Mite-specific IgE antibody response and dermatitis in NC mice infected with *Myobia musculi*

Yosaburo Oikawa\(^{1)}\), Teruaki Ikeda\(^{1)}\), Yutaka Kawakami\(^{2)}\), Masami Kojima\(^{2)}\), Hiroyuki Matsuoka\(^{3)}\) and Akira Ishii\(^{3)}\)

\(^{1)}\) Department of Medical Zoology, Kanazawa Medical University, Ishikawa, 920-0293 Japan
\(^{2)}\) Department of Ophthalmology, Kanazawa Medical University, Ishikawa, 920-0293 Japan
\(^{3)}\) Department of Medical Zoology, Jichi Medical School, Tochigi, 329-0498 Japan

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**Abstract:** *Myobia musculi*-infected NC mice produced IgE antibody specific for *M. musculi*, and this IgE antibody was not reactive to the antigens of other species of Acari. NC mice did not develop dermatitis only when reared in the conventional environment, but did dermatitis following infection with *M. musculi*.

**INTRODUCTION**

NC mice develop dermatitis spontaneously when reared in the conventional environment. Since this dermatitis is histopathologically similar to human atopic dermatitis (Suto et al., 1999; Matsuda and Tanaka, 2000), the NC mouse is expected to be useful as an experimental model of this disease. In fact, this model has been used in drug screening for atopic dermatitis (Sengoku et al., 1998; Kotani et al., 2000). Recently, it was suggested that some species of mites might be concerned in the crisis of this dermatitis. When the mites *Myoceptes musculinus* (Morita et al., 1999) or *Myobia musculi* (Iijima et al., 2000) were exterminated from the mice, the dermatitis improved with a decrease in the total IgE level. As the environmental factors are complicated, the correlation is not always positive between total IgE level and the cutis symptoms in human atopic dermatitis (Yoshiike and Takamori, 1996; Osuna et al., 1996). However, it is easy to control environmental factors and the mites in the experimental model of NC mouse dermatitis. It may be possible to examine the effects of mite-specific IgE antibodies in the dermatitis in detail. In this report, we observed the relationship between formation of *M. musculi*-specific IgE and IgG antibodies and dermatitis in NC mice.

**MATERIALS AND METHODS**

**Mice**

NC/Num mice from Institute for Experimental Animal, Faculty of Medicine, Kanazawa University (20–25 g body weight) by bred in a specific pathogen-free (SPF) environment were transferred to the conventional environment, and infected with mites by cohabitation with *M. musculi*-infected NC/Nga mice (Kojima et al., 1998) from Department of Applied Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University. A group of SPF mice were reared in the same conventional environment without infection as the uninfected controls.

After 6 months of cohabitation or rearing in conventional environment, 5 num-
bers of NC mice of two groups were euthanized by intraperitoneal injection of pentobarbital and the blood was collected from the right atrium by a syringe. Then the centrifugal separated sera were stored at −20°C until used.

**Antigen**

*M. musculi*-infected NC mice were euthanized by intraperitoneal injection of pentobarbital. The mites (3–5×10³) liberated from the bodies of the mice were collected using a paintbrush. Collected mites were added to 1 ml of physiological saline and homogenized using a glass homogenizer, and were then frozen and thawed. This extract was centrifuged at 5,000 rpm for 10 min, and the supernatant was used as the *M. musculi*-antigen solution (0.3 mg/ml protein content). Antigen solutions of *Leptotrombidium scutellare* larvae and *Ornithodorus mubata* nymphs were produced by similar procedures, and the antigen solution of *Dermatophagoides farinae* was produced by extracting the mixture of mites and culture medium. These were used for comparison with the *M. musculi* antigen solution.

**Enzyme-linked immunosorbent assay (ELISA)**

Ninety-six-well plates (Immuno Module, Nunc, Denmark) were sensitized overnight at 4°C with antigen-solution (10 µg protein/ml) in carbonic acid buffer solution (pH 9.6). After washing with phosphate buffered saline containing 0.05% polyoxyethylene sorbitan monolaurate (PBS/T), the plates were used as sensitized plates.

For IgG-ELISA, the wells were incubated for 45 min at room temperature with 100 µl of mouse serum diluted 1 : 200 with PBS/T containing 10% normal goat serum. After washing with PBS/T, the wells were incubated for 45 min at room temperature with 100 µl of peroxidase-conjugated goat anti-mouse IgG antibody (Cappel, PA) diluted 1 : 1,000 with PBS/T containing 10% normal goat serum.

For IgE-ELISA, the wells were incubated for 45 min at room temperature with 100 µl of mouse serum diluted 1 : 10 with PBS/T containing 10% normal goat serum. After washing with PBS/T, the wells were incubated for 45 min at room temperature with 100 µl of peroxidase-conjugated rat anti-mouse IgE monoclonal antibody (Clone Lo-Me-3, Technopharm Biotechnology) diluted 1 : 1,000 with PBS/T containing 10% normal goat serum.

Finally, the wells were incubated for 45 min at room temperature with 100 µl of the substrate (0.002% 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) di-ammonium salt, Sigma, MO) in citrate buffer (pH 4.5) containing 0.0003% hydrogen peroxide. The optical density was measured at 405 nm and the correction value (called ELISA OD) was calculated by subtraction of the optical density at 620 nm.

**Passive cutaneous anaphylaxis (PCA)**

Aliquots of 0.1 ml of mouse sera diluted 1 : 10 were injected intracutaneously in the shaved back of Wistar rats anaesthetized with ether. After 4 hours, 1 ml of antigen saline solution (containing 0.3 mg of protein) and 1% Evans blue was injected intravenously to rats anaesthetized with ether. After 20 minutes, rats were sacrificed by overdose of ether, and the diameters of macula cerulea on the back skin were measured.

**Statistical analysis**

Student's *t*-test was done for statistical approval of the data.

These experiments using animals were conducted by complying with “Guidelines for Animal Experimentation” (Japanese Association for Laboratory Animal Science, 1987).

**Results**

**State of infection**

The NC mice showed frequent scratch-
Fig. 1. Parasitism by \textit{M. musculi} in NC mice. 1a, States of the dermatitis in those mice; 1b, Mites gathered on the hair tip.

Fig. 2. \textit{M. musculi} embedded in the gum-chloral.

\begin{itemize}
\item[\textbullet] Mites parasitized not only the proterosoma with intense dermatitis but also the normal hysterosoma of the hair. The species was identified examining 100 mites, and all were \textit{M. musculi} (Fig. 2). Estimation number of mites was $6-9 \times 10^3$ per mouse. The control mice transferred to the conventional environment from the SPF environment at the same time but not infected with \textit{M. musculi} showed no dermatitis and no mites were isolated from the bodies of mice after euthanasia.
\end{itemize}

\textbf{IgG antibody titer}

The titers of anti-\textit{M. musculi} IgG antibody were compared between sera from \textit{M. musculi}-infected mice and uninfected control mice (both $n=5$). The mean± standard deviation of ELISA OD in sera diluted 1:200 was 0.46±0.25 in infected mice and 0.045±0.003 in controls. This difference between the sera of infected and uninfected mice was significant ($P<0.01$) (Fig. 3). In ELISAs using \textit{L. scutellare} larvae and \textit{O. mubata} nymphs as antigens, ELISA OD values for all sera were lower than 0.04, and were no differences between \textit{M. musculi}-infected and uninfected mice (Fig. 3).
Fig. 3. IgG antibody titers against the antigens of *M. musculi* and other species of Acari. *O. m., O. mubata; L. s., L. scutellare; M. m., M. musculi; Inf., NC mice infected with *M. musculi; Uninf., uninfected control NC mice; ELISA OD, compensated ELISA optical density. Bars in figures show the means of five mice.

**IgE antibody titer**

The titers of anti-*M. musculi* IgE antibody were compared between sera from *M. musculi*-infected mice and uninfected control mice (both *n*=5). The mean±standard deviation of ELISA OD in sera diluted 1:10 was 0.54±0.069 in infected mice and 0.13±0.009 in controls. This difference between the sera of infected and uninfected mice was significant (*P*<0.01) (Fig. 4).

**PCA antibody titer**

The titers of anti-*M. musculi* PCA antibody were compared between sera of *M. musculi*-infected mice and uninfected control mice (both *n*=5). The diameters of macula cerulea by PCA using sera diluted 1:10 were 2.2±0.75 cm (the mean diameter of five mice±standard deviation) in the infected mice, but the macula cerulea was not recognized in the sera of uninfected mice (Fig. 5). In PCA using *D. far-
**Fig. 5.** PCA antibody titers against the antigens of *M. musculi* and other species of Acari.

*O. m., O. mubata; D. f., D. farinae; M. m., M. musculi; Inf., NC mice infected with *M. musculi*; Uninf., uninfected control NC mice; DIAMETER (cm), diameter of macula cerulea. Bars in figures showed the means of five mice.

**inae** or *O. mubata* nymphs as the antigen, no macula cerulea was observed in the sera of infected or uninfected mice (Fig. 5).

**DISCUSSION**

It has been known for about 40 years that dermatitis in mouse caused by mites often occurs in aged (over one year old) mice of the *dd* strain (Fukui et al., 1961). In the contrast, in the NC mice it was reported recently that dermatitis began at 7–15 weeks old and the total IgE level rose before development of dermatitis (around 4 weeks old) (Matsuda et al., 1997). Two species of mites have been reported to be the cause of dermatitis in NC mice; *M. musculinus*, which seemed to not feed on the body fluid, and *M. musculi* that dose feed on the body fluid. Morita et al. (1999) reported for *M. musculinus* that histamines were released antigen-specifically from mast cells sensitized with the serum of infected NC mice. Iijima et al. (2000) reported for *M. musculi* that total IgE decreased in mite-infected NC mice following ridding of the animals of mites, and that the dermatitis also disappeared simultaneously. And also Morita et al., (1999) reported that a decrease in the total IgE level by elimination of *M. musculinus* by ivermectin spray. They reported a close relationship among the mite infection, total IgE level and the severity of dermatitis.

In our experiment, mite-infected NC mice produced IgE antibodies that were specific to *M. musculi*, and as demonstrated by PCA test, there was no cross-reactivity of sera of *M. musculi*-infected mice to the antigens of the other species of Acari. That is, the NC mice were sensitized to the mite antigen by mite-infection and produced a specific IgE antibody continuously. Specific IgG antibody response in Acari-infected animal is well known and also that in *M. musculi*-infected mice was observed in this study. However, the correlation between the titer of IgG and IgE antibodies was negative (data not shown) and this may suggest the difference of antibody-inducing antigens in both classes of antibody.

Koizumi and Hayakawa (1986) reported that NC mice developed dermatitis only when reared in the conventional environment. However, in our study, not only the rearing in the conventional environment but also the infection with *M. musculi* was necessary for the dermatitis of NC mice.
These results suggest that the mite antigen is an important allergen among those existing in the environment, and indicates the specificity of the mite antigen as an allergen involved in dermatitis in this model. This may be a useful animal model for examination of the relationship between mite antigens and dermatitis as the relationship between *Dermatophagoides* antigens and atopic dermatitis in humans.

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