

ORIGINAL PAPER

Topical application of F991, an immunoglobulin free light chain antagonist, prevents development of contact sensitivity in mice

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Summary

Background Exposure to reactive chemicals or environmental allergens can lead to hypersensitivity reactions in the skin of predisposed people. Most of these reactions are of atopic origin, but a subgroup of patients exhibits skin hypersensitivity reactions without features of atopy.

Objective This study was undertaken to examine the effect of inhibiting the action of Ig-free light chains in a murine model for non-atopic skin hypersensitivity by dermal application of the free light chain antagonist F991.

Methods To study the efficacy of F991, BALB/c mice were either passively immunized with trinitrophenyl (TNP)-specific immunoglobulin light chains (IgLC) and challenged with the hapten picryl chloride (PCI) or actively skin-sensitized and challenged with dinitrofluorobenzene (DNFB). The effect of F991 or control treatment was investigated by measuring local edema formation and the production of pro-inflammatory cytokines.

Results Passive immunization with TNP-specific IgLC resulted in an increase in ear swelling 2 h after PCI challenge. F991 inhibited this enhanced ear swelling in a dose-dependent manner when applied 4 h before the sensitization with IgLC. F991 also inhibited DNFB-induced contact hypersensitivity reaction in the mouse skin 2 and 24 h after challenge when applied before challenge. Besides the prophylactic action, F991 when applied 2 h after DNFB-challenge, it was also able to attenuate symptoms of the DNFB-induced hypersensitivity reaction at 24 h after challenge. We showed that the beneficial effects of F991 are restricted to the side of application.

Conclusion F991 is able to effectively alleviate symptoms of contact sensitivity in mice. Our study suggests that local interference with IgLC-induced allergic symptoms may be attractive in the treatment of hypersensitivity responses.

Keywords contact sensitivity, ear swelling, Ig-free light chain, IL-6, mast cell, TNF- α

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Introduction

Contact dermatitis or contact hypersensitivity is a chronic relapsing inflammatory skin disease characterized by eczematous skin rashes, intense pruritis and dry skin. It is elicited by skin exposure to environmental or industrial allergens. The underlying mechanisms involve the participation of a wide variety of cell types and their mediators, including Langerhans cells, T lymphocytes and mast cells

[1, 2]. Although most of the dermatitis patients have atopic or IgE associated dermatitis characterized by high serum IgE levels and positive skin prick tests, a subgroup of dermatitis patients suffer from non-atopic skin disease with features that clinically resemble those observed in atopic dermatitis. In general, it is estimated that between 16% and 40% of all cases of dermatitis are of this non-atopic form of dermatitis [3]. Compared with atopic dermatitis, considerable less information is present on the underlying mechanisms of non-atopic dermatitis.

Contact hypersensitivity reactions to reactive small compounds (haptens) can be experimentally induced in

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rodents, especially mice, by topical exposure to the allergen. Many investigators have used such models to study the pathological mechanisms underlying atopic contact dermatitis. Contact hypersensitivity responses comprise a sensitization and elicitation phase. Epicutaneous challenge of sensitized animals generally results a biphasic tissue edema response [4, 5]. In previous studies, we have demonstrated that immunoglobulin-free light chains (IgLC) are crucial in the development of these contact hypersensitivity responses [6–8]. Moreover, using F991—a selective IgLC antagonizing peptide—we were able to specifically inhibit these hypersensitivity responses [6, 9].

In this study, we have investigated both prophylactic and therapeutic effects of topical application of F991 in passive and active immunized mice. Our results indicate that topical treatment with F991 is beneficial in the treatment of contact hypersensitivity responses.

Materials and methods

Animals

Male BALB/c mice, 5–6 weeks of age, were supplied by the Central Animal Laboratory, Utrecht, the Netherlands. All mice had free access to tap water and commercially available chow food. All experiments were conducted in accordance with the Animal Care Committee of the Utrecht University.

Dermal application of the immunoglobulin light chains antagonist F991

The IgLC-binding compound, F991, was synthesized by Fmoc Chemistry (Ansynth, Roosendaal, the Netherlands). It was applied topically in a cetomacrogol cream on the inside of the pinnae on different time-points before and after ear challenge. The cream (1 cm stripe equaling 20 mg of cream) was dispensed using an eye-cream tube and spread out using an inert spatula. The amount of F991 applied in the cream onto the ears is indicated in the figures. Cetomacrogol cream FNA is a hydrophilic cream that is normally used in indifferent treatment of skin conditions and was readily taken up by the mouse skin. To study the prophylactic effect F991 cream was applied 4 h before challenge. In case of the therapeutic effect, F991 cream was applied 2 h after challenge.

Passive trinitrophenol-specific immunoglobulin light chains immunization

TNP-specific IgLC were isolated from TNP-specific IgG1 as described previously [6]. Four hours before the mice were injected intravenously (i.v.) with 2 µg isolated TNP-specific IgLC, F991-cream or control cream was applied on the inside of the ears of anaesthetized mice

[ketamine–xylazine–saline solution (4 : 3 : 7), 30 µL, intramuscularly (i.m.)]. Thirty minutes after injection of the TNP-specific light chains, the animals were anaesthetized with halothane and the ears were challenged either with vehicle solution (acetone:olive oil = 4 : 1, 20 µL) or with 0.8% picryl chloride (PCI, 20 µL) dissolved in vehicle solution. Challenge solution was only applied on the outside of the pinnae. Two hours after challenge, ear thickness was determined.

To study the possible systemic exposure of F991, two approaches were followed. Firstly, F991 was applied on the shaven spinal position of the animal and ear swelling was measured after an ear challenge. Secondly, F991 cream was applied on the left ear and the effect of a challenge was measured on the right one, which was treated with control cream.

Active dinitrofluorobenzene immunization

Skin sensitization was accomplished by 0.5% DNFB as described by Buckley and Nijkamp [10]. Briefly, mice were sensitized on days 0 and 1 with a 0.5% DNFB dissolved in a mixture of acetone and olive oil (4 : 1). On day 0, DNFB or vehicle solution was applied to the shaved abdomen (50 µL) and paws (50 µL). On day 1, DNFB or vehicle was only applied to the abdomen (50 µL). During the sensitization procedure the animals were anaesthetized with ketamine–xylazine. On day 5, the animals were anaesthetized with halothane and the left ears were challenged with vehicle solution and right ears with 0.2% DNFB solution. Challenge solution was applied to the outside of the pinnae. Ear thickness was determined 2 and 24 h after challenge. In stated experiments, F991 cream or control cream was applied to the inside of the ears 4 h before the challenge or 2 h after challenge.

Measurement of ear thickness

Ear thickness of both left and right ears was determined by an engineer's micrometer (Mitutoyo, Veenendaal, the Netherlands). Results are expressed as absolute ear thickness or as difference in ear thickness (Δ ear swelling) between the left and right ears.

Measurement of tissue cytokines

Immediately after ear thickness measurement at 24 h, whole ears were collected and snap frozen in liquid nitrogen. Frozen ears were crushed in liquid nitrogen using a mortar and pestle and taken up in 0.5 mL phosphate buffer solution (PBS)/0.5% TX-100 supplemented with protease inhibitor cocktail (Roche, Almere, the Netherlands). Homogenates were centrifuged for 5 min at 14 000 g before analysis of cytokine content. Cytokine concentrations were measured using a cytometric bead array for mouse inflammatory

cytokines (Becton Dickinson, Alphen a.d. Rijn, the Netherlands) according to the manufacturer's protocol. Beads were analysed at a FACSCalibur flow cytometer (Becton Dickinson). Data analysis was performed with the analysis software provided with the cytometric bead array.

Statistical analysis

The results are expressed as mean $\pm$ SEM. Differences between groups were analysed by a Student's *t*-test or ANOVA. If appropriate, the ANOVA was followed by the Bonferroni multiple comparison test (GraphPad Prism version 2.01; GraphPad, San Diego, CA, USA). All *P*-values smaller than 0.05 were considered to reflect a statistically significant difference.

Results

Dose-dependent inhibition by dermal application of F991 of an Immunoglobulin light chains-induced ear swelling

Passive transfer of TNP-specific IgLC followed by a topical challenge with PCI induced a significant increase in ear swelling at 2 h. Dermal application of F991 in a cream at 4 h before PCI challenge inhibited the IgLC-induced increase in ear swelling in a dose-dependent manner (Fig. 1). F991 did not inhibit the irritant effect of DNFB in non-sensitized animals (compare open bars in Figs 1 and 3). Vehicle cream had no effect on IgLC-induced ear swelling.

Local dermal application of F991 has no systemic effects in immunoglobulin light chains-sensitized mice

To investigate whether local administration of F991 would lead to systemic inhibition, two different methods were

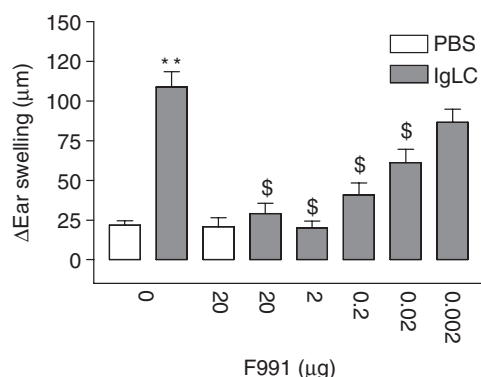


Fig. 1. Dose-dependent inhibition of picryl chloride (PCI)-induced ear swelling by F991. F991 or control cream was applied 4 h before immunization with immunoglobulin light chains (IgLC; closed bars) or phosphate buffer solution (PBS; open bars). Data are reported as means $\pm$ SEM of four to five mice per group. ***P* < 0.01 compared with control cream-treated PBS-sensitized animals. \$*P* < 0.05 compared with control cream-treated IgLC-sensitized mice.

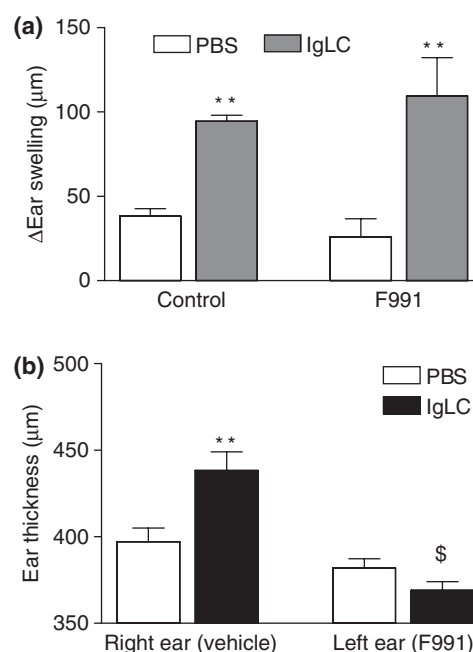


Fig. 2. No systemic effect of F991 (20 μg) when applied on the shaven back (a) or on left ear only (b) 4 h before immunoglobulin light chains (IgLC; closed bars) or phosphate buffer solution (PBS; open bars) sensitization. Ear swelling (a) or absolute ear thickness (b) was measured 2 h after picryl chloride (PCI) ear challenge. Data are expressed as means $\pm$ SEM of four to six mice per group. ***P* < 0.01 compared with PBS sensitized mice; \$not significant compared with PBS-sensitized mice.

followed. First, F991 or control cream was applied on the shaven back of the animals 4 h before passive immunization with IgLC. Dorsal application of F991 cream had no inhibitory effect on ear swelling 2 h after challenge (Fig. 2a). Secondly, F991 was applied on one ear and vehicle on the other ear, 4 h before passive immunization. Two hours after challenge, there was an increase in absolute thickness of the vehicle-treated ears of IgLC-sensitized mice compared with control cream-treated ears of non-sensitized mice (Fig. 2b). This increase in ear thickness was only inhibited in the ears treated with F991 and not in the control cream-treated ears (Fig. 2b). From these experiments can be concluded that the F991 cream acts only at the site of application.

Local dermal application of F991 inhibits hapten-induced contact sensitivity

In animals, especially rodents, contact dermatitis can be actively induced by low molecular weight haptens such as DNFB. To study whether local administration of F991 has a prophylactic effect on DNFB-induced contact sensitivity, mice were treated with F991 cream (20 μg/ear) or control cream 4 h before DNFB-challenge on day 5. Application of F991 cream 4 h before hapten challenge of DNFB-sensitized animals completely inhibited ear

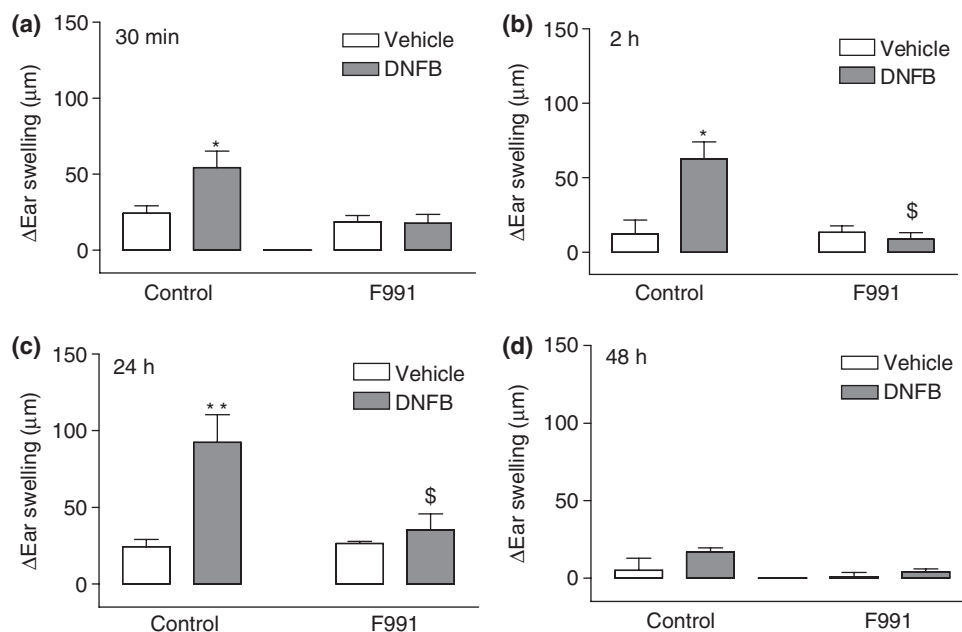


Fig. 3. Prophylactic effect of F991 (20 μg/ear) on a dinitrofluorobenzene (DNFB)-induced hypersensitivity reaction in vehicle sensitized (white bars) or DNFB-sensitized (black bars) mice 30 min (a), 2 h (b), 24 h (c) and 48 h (d) after DNFB-challenge. Cream was applied 4 h before the challenge on day 5 of sensitization. Data are expressed as means ± SEM of four to six mice per group. * $P < 0.05$ compared with control-treated vehicle-sensitized mice. ** $P < 0.01$ compared with control-treated vehicle-sensitized mice. \$ $P < 0.05$ compared with control cream-treated DNFB-sensitized mice.

swelling in the early phase (30 min and 2 h) and late phase (24 and 48 h) (Figs 3a–d). Treatment with control cream did not inhibit early- and late-phase contact sensitivity responses.

Local dermal application of F991 has therapeutical effects on a dinitrofluorobenzene-induced hypersensitivity reaction in the skin

To study whether F991 has therapeutical effects, F991 cream (20 μg/ear) or control cream was applied onto the ears of sensitized mice 2 h after DNFB challenge. As can be observed in Fig. 4a, at time of application of F991 cream or control cream (e.g. 2 h after challenge), there was an increase in ear swelling found in mice which were DNFB-sensitized and DNFB-challenged compared with vehicle-sensitized and DNFB-challenged vehicle-sensitized and DNFB-challenged mice (Fig. 4a). This increase in ear swelling was more pronounced 24 h after challenge in control cream-treated DNFB-sensitized mice. F991 treatment partially reduced the increase in ear swelling in DNFB-sensitized and challenge mice (Fig. 4b).

Dermal application of F991 inhibits local production of interleukin-6 and tumour necrosis factor-α during dinitrofluorobenzene-induced hypersensitivity response

As local application of F991 inhibited the early- and late-phase ear swelling response, we further examined its

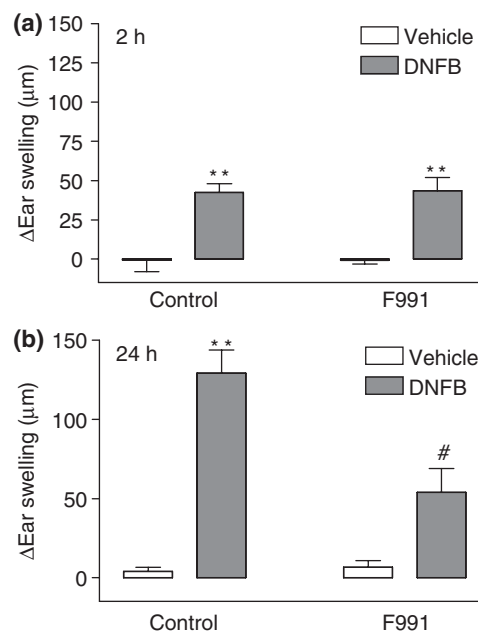


Fig. 4. Therapeutic effect of F991 (20 μg/ear) on an established dinitrofluorobenzene (DNFB)-induced hypersensitivity response at 2 h (a) and 24 h (b) after challenge in vehicle-sensitized (open bars) or DNFB-sensitized (closed bars) mice. Cream was applied 2 h after challenge and ear thickness was measured at time of application and 24 h after challenge. Data are expressed as means ± SEM of four to six mice per group. ** $P < 0.01$ compared with control cream-treated vehicle-sensitized mice. # $P < 0.05$ compared with control cream-treated DNFB-sensitized mice.

effect on the production of pro-inflammatory cytokines. At 24 h after hapten challenge IL-6 and TNF- α were measured in homogenized ear tissue. Hapten challenge resulted in a significant increase in both IL-6 and TNF- α production in sensitized animals. The production of both cytokines was greatly reduced by F991. Hapten challenge of non-sensitized animals (irritant effect) resulted in a significant increase of only IL-6 production. The latter was not inhibited by application of F991.

Discussion

This study describes the beneficial effect of dermal application of F991, a new peptide antagonist, in cutaneous early- and late-phase DNFB-induced hypersensitivity reactions. Previous work showed that F991, a 9-mer peptide (AHWSGHCLL) derived from the amino acid sequence in THP, is selective for IgLC and does not interfere with hypersensitivity reactions induced by either IgE, IgG1 [6] or IgM (unpublished). The present study shows that topical applied F991 at the time of challenge completely inhibited the increased ear swelling in both passively and actively immunized mice. Besides these prophylactic effects, F991 also attenuated an established DNFB-induced hypersensitivity response in the mouse skin when applied 2 h after antigen challenge.

In earlier studies, we have demonstrated that passive immunization with TNP-specific IgLC elicits immediate-hypersensitivity reactions in the skin and airways upon local antigen challenge [6, 9]. Furthermore, skin sensitization with low-molecular weight compounds such as DNFB, oxazolone or PCl results in production of antigen-specific IgLC [6]. These light chains may even be produced within one day post-immunization [11]. Our earlier studies showed that IgLC can bind to mast cells and that cross-linking of the mast cell surface proteins by IgLC-induced activation and degranulation of mast cells. *In vivo*, cross-linking of IgLC is accompanied with morphological signs of mast cell activation and concomitant release of mast cell mediators [4, 6], while no macroscopic swelling was detected in mast cell-deficient animals.

Mast cells are important inflammatory cells in the classical IgE-associated atopic skin reactions. In mice, repeated applications of DNFB (e.g. long-term sensitization) onto the ears led to an increase in allergen-specific IgE and resulted in an immediate reaction in ears after challenge of previously DNFB-sensitized mice [2, 12]. Mast cell deficient WBB6F1 mice show a reduced hypersensitivity response [2] and increased numbers of mast cells could be found after repeated DNFB application [13]. In addition, administration of a mast cell chymase inhibitor attenuated spontaneously induced contact dermatitis in NC/Nga mice [14]. The role for mast cells in non-atopic contact sensitivity responses is controversial. Some experimental models of non-atopic contact hyper-

sensitivity have strongly implicated mast cells as important contributors to the responses [15, 16], whereas others have detected no such role [17]. This disparity may be related to the differences in experimental protocol with respect to concentration of hapten solution used for sensitization and challenge and the solvent used to apply the hapten. Several studies have shown the importance of TNF- α in contact hypersensitivity reactions [16, 18]. Biedermann et al [16] showed that TNF- α produced during the effector phase of a hapten-induced delayed-type hypersensitivity response is exclusively derived from mast cells and is essential for the recruitment of polymorphonuclear cells and tissue swelling. Indeed, we found that the reduction of ear swelling response after application of F991 was paralleled with a greatly reduced TNF- α production during the contact hypersensitivity response. Also, production of the pro-inflammatory cytokine IL-6 was inhibited after F991 treatment. Importantly, F991 only inhibited the hapten-induced immunologic response and not the irritant reaction indicating the specificity of the antagonist (Fig. 5a compare vehs/DNFBc with DNFBs/DNFBc).

The relevance of this mouse model to human non-atopic contact dermatitis remains to be further established.

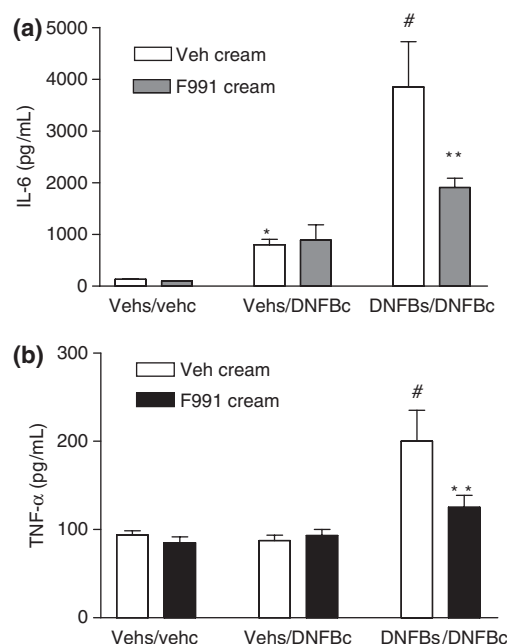


Fig. 5. Dermal application of F991 inhibits production of pro-inflammatory cytokines [IL-6 (a) and TNF- α (b)] in a dinitrofluorobenzene (DNFB)-induced contact sensitivity reaction. Three groups of mice (vehicle sensitized and vehicle challenged (vehc/vehc); vehicle sensitized and DNFB challenged (vehc/DNFBc); DNFB sensitized and DNFB challenged (DNFBs/DNFBc)) were treated with either control cream (open bars) or F991 cream (closed bars). At 24 h after hapten challenge, ears were collected and processed for cytokine measurement as described in Materials and methods. * $P < 0.05$ compared with vehc/vehc group; # $P < 0.05$ compared with vehc/DNFBc group; ** $P < 0.05$ compared with vehicle cream-treated animals in DNFBs/DNFBc group.

Increases in serum Ig-free light chains have been determined in various diseases including autoimmune diseases, allergy and asthma [9, 19, 20]. In a previous study, we found that serum κ free light chain concentrations were increased in non-atopic and atopic asthma patients when compared with non-atopic, non-asthmatics. We further showed in an animal model for non-atopic asthma that F991 prevented airway inflammation and the development of airway hyper-reactivity [9] demonstrating a crucial role for free light chains in the pathophysiology of asthma. Although we have demonstrated that antigen-specific free light chains are produced in animal models for contact sensitivity [6], no such information is presently available for human contact dermatitis patients.

In conclusion, we found that topical treatment with F991 before or after hapten challenge is effective in reducing the contact hypersensitivity reaction in mice. Interfering in free light-chain effector functions may be an interesting novel target in the treatment of non-atopic hypersensitivity responses.

Acknowledgements

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