— Note —

IgE Responses to *Fasciola hepatica* Extract in Several Inbred Mouse Strains

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IgE responses to *Fasciola hepatica* extract in 6 strains of mice (C3H/HeN, CBA/JN, DBA/2N, BALB/cAnN, C57BL/6N and NZBWF₁) were investigated. Serum anti-*F. hepatica* IgE antibodies were determined by passive cutaneous anaphylaxis (PCA). DBA/2N and BALB/cAnN mice showed higher IgE responses than other strains.—KEY WORDS : *Fasciola hepatica*, IgE response, mice

IgE antibodies are thought to play important roles in biodefence against parasites, as well as serious allergic diseases including polinosis and atopic dermatitis. Generally, parasitic infection causes a high IgE response in both humans and animals [1]. Therefore, several parasite-animal systems for studying IgE responses have been developed, and have been used in immunological and allergological investigations. Some of these systems use parasite antigens as the immunizing agents.

In this study, we investigated the IgE response to *Fasciola hepatica* extract in various mouse strains in order to obtain basic research information on the IgE-inducing mechanisms of parasite antigens. The IgG1 responses to *F. hepatica* extract were also investigated because IgE-secreting plasma cells were induced via an immunoglobulin class switch to C ϵ , mediated by C γ 1, during the differentiation of B cells [6]. Both class switches, from C μ to C γ 1 and from C γ 1 to C ϵ , were controlled by the same cytokine, interleukin-4 (IL-4) [4].

Four-week-old male and female mice (6 strains) were kindly donated by Charles River Japan Inc. The strains used were C3H/HeN, CBA/JN, DBA/2N, BALB/cAnN, C57BL/6N and NZBWF₁. Each strain of healthy mice was allocated to groups of 5 animals by stratified

randomization. Mice were housed in a room maintained at a temperature of $23\pm 2^{\circ}\text{C}$ and a relative humidity of $55\pm 10\%$. The animal room was ventilated at a frequency of 10 to 15 cycles/h, and artificially lighted 12 h per day. The animals were maintained on a pellet diet, MF (Oriental Yeast Co., Ltd.), and tap water *ad libitum*.

Adult worms of *F. hepatica* were homogenized with 20 volumes of 0.05 M ammonium bicarbonate containing 0.01 M ethylenediaminetetraacetic acid-2Na (EDTA-2Na). The homogenate was allowed to stand for 24 h at 4°C , and the supernatants were then obtained after centrifugation at $10,000\times g$, and 80% saturated with ammonium sulfate. The precipitate was redissolved in physiological saline and dialyzed against the same medium.

Each mouse was immunized with two intraperitoneal injections of $10\mu\text{g}$ protein/animal of *F. hepatica* extract with 1mg aluminum hydroxide adjuvant (alum), with a week between injections. Mice were bled 3 weeks after the second injection of antigen and the serum was separated by centrifugation. Serum samples were stored at -80°C until use.

The antigen-specific IgE antibodies were detected by heterologous passive cutaneous anaphylaxis (PCA) reaction using rats as the

Table 1. IgE responses to *F. hepatica* in mice

Strain	Male	Female
	Mean $\pm$ S.D.	Mean $\pm$ S.D.
C3H/HeN	2.4 $\pm$ 1.1	2.8 $\pm$ 0.8 ^{a, b, c, d}
CBA/JN	2.4 $\pm$ 0.5	1.2 $\pm$ 1.1 ^{a, E, F}
C57BL/6N	2.4 $\pm$ 0.9	1.8 $\pm$ 0.4 ^{b, G, H}
NZBWF ₁	1.0 $\pm$ 1.4 ^a	1.8 $\pm$ 0.4 ^{c, I, J}
DBA/2N	3.2 $\pm$ 0.8 ^a	3.8 $\pm$ 0.4 ^{d, E, G, I, k}
BALB/cAnN	2.4 $\pm$ 0.5	3.0 $\pm$ 0.0 ^{f, H, J, k}

Data converted by $\log_3$ (dilution/10) are shown as the mean PCA titer $\pm$ S.D. of 5 animals. Means are significantly different at the 1% level as indicated by capital superscripts in common and at the 5% level with small letters within each sex.

Table 2. IgG1 responses to *F. hepatica* in mice

Strain	Male	Female
	Mean $\pm$ S.D.	Mean $\pm$ S.D.
C3H/HeN	0.144 $\pm$ 0.036 ^a	0.180 $\pm$ 0.046 ^{A, B, c, d}
CBA/JN	0.138 $\pm$ 0.025 ^{b, c, d}	0.072 $\pm$ 0.005 ^{A, E, f, G}
C57BL/6N	0.087 $\pm$ 0.009 ^{a, b, E, f, G}	0.086 $\pm$ 0.018 ^{B, H, I, J}
NZBWF ₁	0.179 $\pm$ 0.026 ^{c, E, H}	0.253 $\pm$ 0.051 ^{c, E, H, K}
DBA/2N	0.121 $\pm$ 0.021 ^{f, H, I}	0.139 $\pm$ 0.039 ^{f, I, K, L}
BALB/cAnN	0.196 $\pm$ 0.044 ^{d, G, I}	0.240 $\pm$ 0.024 ^{d, G, J, L}

Data are shown as the mean O.D. $\pm$ S.D. of 5 animals. Means are significantly different at the 1% level as indicated by capital superscripts in common and at the 5% level with small letters within each sex.

recipients [3]. For the PCA test, the antisera were serially (3-fold) diluted starting with a 1:10 dilution. Serum anti-*F. hepatica* IgG1 antibody levels were determined by enzyme-linked immunosorbent assay (ELISA) according to a previously described method [2,5]. In this ELISA system, 1:16,000-diluted peroxidase-conjugated goat anti-mouse IgG1 (γ 1 chain specific, The Binding Site Inc.) in PBS was used for the second antibody. Data were statistically analyzed among all groups within each sex using Student's t-test. In the case of PCA titers, data were analyzed after base 3 logarithmic conversion.

The IgE responses measured by PCA reaction are shown in Table 1. DBA/2N mice showed the highest PCA titers in both sexes. Although DBA/2N mice showed a significantly

elevated IgE response only in comparison to NZBWF₁ males, the mice of this strain showed a significantly higher IgE response than all other strains and BALB/cAnN mice showed the second-highest IgE response in females. CBA/JN, C57BL/6N and NZBWF₁ mice showed lower IgE response in females than the other strains. Although it will be necessary to carry out more detailed investigations, DBA/2N and BALB/cAnN mice may be high IgE responder strains to *F. hepatica*. Therefore, these mouse strains may be useful for investigating the IgE response to antigens.

ELISA values for specific IgG1 antibodies against *F. hepatica* are shown in Table 2. NZBWF₁ and BALB/cAnN mice showed higher IgG1 antibody levels than other mouse strains in both sexes. CBA/JN, C57BL/6N and

DBA/2N mice showed comparatively low IgG1 responses.

In this study, some strains of mice showed a difference between the IgG1 and IgE responses to the same antigen. That is, the NZBWF₁ mice, which showed the highest IgG1 response, had the lowest IgE response, and DBA/2N mice, which showed the highest IgE response, had a comparably low IgG1 response. Although the mechanisms responsible for these opposite responses should be examined in a future study, they may provide important information elucidating the mechanisms regulating the IgE and IgG1 responses.

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近交系マウスの肝蛭 (*Fasciola hepatica*) 抽出液に対する IgE 応答

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肝蛭 (*Fasciola hepatica*) 抽出液に対する 6 系統のマウス (C3H/HeN, CBA/JN, DBA/2N, BALB/cAnN, C57BL/6N および NZBWF₁) の IgE 応答を,

受身皮膚アナフィラキシー (PCA) 反応を用いて検討した。その結果, DBA/2N および BALB/cAnN が他の系統に比べて高い IgE 応答を示した。