Changes in Immune Responses to Mite Antigen Sensitized through Barrier-Disrupted Skin with CpG-Oligodeoxynucleotide in Mice

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CpG-oligodeoxynucleotide (CpG-ODN) plays a critical role in immunity via the augmentation of Th1 and suppression of Th2 responses. We examined here the effect of CpG-ODN on the immune response to mite antigen sensitized through barrier-disrupted skin of human atopic dermatitis (AD) model mouse. Although sensitization with mite antigen induced Th2-dominant immune response, co-administration of CpG-ODN elicited Th1-dominant immune response. In terms of antigen-specific antibody production, the level of IgG2a was increased by CpG-ODN, but not in IgE. These results suggested that administration of CpG-ODN via skin is a simple strategy for patients with diseases like AD, which is characterized by Th2-dominated inflammation.

Key words CpG-oligodeoxynucleotide (CpG-ODN); Th1/Th2 balance; transdermal administration; mite antigen; atopic dermatitis; NC/Nga mouse

Atopic disorders have a complex and chronic pathogenesis that provides many potential cellular and molecular targets for therapeutic intervention, but may also include redundant pathways mediating disease. The stratum corneum normally provides a cutaneous permeability barrier against environmental allergens such as mite antigen and house dust. In atopic dermatitis (AD) patients’ skin, however, there is a significant deficiency in the barrier function of the skin due to a reduction in ceramides, a major constituent of the stratum corneum nor-#mally provides a cutaneous permeability barrier against envi-

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CD4+ helper T (Th) cells have been subdivided into at least two subsets, Th1 and Th2, on the basis of the cytokines they secrete. The balance of Th1 and Th2 cells is relevant to the outcome of immunologically mediated clinical syndromes, and dysregulated Th1 or Th2 responses are thought to be central to the pathology of diseases such as AD and asthma, which is characterized by Th2-dominated allergic inflammation.

Both the bacterial dinucleotides flanked by certain bases (Cpg-motif) and synthetic oligodeoxynucleotides containing a CpG-motif (CpG-ODN) activate cells such as B-cells, macrophages and dendritic cells, through toll-like receptor-9 (TLR-9). Signaling through TLR-9 leads to the secretion of proinflammatory cytokines such as IL-1, IL-6, TNF-α, and IL-12. IL-12 acts on T and NK cells inducing the production of cytokines, primarily IFN-γ. Consequently, CpG-ODN could be useful as an adjuvant for cellular and humoral immunizations. The induction of such innate immune responses and production of Th1-related cytokines is very important in controlling in determining the type of antigen-specific immune responses that are induced.

It is reported that the topical application of mite antigen to NC/Nga mouse, an inbred strain established from Japanese fancy mice, could induce an AD-like skin response. It is also reported by Kondo et al. and Yamada et al. that tape-stripping is enough to remove the stratum corneum and mimic the structure of AD skin lesions. So, we used the mite antigen and tape-stripped skin as a model of AD skin and in our experiments.

In this paper, we examined the alterations in immune response to mite antigen applied to tape-stripped skin of NC/Nga mice as evaluated by cytokine and antibody levels.

MATERIALS AND METHODS

Materials The sequences for CpG-ODN and non-CpG-ODN were 5'-TCCATGACGGTTCCTAGGCT-3' and 5'-TCAGTACCTCTTCCTAGGCT-3', respectively, and both HPLC-purified phosphorothioate ODNs were obtained from Amersham Pharmacia Biotech Co. (Tokyo, Japan). All ODNs were dissolved in distilled water. Mite antigen was generously provided by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan). The mite antigen was a mixture of antigen from Dermatophagoides farinae 1 (Der f1) and Dermatophagoides farinae 2 (Der f2), representative of allergens of human AD. Mite antigen was dissolved in sterilized PBS.

Animal Experiments NC/Nga mice (male, 6—8 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan). Animal use and relevant experimental procedures were approved by the Tokyo University of Pharmacy and Life Science Committee on the Care and Use of Laboratory Animals (permission No: 2004-003). Mice housed under specific pathogen-free (SPF) conditions were anesthetized with an i.p. injection of Nembutal (1.5 mg/mouse), the abdominal skin was shaved, and then the stratum corneum was removed by stripping with adhesive tape 10 times. PBS, OVA or OVA plus CpG-ODN (50 μl) were applied to the barrier-disrupted skin.

Preparation of Mouse Splenic Cells Following the application of CpG-ODN and mite antigen to the barrier-disrupted skin, the spleen was removed on day 5, and splenic single cell suspensions were prepared. To examine the production of cytokines, spleen cells (5×10^7/well) were incubated in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin,
2 mm l-glutamine and 5×10^{-5}M 2-mercaptoethanol for 72 h in the presence of mite antigen (2 mg/ml).

Cytokine Determination The levels of IL-4 and IFN-γ in the culture supernatant of splenic cells were determined with a sandwich ELISA using pairs of purified capture and biotinylated detection monoclonal antibodies (all obtained from Pharmingen) recognizing murine IL-4, and IFN-γ, according to the manufacturer’s protocols.

Changes in Mite Antigen-Specific Antibody Levels Blood samples from mice treated with mite antigen and CpG-ODN were collected on specific days from the retro-orbital plexus and sera were pooled. A doubling dilutions of sera (50 μl) were poured into a mite antigen-coated ELISA-plate, and changes in antibody levels were determined by ELISA.

Statistical Analysis The paired Student’s t test was used to compare paired groups. An analysis of variance (ANOVA) was used for multi-group analysis. Values of p>0.05 were considered to indicate lack of significance.

RESULTS

Changes in Cytokine Levels in NC/Nga Mouse Splenocytes We examined the effect of CpG-ODN on the immune response in NC/Nga mice, an animal model for human AD. The mite antigen was co-applied with CpG-ODN on the tape-stripped skin, and the production of IFN-γ and IL-4 in splenic cells was investigated in vitro. Spleen cells were prepared from NC/Nga mice on day 5 following the transdermal administration of mite antigen and CpG-ODN, and were incubated in the presence of 2 mg/ml of mite antigen for 72 h. Supernatants were collected and concentrations of IFN-γ and IL-4 were evaluated by ELISA. As shown in Fig. 1, the production of IFN-γ increased on the co-application of mite antigen and CpG-ODN. On the other hand, the level of IL-4 decreased drastically when the mice were treated with mite antigen and CpG-ODN (Fig. 1).

Changes in Antibody Levels in NC/Nga Mice We next examined the effect of CpG-ODN on the immune responses to mite antigen based on the production of immunoglobulin isotypes. Sera were collected 2 weeks after the last transdermal administration of mite antigen with CpG-ODN, and the antigen-specific immunoglobulin isotypes were determined by ELISA. The changes in immunoglobulin isotype levels in NC/Nga mice are shown in Fig. 2. The production of antigen-specific IgG2a drastically increased when mite antigen was applied to the tape-stripped skin with CpG-ODN, but no production of IgG2a was observed in mice sensitized to mite antigen only. Furthermore, the production of antigen-specific IgE, a Th2-like isotype and one of the major causative factors of atopic inflammation, was strikingly suppressed by the co-application of CpG-ODN (Fig. 2). When mice were treated with non-CpG-ODN and mite antigen, the production of IgG2a and IgE was almost the same as that in mice given mite antigen alone. Taking these findings into consideration, CpG-ODN co-applied with the antigen to tape-stripped skin, a model of AD skin, changes the immune response from Th2 to Th1 in NC/Nga mice.

DISCUSSION

The balance of Th1 and Th2 cells is relevant to the outcome of immunologically mediated clinical syndromes, and asthma and AD are characterized by Th2-dominated allergic inflammation. \(^5\)–\(^8\) CpG-ODN is able to shift the immune response from a Th2 to Th1-like response. Therefore CpG-ODN is expected to be useful for immunotherapy among patients with these allergic diseases. It is considered that the stratum corneum acts as a barrier to antigens because of their high molecular weight and high water-solubility. In AD patients’ skin, there is a significant deficiency in ceramides, a major constituent of the stratum corneum, in both lesional and non-lesional skin, and the skin is highly susceptible to antigen penetration. \(^9\) Therefore, environmental allergens can easily penetrate such susceptible skin. Tape-stripping is a simple method to remove the epidermal barrier of the stratum corneum. \(^10\) We thus used this tape-stripping method to disrupt the cutaneous barrier and constructed a model of AD skin in mice. Following stripping with adhesive tape 10 times, the stratum corneum was completely removed and the naked epidermal layer was exposed the same as human AD skin lesions (data not shown). These findings suggest that tape-stripping is a very simple method to create AD-like skin in mice.
CpG-ODN induces a Th1-like pattern of cytokine production that is dominated by IL-12 and IFN-γ with little secretion of Th2 cytokines, and then shifts the immune response from Th2 to Th1. Indeed, injection of CpG-ODN prevented the development of airway eosinophilia and bronchial hyperreactivity in mice sensitized to an antigen. AD is a disease where allergens such as mites or house dust penetrate the skin and cause a strong Th2-type immune response. We thus investigated whether topical application of CpG-ODN to tape-stripped skin changes the immune response to mite antigen from Th2 to Th1 by evaluating the production of cytokines and antibodies in NC/Nga mice. In this strain, CpG-ODN induced the secretion of Th1-type cytokines (Fig. 1) and immunoglobulin isotypes (Fig. 2).

Th2-type immune responses mediated by the secretion of IL-4, IL-5 and IL-13 are the key to the pathogenesis of atopic disorders. NC/Nga mice showed Th2-dominant immunoglobulin isotype expression against mite antigen and immunoglobulin isotypes (Fig. 2). The regulation of IgE synthesis has been extensively investigated in recent years, and it has been found that IFN-γ and IL-4 strongly contribute to this regulation. IFN-γ and IL-4 promote IgG2a and IgG1 class switching, respectively. Further, it has been reported that IL-4 and IFN-γ strongly regulate IgE synthesis, and the Th1-type cytokine IFN-γ acts as a counter-part of IL-4 and suppresses the production of IgE. Taken together, IgE synthesis in response to an antigen that has penetrated the skin is also regulated by the balance of IL-4 and IFN-γ, and co-application of CpG-ODN reduced IgE production in NC/Nga mice.

This paper shows the preliminary data that CpG-ODN remarkably changed the immune response to mite antigen from a Th2 to Th1-type response. When NC/Nga mice were kept under conventional condition, they started to scratch themselves at about 8 weeks, at which time their skin becomes dry and scaly. These phenomena resemble human AD, and indeed show the high IgE levels in sera. We are now investigating whether CpG-ODN has an ability to reduce the symptoms that are induced by mite in NC/Nga mice.

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