

affective rather than sensory experience. It is as yet difficult to explain the pathophysiology of the observed effects because acupuncture studies in this field are still rare. Some data indicate that acupuncture might influence itch-associated mediator effects¹⁰: in the central nervous system opioids and serotonin, on the spinal level prostaglandin E₂, and in the periphery substance P, calcitonin gene-related peptide, vasoactive intestinal peptide, neuropeptide Y, TNF- α , IgE, IL-1, IL-6, IL-8, and IL-10.

Further studies are needed to specifically evaluate a possible role of these mediators in acupuncture prophylaxis or treatment of itch and to evaluate the clinical effect in histamine-mediated diseases. We conclude that prophylactic acupuncture at point Quchi can significantly reduce histamine-induced itch and wheal formation compared with placebo-point acupuncture and no intervention, showing the relevance of point specificity for both procedure and underlying condition.

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Conjugation of 3' hexameric deoxyribo-guanosine run to phosphodiester CpG oligodeoxynucleotides can inhibit allergen-specific IgE synthesis with less risk of splenomegaly

To the Editor:

CpG oligodeoxynucleotides (ODNs) have the CpG motif, which displays a 5'-purine-purine-unmethylated cytosine-guanine-pyrimidine-pyrimidine-3' base sequence and enhances T_H1 response and regulatory immune response through a pattern recognition receptor, Toll-like receptor 9.¹ CpG-ODNs are expected to be a new therapeutic modality for allergic diseases, cancer, and infectious diseases.¹ CpG-ODNs have 2 different kinds of backbone structure.^{2,3} One is the natural phosphodiester backbone (PE), which is very susceptible to exonuclease. PE CpG-ODNs have very low immunostimulatory effect *in vivo* because of their susceptibility to exonuclease. The other is the synthetic phosphorothioate backbone (PS) structure, which is resistant to exonuclease and has a high immunostimulatory effect *in vivo*. We reported that the conjugation of a hexameric deoxyriboguanosine run (dG₆ run) to CpG-ODNs enhanced the protective effect against allergic asthma in mice. The protective effect of 3' dG₆ run-conjugated PE CpG-ODNs was almost equivalent to that of PS CpG-ODNs.²

Possible side effects of CpG-ODNs were suggested to be as follows: CpG-ODNs might cause local inflammation (eg, arthritis after intra-articular injection), can promote autoimmune disease as an adjuvant, and can trigger systemic inflammatory response syndrome on endotoxin exposure or after TNF- α exposure.⁴ PS CpG-ODNs induce massive splenomegaly in mice⁵ and other toxicities related to excessive immune stimulation, including the death of the immunized hosts, when used in large amounts.²

We evaluated the effect of 3' dG₆ run conjugation to PS or PE CpG-ODNs on splenomegaly in mice. Ten BALB/c mice for each group were sensitized with 20 μ g of ovalbumin and 2 mg of alum by means of intraperitoneal injection on days 1 and 14. The mice were challenged with 1% ovalbumin on days 21, 22, and 23. CpG-ODNs or PBS was injected at the time of sensitization with the allergen. CpG-ODNs used were as follows: PS-CpG (tccatgacgttctctgacgtt), PE-CpG (TCCATGACGTTCTCTGACGTT), PS-CpG-dG₆ (tccatgacgttctctgacgttggggg), PE-CpG-dG₆ (TCCATGACGTTCTCTGACGTTGGGGG), and PE*-CpG-dG₆ (ie, PE-CpG-dG₆ with 2 phosphorothioate backbone at the 5' terminus [tccATGACGTTCTCTGACGTTGGGGG]). On day 25, body weight and weight of the spleen, which was cautiously separated without bleeding, were measured. Serum ovalbumin-specific antibodies and cytokine production from the spleen were evaluated as

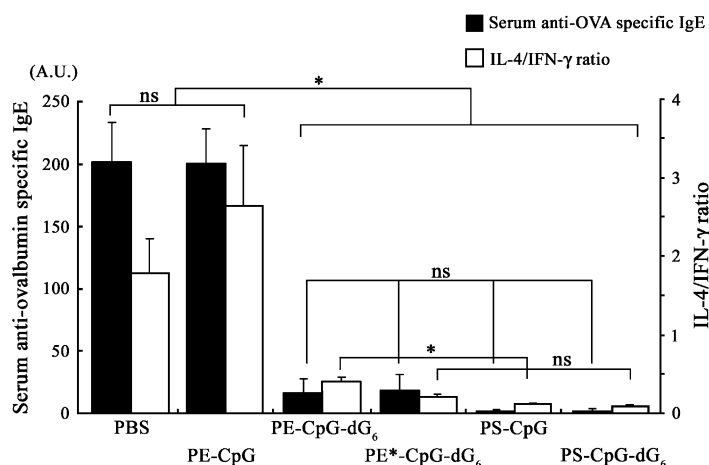


FIG 1. Serum allergen-specific IgE synthesis and IL-4/IFN- γ ratio from spleens were almost equally inhibited in the groups treated with PS CpG-ODNs (PS-CpG and PS-CpG-dG₆) and in the groups treated with 3' dG₆ run-conjugated PE CpG-ODN (PE-CpG-dG₆ and PE*-CpG-dG₆). Treatment with PE CpG-ODNs without 3' dG₆ run (PE-CpG) failed to inhibit IgE synthesis. * $P < .05$. OVA, Ovalbumin; ns, no significant difference.

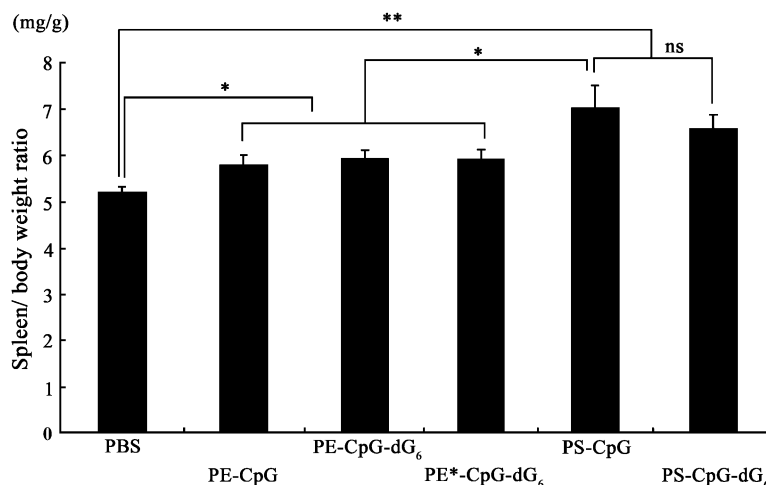


FIG 2. The ratio of spleen/body weight was higher in the group treated with PS CpG-ODNs without 3' dG₆ run (PS-CpG) than in the groups treated with PE CpG-ODNs (PE-CpG, PE-CpG-dG₆, and PE*-CpG-dG₆) or in the control (PBS) group ($P < .05$). * $P < .05$, ** $P < .005$. ns, No significant difference.

previously described.² Statistical analysis was performed by using the Kruskal-Wallis and Mann-Whitney tests. Serum allergen-specific IgE synthesis and IL-4/IFN- γ ratio from the spleen were almost equally inhibited in the groups treated with PS CpG-ODNs (PS-CpG and PS-CpG-dG₆) and the groups treated with 3' dG₆ run-conjugated PE CpG-ODN (PE-CpG-dG₆ and PE*-CpG-dG₆) compared with those of the PBS-treated control group ($P < .05$). Those treated with PE CpG-ODNs without 3' dG₆ run (PE-CpG) showed similar serum allergen-specific IgE levels as the PBS-treated control group ($P > .05$, Fig 1). The ratio of spleen/body weight was higher in the group treated with PS CpG-ODNs without 3' dG₆ run (PS-CpG: 7.01 ± 0.49) than the groups treated with PE CpG-ODNs (PE-CpG: 5.82 ± 0.17 , PE-CpG-dG₆: 5.91 ± 0.20 ,

PE*-CpG-dG₆: 5.88 ± 0.27) or in the PBS-treated control group (5.19 ± 0.13 , $P < .05$). The ratio of spleen/body weight in mice treated with 3' dG₆ run-conjugated PS CpG-ODNs (6.57 ± 0.31) was not different from that of PS CpG-ODNs without 3' dG₆ run or that of PE CpG-ODN-treated mice ($P > .05$, Fig 2).

Splenomegaly is a well-recognized phenomenon accompanying some oligonucleotide injections.⁵ A dramatic but transient splenomegaly with a maximum increase of spleen weight after 6 days of PS CpG-ODN injection has been reported. This increase of spleen weight by more than 3 times the normal weight was dose and sequence dependent and normalized after 12 to 14 days.⁵ CpG-ODN-induced splenomegaly was reported to be due to extramedullary hematopoiesis.⁵ The innate

immune system activated by pattern-recognition receptors controls the initiation of the adaptive immune response by regulating the expression of costimulatory activity on antigen-presenting cells and the release of effector cytokines. It also elaborates hematopoietically active cytokines and growth factors by macrophages and dendritic cells.⁵ The 3' dG₆ run forms a tetraplex structure, called a G quartet, which is proved to confer exonuclease resistance and to enhance the receptor-mediated endocytosis of CpG-ODNs.^{2,3} Therefore conjugation of 3'-dG₆ run to PE-CpG-ODNs could enhance T_H1 response while inhibiting T_H2 response and protect against the development of asthma in a murine model.² It has been reported that PS backbone itself has not only nuclease resistance but also chemotactic activity for antigen-presenting cells, such as macrophages.⁶ This might be a possible explanation for the difference in the induction of splenomegaly between PS CpG-ODNs and 3' dG₆ run-conjugated PE CpG-ODNs, which have shown similar inhibitory effects on allergen-specific IgE synthesis. However, the exact mechanism is yet to be elucidated. It has been reported that conjugation of 3' dG₆ run to PS CpG-ODNs reduced the production of TNF- α and IL-12 and that the enhancement of immunostimulatory effect by 3' dG₆ run depends on the chemical property of the ODN backbone.³ This might explain why the ratio of spleen/body weight was not different in 3' dG₆ run-conjugated PS CpG-ODN-treated and PE CpG-ODN-treated mice.

In conclusion, conjugation of 3' dG₆ run to PE CpG-ODNs could inhibit allergen-specific IgE synthesis with less risk of splenomegaly.

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Fixed food eruption caused by asparagus

To the Editor:

A 50-year-old white woman working as a commercial clerk presented with 2 sharply margined, round, slightly elevated erythemas on her right forearm and left chest wall that appeared a few hours after ingestion of tinned asparagus (Fig 1, A). These erythemas persisted for more than 4 weeks and faded slowly without treatment, leaving circumscribed areas of hyperpigmentation. In spring 2003 and 2004, the patient experienced another 2 episodes, with erythemas at exactly the same locations after eating either fresh or tinned asparagus. These erythemas never developed independent of asparagus intake. The patient had

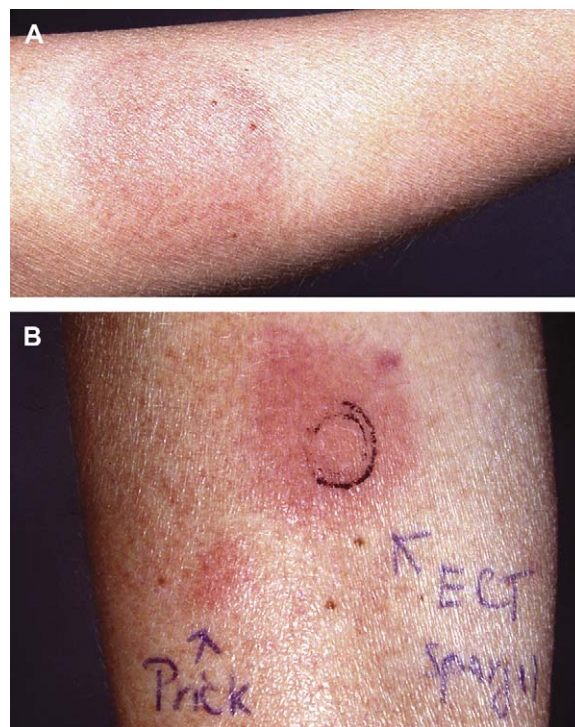


FIG 1. A, Recurrent localized erythemas: sharply margined, round erythema on the patient's right forearm. **B**, Skin test reactions after intraleisional application of asparagus. Intraleisional tests were performed at the patient's right forearm. Results of the prick (left) and patch (right) test reaction 72 hours after application of asparagus are shown.