CpG motifs) of DNA are postulated to be the active ingredients in bacterial DNA extracts able to induce immune responses [15,16] and display adjuvant effects [17] which may have applications in human vaccines [18]. These immune activating effects may occur through acquired bacterial or viral DNA, oligodeoxynucleotide (ODN) fragments or through self-derived DNA fragments [19]. However, repeated exposure to CpG ODN has also been shown to have adverse consequences including lymphoid follicle destruction and immunosuppression in mice [20].

Microbial pathogens containing CpG fragments are known to bind Toll-like receptors and/or stimulate microbe-specific T cells to express CD40 ligand, thereby licensing antigen presenting cells that bear both microbial and autoantigens to break tolerance and precipitate autoimmune disease [21,22]. A similar mechanism is proposed to induce autoreactive T cell responses to pyruvate dehydrogenase complex (PDC) in a murine model skewing CD4(+) T cell responses toward the Th1 phenotype [23]. In lupus-prone mice, abnormal innate responses through their pattern-recognition TLR9 receptors implies that response to infectious danger in these mice is inappropriate and may be linked to lupus pathogenesis [24,25]. Hence autoimmune and inflammatory processes are known to be induced through these mechanisms.

Heat shock proteins (hsps) may also be implicated in the recognition of bacterial or mammalian CpG DNA by acting as a ligand transfer molecule and/or play a central role in the signalling cascade induced by CpG DNA [26]. Moreover, innate and

adaptive immune mechanisms may act through a cross priming adjuvant mechanism to engage heat shock protein in autoreactive responses [27]. Hsps also activate Toll-like receptors in triggering innate immunity, perhaps through adjuvant-like signals [28,29]. Hsps thus have an established place in regulation of the immune response [30]. Hsps also bind with other antigenic peptides to form immunostimulatory complexes [31] and interestingly may take the role of antigenic presentation and processing in immunoprotected regions such as the central nervous system [32]. Indeed aberrant self hsp expression may lead to enhancement/modulation of autoimmune responses in the context of myelin basic protein and MHC class II type interactions [33].

Mammalian DNA normally has lower than predicted CpG dinucleotide fragments and these are also usually methylated [34]. These characteristics differ from bacterial and viral DNA which contains higher percentages of CpG fragments and these are more likely to be hypomethylated, providing a biological 'friend or foe' identification system.

Some autoimmune disorders are thought to derive from dysfunction of endogenous DNA through CpG hypomethylation and subsequent autoreactivity. Ancient DNA sequences mimicking bacterial and viral genomes containing higher proportions of CpG elements have become incorporated into mammalian DNA as human endogenous retrovirus (HERV). These genetic components have become methylated over time making them mostly benign components of mammalian DNA. However, these DNA components may undergo hypomethylation through a range of stimulating factors, making them able to regulate transcriptional activity and expression of the HERV family [35] with implications for a range of pathologies.

Spontaneous hypomethylation of susceptible endogenous CpG sequences, or exposure to bacterial CpG DNA and subsequent stimulation of cellular processes may mediate innate and acquired immune pathways including class switching from IgM to more pathogenic IgG immunoglobulin types [36]. IgM and IgG reactivity to key fragments of certain VNs or related hsps thus might theoretically occur. Such postulated mechanisms could establish perverse autoreactive loss of immunological tolerance and effectively create immunisation against these VNs. The known susceptibility of CpG fragments to hypomethylation from toxic causes such as biological poisons and radiation might predispose to the development of these and other pathologies [37,38]. These postulated mechanisms might also link fatigue-related VN autoimmune disorders to exposure to radiological, biological and chemical warfare agents.

References

- [1] Arimura A. Perspectives on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine and nervous systems. *Jpn J Physiol* 1998;48(5):301–31.
- [2] Ganea D, Delgado M. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) as modulators of both innate and adaptive immunity. *Crit Rev Oral Biol Med* 2002;13(3):229–37.
- [3] Delgado M. Inhibition of interferon (IFN) gamma-induced Jak-STAT1 activation in microglia by vasoactive intestinal peptide: inhibitory effect on CD40, IFN-induced protein-10, and inducible nitric oxide synthase expression. *J Biol Chem* 2003;278(30):27620–9.
- [4] Kinhult J, Uddman R, Cardell LO. The induction of carbon monoxide-mediated airway relaxation by PACAP 38 in isolated guinea pig airways. *Lung* 2001;179(1):1–8.
- [5] Watkins CC, Boehning D, Kaplin AI et al. Carbon monoxide mediates vasoactive intestinal polypeptide associated non-adrenergic noncholinergic neurotransmission. *Proc Natl Acad Sci USA* 2004;101(8):2631–5.
- [6] Hinkle RT, Donnelly E, Cody DB et al. Activation of the vasoactive intestinal peptide 2 receptor modulates normal and atrophying skeletal muscle mass and force. *J Appl Physiol* 2005;98(2):655–62.
- [7] Shintani N. Mice lacking PACAP: a mini-review focussing on brain function. *Yakugaku Zasshi* 2004;124(10):667–71.
- [8] Hannibal J. Pituitary adenylate cyclase-activating peptide in the rat central nervous system: an immunohistochemical and in situ hybridisation study. *J Comp Neurol* 2002;453(4):389–417.
- [9] Dohi K, Mizushima H, Nakajo S et al. Pituitary adenylate cyclase-activating polypeptide (PACAP) prevents hippocampal neurons from apoptosis by inhibiting JNK/SAPK and p38 signal transduction pathways. *Regul Pep* 2002;109(1–3):83–8.
- [10] Vaudry D, Gonzalez BJ, Basille M et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol Rev*(2):269–324.
- [11] Keino H, Kezuka T, Takeuchi M et al. Prevention of experimental autoimmune uveoretinitis by vasoactive intestinal peptide. *Arch Ophthalmol* 2004;122(8):1179–84.
- [12] Kato H, Ito A, Kawanokuchi J et al. Pituitary adenylate cyclase-activating polypeptide (PACAP) ameliorates experimental autoimmune encephalomyelitis by suppressing the functions of antigen presenting cells. *Mult Scler* 2004;10(6):651–9.
- [13] Staines DR. Is sudden infant death syndrome (SIDS) an autoimmune disorder of endogenous vasoactive neuropeptides? *Med Hypoth*(62):653–7.
- [14] Staines DR. Is chronic fatigue syndrome an autoimmune disorder of endogenous neuropeptides, exogenous infection and molecular mimicry. *Med Hypoth*(62):646–52.
- [15] Krieg AM, Yi AK, Matson S et al. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 1995;374(6522):546–9.
- [16] Krieg AM. CpG motifs: the active ingredient in bacterial extracts. *Nat Med* 2003;9(7):831–5.
- [17] Tsuchiya H, Matsuda T, Harashima H, Kamiya H. Cytokine induction by a bacterial DNA-specific modified base. *Biochem Biophys Res Commun* 2005;326(4):777–781.
- [18] Ada G, Ramshaw I. DNA vaccination. *Expert Opin Emerg Drugs* 2003;8(1):27–35.
- [19] Gursel I, Gursel M, Yamada H et al. Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. *J Immunol* 2003;171:1393–400.
- [20] Heikenwalder M, Polymenidou M, Junt T et al. Lymphoid follicle destruction and immunosuppression after repeated CpG oligodeoxynucleotide. *Nat Med* 2004;10(2):187–92.
- [21] Ebert S, Gerber J, Bader S et al. Dose-dependent activation of microglial cells by Toll-like receptor agonists alone and in combination. *J Neuroimmunol* 2005;159(1–2): 87–96.
- [22] Ichikawa HT, Williams LP, Segal BM. Activation of APCs through CD40 or Toll-like receptor overcomes tolerance and precipitates autoimmune disease. *J Immunol* 2002;169(5):2781–7.
- [23] Jones DE, Palmer JM, Burt AD et al. Bacterial motif DNA as an adjuvant for the breakdown of immune self-tolerance to pyruvate dehydrogenase complex. *Hepatology* 2002;36(3): 679–86.
- [24] Krieg AM. CpG DNA: a pathogenic factor in systemic lupus erythematosus. *J Clin Immunol* 1995;15(6):284–92.
- [25] Lenert P, Goeken A, Handweger BS, Asman RF. Innate immune responses in lupus-prone Palmerston North mice: differential responses to LPS and bacterial DNA/CpG oligonucleotides. *J Clin Immunol* 2003;23(3):202–13.
- [26] Bandholtz L, Guo Y, Palmberg C et al. Heat shock protein binds CpG oligonucleotides directly: implications for hsp90 as a missing link in CpG signalling and recognition. *Cell Mol Life Sci* 2003;60(2):422–9.
- [27] Kumaraguru U, Pack CD, Rouse BT. Toll-like receptor ligand links innate and adaptive immune responses by the production of heat-shock proteins. *J Leukoc Biol* 2003;73(5):574–83.
- [28] van Eden W, Koets A, van Kooten P, Prakken B, van der Zee R. Immunopotentiating heat shock proteins: negotiators between innate danger and control of autoimmunity. *Vaccine* 2003;21(9–10):897–901.
- [29] Millar DG, Garza KM, Odermatt B et al. Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. *Nat Med* 2003;9(12):1465–6.
- [30] Pockley AG. Heat shock proteins as regulators of the immune response. *Lancet* 2003;362(9382):469–76.
- [31] Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. *Annu Rev Immunol* 2002;20:395–425.
- [32] Oglesbee MJ, Pratt M, Carsillo T. Role for heat shock proteins in the immune response to measles virus infection. *Viral Immunol* 2002;15(3):399–416.
- [33] Mycko MP, Cwiklinska H, Szymanski J et al. Inducible heat shock protein promotes myelin autoantigen presentation by the HLA class II. *J Immunol* 2004;172(1):202–13.
- [34] Shiota K. DNA methylation profiles of CpG islands for cellular differentiation and development in mammals. *Cytogenet Genome Res* 2004;105(2–4):325–34.
- [35] Lavie L, Kitova M, Maldener E et al. CpG methylation directly regulates transcriptional activity of the human endogenous retrovirus family HERV-K (HML-2). *J Virol* 2005;79(2):876–83.
- [36] He B, Qiao X, Cerutti A. CpG DNA induces IgG class switch DNA recombination by activating human B cells through an innate pathway that requires TLR9 and cooperates with IL-10. *J Immunol* 2004;173(7):4479–91.
- [37] Chen H, Li S, Liu J et al. Chronic inorganic arsenic exposure induces hepatic global and individual gene hypomethylation: implications for arsenic hepatocarcinogenesis. *Carcinogenesis* 2004;25(9):1779–86.

- [38] Pogribny I, Raiche J, Slovack M, Kovalchuk O. Dose-dependence, sex- and tissues-specificity, and persistence of radiation-induced genomic DNA methylation changes. *Biochem Biophys Res Commun* 2004;320(4):1253–61.

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