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A rodent model for allergic dermatitis induced by flea antigens

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Abstract

There have been very few reports of experimentally induced animal models of allergic dermatitis, an immunologic disorder. This report describes the induction of histopathology confirmed allergic dermatitis in C57BL/6 mice along with the consistent clinical sign of alopecia following the administration of flea antigens emulsified in complete Freund's adjuvant (CFA). By comparing different strains of mice, routes of injection, types of adjuvants and different dosages of flea antigens, C57BL/6 mice were found to be most susceptible to flea antigens administered intramuscularly (i.m.) and subsequently developed dermatologic excoriations and local alopecia. The level of specific IgE reactive to flea antigens in C57BL/6 mice after the onset of clinical signs was significantly higher than such levels in mice without clinical signs, suggesting that flea antigen-specific IgE level can be correlated to the severity of allergic hyper-reaction. $CD4^+$ T lymphocytes and IL-4 rather than IL-10, or IFN-γ were found to be the predominant cytokines associated with the clinical onset of allergic symptoms in C57BL/6 mice. Further, histopathologic analysis indicated that not only mast cells had infiltrated into the area of the skin lesion, but the damage was found to be at a stage where mast cells were degranulating causing considerable exacerbation of the local injury. In conclusion, this murine allergic dermatitis model induced by flea antigens may provide a useful means to evaluate vaccines or immunodulatory drugs; thus providing researchers with a tool to study allergy-related disorders and other parameters needed in the area of allergic investigations.

Keywords: Mouse allergic model; Flea antigens; Complete Freund's adjuvant; Hypersensitivity

1. Introduction

There have been very few reports of experimentally induced animal models of allergic dermatitis, an immunologic disorder. Flea allergy dermatitis (FAD) is the most common dermatological disease of domestic pets such as cats and dogs that live in flea endemic areas. Susceptible animals develop an intense pruritic papular reaction to bites of the cat flea, *Ctenocephalides felis* (Rust and Dryden, 1997). It is the most common ectoparasite of domestic pets, responsible for production and transmission of several diseases of animals,

Abbreviations: RT-PCR, reverse transcriptase PCR; HPRT, hypoxanthine phosphoribosyl transferase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PMS, phenazine methosulfate; PBST, phosphate buffer saline plus Tween 20; BSA, bovine serum albumin; FACS, fluorescence activated cell sorter; OD, optical density; SI, stimulation index; TMB, tetramethylbenzidine

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including flea allergy dermatitis and anemia (Olivry and Mueller, 2003). Subsequent trauma results in excoriations, alopecia, and frequently, secondary bacterial or fungal infections of the damaged skin. Using FAD as an animal model to study flea induced dermatitis requires not only a containment facility and expertise in flea colony management, but also an extended period of time to establish the clinical onset of disease after repeated flea infestations of dogs or cats. In particular, the use of small number of cats or dogs in such flea-challenge studies are often ineffective as a method to evaluate the efficacy of candidate vaccines or other pharmaceuticals (Halliwell, 1984a; Halliwell et al., 1987; Halliwell, 1984b; Wilkerson et al., 2004). It is therefore desirable to establish a rodent model to evaluate potential vaccines or other pharmaceuticals which can prevent or treat FAD or similar dermatologic disorders prior to testing them in cats or dogs.

In this study, we evaluated the use of C57BL/6 mice as a model for allergic dermatitis after induction of clinical symptoms following the administration of the whole killed flea antigens. All the immunologic and histopathologic parameters resembled and were comparable to FAD observed in cat or dog, which had been induced by live flea infestation. Even though FAD clinical symptoms, such as erythema, papules, crusts and scale, are not as easily assessed as those in dogs, the signs of alopecia and excoriation are reproducible and observed consistently in this model. Thus, the rodent model represents a valuable representation for the development and preclinical assessment of vaccines or other pharmaceuticals for allergic dermatitis induced by flea antigens. Moreover, this model may provide a novel paradigm which could benefit other similar allergic or correlative dermatological studies.

2. Materials and methods

2.1. Mice

Six-week-old female mice of C57BL/6, BALB/C or DBA/2 were purchased from the animal facility at the Center for Experimental Animal, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China. The animals were maintained under pathogen-free conditions. Mice were generally used in experiments at 8–12 weeks of age.

2.2. Antigen

Killed flea antigens (cat no. B22-66-1C9) for veterinary use was purchased from Greer Laboratories, Inc. (Lenoir, NC). Complete Freund's adjuvant (CFA), and incomplete Freund's adjuvant (IFA) were obtained from Sigma–Aldridge Inc. (St. Louis, MO) and stored at 4 °C.

2.3. Allergy induction and clinical scoring

Female BALB/C, or DBA/2 mice were randomly divided into groups of six and administered with 100 μl (100 μg/mouse) killed flea antigens emulsified (v/v, at 1:1 ratio) in CFA and injected intramuscularly (i.m.). For the evaluation of injection routes, C57BL/6 mice were administered intramuscularly, intraperitoneally (i.p.), subcutaneously (s.c.) and in the footpads (i.f.) with 100 μl of killed flea antigen (100 μg/mouse) emulsified (1:1) in CFA. For assessment of the effect of adjuvants, C57BL/6 mice were administered i.m. with killed flea antigen alone, or with killed flea antigen in CFA, or in incomplete Freund's adjuvant. For the dose dependent study, C57BL/6 mice were administered with various doses of flea antigen in CFA (50 μg, 100 μg, 200 μg, 300 μg per mouse/100 μl). All animals were immunized twice, at an interval of 2 week. The degree of allergic reactions were assessed and graded on a scale of 0–3 as previously described by Wilkerson (Wilkerson et al., 2004) with minor modifications as indicated in Table 1. The lesional clinical signs were assessed every 3 days starting from Day 0 to Day 30. A lesional score was given by adding the number of the score of alopecia. Each lesion was graded on a scale from 0 to 3: a score of 0 was given for no signs; 1, for mild; 2, for moderate; and 3, for severe. Three body sites were assessed from each side: from the injected right thigh; (1) right side hip; (2) right side of dorsum to abdomen; (3) right side of neck to head; (4) left side hip; (5) left side of dorsum to abdomen; (6) right side of neck to head. The scores from the location on the left side of body were multiplied by two and summed for each animal at each observation period and the grand total was calculated.

All experimental protocols concerning the handling of mice were in accordance with the requirements of the Institutional Animal Care and Use Committee at the China Agricultural University.

2.4. T cell proliferation

Spleens were isolated from mice and single-cell suspensions were prepared by mechanical dissociation 21 days after antigen administration. Cells (3×10^6 cells/ml) were resuspended in complete RPMI 1640 medium supplemented with 3% heat-inactivated