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Short communication

Suppression of atopic-like dermatitis by treatment with antibody to lymphocyte function-associated antigen-1 in NC/Nga mouse

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Abstract

The effect of a blocking-antibody specific for lymphocyte function-associated antigen-1 (LFA-1) was studied in an atopic-like dermatitis model, which was induced by the repeated application of picrylchloride in NC/Nga mice. Prophylactic treatment with anti-LFA-1 monoclonal antibody (mAb), not therapeutic treatment, significantly inhibited the skin severity score and the acanthosis with ulceration and infiltration of mast cells. Furthermore, the serum immunoglobulin E levels and cytokine production (interleukin-4 and interferon-γ) by splenocytes stimulated with anti-CD3 antibody were also inhibited by treatment with anti-LFA-1 mAb. Our results suggest that LFA-1 plays an important role in the induction phase of the atopic-like dermatitis model.

Keywords: LFA-1 antibody; Allergy; Atopic dermatitis

1. Introduction

Atopic dermatitis is a chronically relapsing inflammatory skin disease characterized by episodes of intense pruritus, multiple lesions with erythema, excoriation, erosions, lichenification, papules, dry skin, and susceptibility to cutaneous infection. Histopathological studies have shown that the skin lesions of atopic dermatitis are characterized by acanthosis and spongiosis in the epidermis, which exhibits T cell-induced keratinocyte apoptosis (Trautmann et al., 2001). One experimental model of allergic dermatitis involves the production of immunoglobulin (Ig) E-antibody in mice repeatedly treated with a contact sensitizing agent (Ohmura et al., 2004b). NC/Nga mice showed severe atopic-like dermatitis with Th-cytokine production (Vestergaard et al., 2000) and severe itching (Ohmura et al., 2004a), which is similar to human atopic dermatitis. However, the cytokine network and interactions of adhesion molecules in the model remain to be understood. One of these interactions is mediated by the interaction of lymphocyte function-associated antigen-1 (LFA-1) and its ligand, intercellular adhesion molecule-1 (ICAM-l). LFA-1 has an established role in lymphocyte cell adhesion and exerts its influence on cell–cell contact through binding to ICAM-1 (Haskard et al., 1986; Makgoba et al., 1988). Several in vitro experiments have shown that the interaction between LFA-1 and ICAM-1 not only is required for cell adhesion and migration, but also plays a key role in other lymphocyte functions, such as antigen presentation and T-cell-dependent antibody production in humoral immunoresponses (Springer et al., 1987).

In studies of various models of contact hypersensitivity, others have reported that treatment with a combination of

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monoclonal antibodies (mAbs) against LFA-1 and ICAM-1 inhibited contact hypersensitivity in mice (Scheynius et al., 1996). In this study, we examined the effect of monotherapy with M17/4, a LFA-1-blocking mAb, on an atopic-like dermatitis model in NC/Nga mice to clarify the role of LFA-1 in atopic dermatitis.

We have used monotherapy with anti-LFA-1 to study directly the role of LFA-1 in atopic-like dermatitis without the complications of blocking other ligands of ICAM (e.g. macrophage antigen-1) and to avoid nonspecific antibody interactions. In the present study, the effects of blocking LFA-1 on the severity of the dermatitis (skin score), IgE production in vivo and production of interleukin-4 and interferon (IFN)-γ in hapten-sensitized mouse splenocytes ex vivo were examined in the NC/Nga mouse severe atopic-like dermatitis model.

2. Materials and methods

2.1. Methods

All experimental procedures were approved by the Animal Care Use Committee at Nippon Boehringer Ingelheim. Twenty-four male NC/Nga mice (SPF) aged 5 weeks, weighing 13–19 g, were purchased from Charles River Japan. The fur of the thoracic and abdominal regions of ether-anaesthetized animals was shaved off with a hair clipper 1 week before sensitization. PiCl solution (150 μl) was applied to the thoracic and abdominal areas (sensitization), as well as to the soles of the hind paws. Three days after sensitization, induction was performed by applying 200 μl of PiCl solution to the back and to the left and right ears once a week for 4 weeks (prophylactic effect) or 6 weeks (therapeutic effect). Starting 1 h before sensitization, 10 mg/kg of anti-LFA-1 mAb or rat IgG was administered intraperitoneally three times per week for 4 weeks (prophylactic effect). In the case of therapeutic effect, these antibodies were administered intraperitoneally from the day before the third induction, three times per week for 4 weeks.

2.2. Dermatitis observation (scored evaluation)

Mice were scored weekly, just before each sensitization, for the presence of (1) flare haemorrhage, (2) oedema, (3) excoriation and erosion, and (4) incrustation and xerosis. For each of these signs, a score was assigned as follows: 0, no sign; 1, mild; 2, moderate; or 3, severe. The sum of the individual scores was taken as the dermatitis score.

2.3. Measurement of serum IgE level

Blood was collected from the retro-orbital plexus of ether-anaesthetized animals after the last dermatitis observation. Serum samples were obtained by centrifugation and stored at −80 °C until measurement. Total IgE level was measured by a sandwich enzyme-liked-immunosorbent assay (ELISA).

2.4. Measurement of cytokine levels produced by splenocytes

After blood collection, whole splenocyte suspensions were prepared in RPMI-1640 medium. Splenocytes were cultured in 24-well plates at a density of 2×10^6 cells/ ml (1 ml/well). The cells were cultured for 48 h with or without anti-CD3 coating (10 μg/ml). Culture supernatants were stored at −80 °C until analyzed for cytokines (IFN-γ, interleukin-4) by ELISA.

2.5. Histopathological examination

The portions of the back skin were fixed with 10% neutral-buffered formalin, embedded in paraffin and sectioned at 4 μm. Sections were stained with hematoxylin–eosin and 0.5% toluidine blue to identify the mast cells.

2.6. Reagents

M17/4 (anti-mouse LFA-1 mAb) was generated from cell lines obtained from American Type Culture Collection (Rockville, MD) by Boehringer Ingelheim Pharmaceuticals (Ridgefield, USA). Control rat IgG was purchased from Sigma and purified at Boehringer Ingelheim Pharmaceuticals. 2,4,6-Trinitrochlorobenzene (PiCl) was provided by Tokyo Kasei Chemical (Tokyo, Japan) and used after recrystallization. Mouse IgE EIA kits were purchased from Yamasa (Tokyo, Japan). Murine IFN-γ and interleukin-4 ELISA kits were purchased from Amersham Pharmacia Biotech (UK).

2.7. Data analysis

Data are presented as the mean±S.E. The significance of differences in skin severity score was analyzed using the Wilcoxon rank sum test. The significance of differences in IgE, intreleukin-4 and IFN-γ among rat IgG-treated, anti-LFA-1 mAb-treated and intact groups were tested by a factorial analysis of variance (ANOVA) followed by an unpaired *t*-test. $P<0.05$ was considered significant.

3. Results

Anti-LFA-1 mAb inhibited the increase in clinical skin severity score in the PiCl-induced dermatitis model (Fig. 1A). The score was significantly reduced throughout the 4 weeks of anti-LFA-1 mAb treatment. After 4 weeks of treatment, the scores of the rat IgG (control Ab) group and anti-LFA-1 mAb group were 6.3±0.2 and 2.5±0.2, respectively ($p<0.01$). Moderate acanthosis (increased

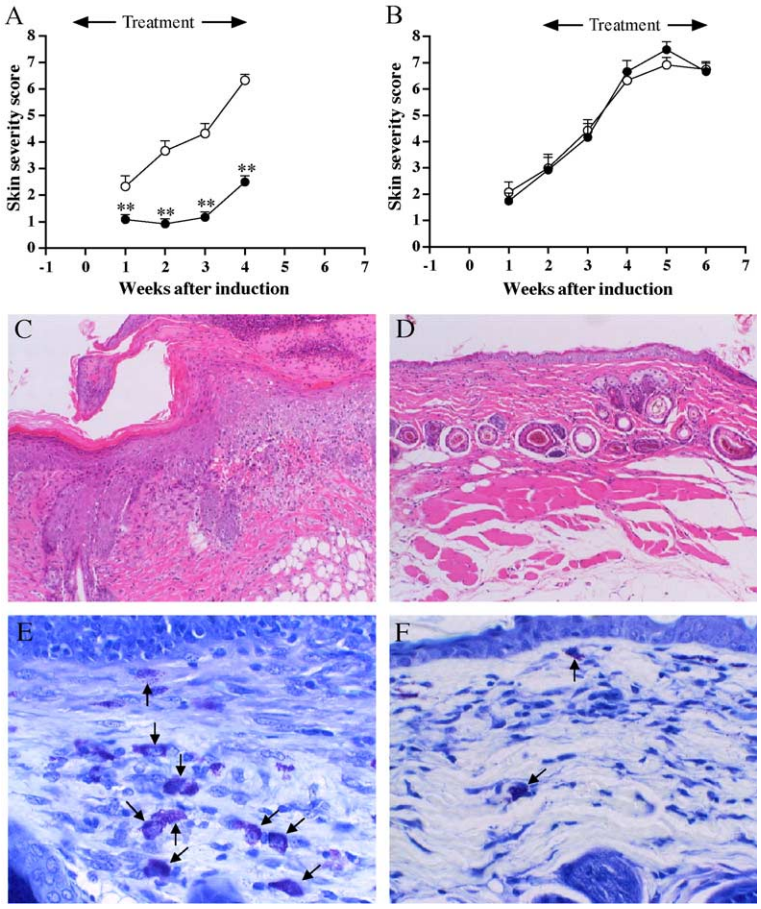

Fig. 1. The effect of anti-LFA-1 mAb on the clinical skin severity score. Dermatitis-like signs were assessed once weekly just prior to each induction. The clinical skin severity score was calculated as described in Methods. (A) Mice were treated with rat IgG, 10 mg/kg i.p. (open circles) or anti-LFA-1 mAb, 10 mg/kg i.p. (closed circles) prophylactically. Values are represented as mean±S.E. (n=12). **p<0.01 compared to rat IgG. (B) Mice were treated with rat IgG, 10 mg/kg i.p. (open circles) or anti-LFA-1 mAb, 10 mg/kg i.p. (closed circles) therapeutically. Values are represented as mean±S.E. (n=12). Dorsal skin from mice in the positive control group sensitized with PiCl (C, E) and mice treated with anti-LFA-1 antibody (D, F). Skin from the positive control shows acanthosis with ulceration (C) and increased numbers of mast cells in the dermis (E, arrows). Prophylactic treatment with anti-LFA-1 antibody inhibited acanthosis (D) and reduced the number of mast cells (F, arrows) (C, D: HE stain, original magnification ×100; E, F: toluidine blue stain, original magnification ×400).

thickness of the epidermis) with ulcers and infiltration of mast cells into the dermis were observed in the skin of the rat IgG group (Fig. 1C and E), whereas these findings were rarely observed in animals dosed with anti-LFA-1 mAb (Fig. 1D and F). We also examined the effect of 4-week treatment with LFA-1 mAb started after the third induction (effector phase); however, no significant differences from the control group were observed in this therapeutic protocol (Fig. 1B). The treatment with anti-LFA-1 antibody did not influence the increase of eosinophils in either phase (data not shown).

The serum IgE levels and the production of interleukin-4 and IFN-γ were examined only in the case of prophylactic treatment, because therapeutic treatment did not affect the skin severity score. The serum IgE levels were significantly inhibited by anti-LFA-1 mAb treatment compared with the rat IgG group (0.37±0.04 and 6.08±1.34 μg/ml, respectively, p<0.01) (Fig. 2A). The production of interleukin-4 and IFN-γ was increased by ex vivo anti-CD3 stimulation of splenocytes from control mice as compared to no stimulation (Fig. 2B and C). The increase in cytokine production was significantly inhibited in anti-CD3-stimulated splenocytes from mice that received prophylactic treatment with anti-LFA-1 mAb (Fig. 2B and C).

4. Discussion

In this study, it was demonstrated that prophylactic monotherapy with anti-LFA-1 mAb, initiated before sensitization with PiCl, leads to inhibition of mouse allergic dermatitis. It has been reported that treatment with a combination of mAbs against LFA-1 and ICAM-1 inhibited allergic inflammation (Iwamoto and Nakao, 1995) and suppressed allergic contact hypersensitivity (Scheynius et al., 1996). The latter authors reported that monotherapy with anti-LFA-1 mAb had no effect on contact hypersensitivity; however, the findings of the

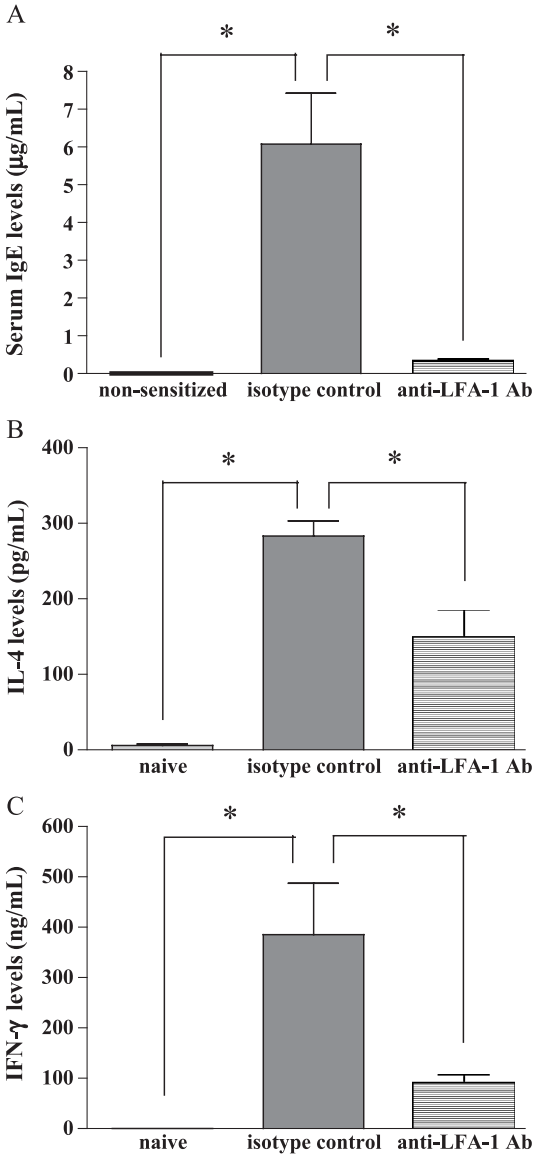

Fig. 2. (A) Serum concentration of IgE. Splenocytes from naïve mice, rat IgG-treated or anti-LFA-1 Ab-treated mice were stimulated with anti-CD3 antibodies for 48 h. The culture supernatants were assayed for interleukin-4 (B) and IFN-γ (C) by ELISA. Values are represented as mean±S.E. (n=6–12). Significant difference between groups (*p<0.01).

present study differ. The reasons for this apparent discrepancy are not clear. However, the present study was done in a disease-relevant model, atopic-like dermatitis, utilizing a different species of mouse and multiple sensitizations. LFA-1 is likely more important in this model than in contact hypersensitivity. Several in vitro experiments have shown that the interaction between LFA-1 and ICAM-1 not only is required for cell adhesion and migration, but also plays a key role in antigen presentation (Springer et al., 1987; Springer 1990). To explore whether the observed effects of anti-LFA-1 in the present study were on the induction phase or on the effector phase, the effect of therapeutic administration of anti-LFA-1 mAb for 4 weeks after the third induction (effector phase) were examined. However in this protocol, no effect of the anti-LFA-1 mAb on the skin severity score was observed. This result suggests that LFA-1 plays an important role during the induction phase of atopic-like dermatitis, but not in its effector phase. In allergic dermatitis, epicutaneously applied haptens with carrier self proteins are recognized by lymphocytes of the epidermis. Macrophages and other antigen-presenting cells (APCs) migrate from the skin to the paracortex of the regional lymph nodes, where the antigen is presented in the context of the major histocompatibility complex (MHC) to naïve T-cells. However, antigen plus MHC alone are insufficient to fully activate T cells. Costimulation via the interaction of cell surface molecules on the APC and lymphocyte is required for T cell proliferation and differentiation. T cells that encounter antigen plus MHC in the absence of costimulation fail to proliferate and become unable to respond to subsequent challenge with the same antigen, i.e., they become anergic (Schwartz, 1992; Gaspari and Katz, 1991). Therefore, it is suggested that the administration of anti-LFA-1 mAb in the present study influences antigen presentation in the induction phase, resulting in T cell anergy and suppression of the dermatitis.

Th1 cells produce interleukin-2 and IFN-γ, whereas Th2 cells produce interleukin-4, interleukin-5 and interleukin-13. It has been reported that NC/Nga mice show a Th2-dominant immune reaction and overexpress interleukin-4. In addition, interleukin-5 was observed in the regional skin (Hiroi et al., 1998). However, recent studies suggest that not only Th2-type cytokines (interleukin-4), but also the Th1-type cytokine (IFN-γ) are expressed in human atopic dermatitis lesions (Grewe et al., 1994; Thepen et al., 1996). Similarly, the present study has shown increased expression of both interleukin-4 and IFN-γ by anti-CD3 stimulated splenocytes from PiCl-treated NC/Nga mice. Anti-LFA-1 mAb completely inhibited the cytokine production by splenocytes and the increase of serum IgE concentration in the model. The observation of increased interleukin-4 and IFN-γ in the response of the control mice and the inhibition of the production of these cytokines by anti-LFA-1 mAb suggests a more complex interaction between Th1 and Th2 processes in this model.

In conclusion, the present study has shown that prophylactic, but not therapeutic, monotherapy with anti-LFA-1 mAb inhibits the development of atopic dermatitis-like skin lesions, IgE production and lymphocyte cytokine production in NC/Nga mice. This most likely occurs through inhibition of antigen presentation.

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