Characterization of ASK Mice, a Strain Highly Sensitive to Anaphylactic Shock

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A mouse strain named ASK that was originally isolated from El (epilepsy) mice has been shown to be highly sensitive to anaphylactic shock. Here, we characterized the bases of the sensitivity of ASK mice in comparison with the parental strain, El. More than 90% of ASK mice, but not El mice that had been sensitized either actively or passively, died within 1 h following an antigen challenge. The anaphylactic death was effectively blocked by diphenhydramine. Plasma histamine levels increased by 30–50 fold in ASK after the antigen challenge, but only a 2–3-fold increase was observed in El mice. All (El×ASK) F₁ mice, either male or female, showed an ASK-like phenotype, suggesting that the impaired plasma histamine response in El mice is due to some recessive mutation(s). Consistent with the plasma histamine responses, cultured mast cells derived from El bone marrow showed impaired potency to degranulate in response to surface IgE engagement, in contrast to ASK mast cells which undergo normal degranulation. Another characteristic feature of ASK mice is their sensitivity to histamine, since 75% of the mice were killed by the subcutaneous administration of 100–200 mg/kg histamine, while C3H and BALB/c mice were resistant to even 600 mg/kg histamine. Taken together, the major bases of the susceptibility to anaphylactic shock in ASK mice are thought to be the enhanced sensitivity to histamine and the recovered degranulation machinery in mast cells that is impaired in El mice.

Key words ASK mouse; epilepsy (El) mouse; anaphylactic shock; histamine; IgE; mast cell

Investigation of the genetic basis which controls the sensitivity to immediate hypersensitivity reactions is of great importance for understanding the mechanism of the onset of allergic diseases. Although mice have been extensively analyzed at genetic levels, they are considered inappropriate for this purpose due to their relative insensitivity to immediate hypersensitivity reactions compared with other experimental animals such as guinea pigs.¹⁾ Thus, murine strains susceptible to anaphylactic shock would be advantageous for analyzing mechanisms which control the sensitivity to acute allergic reactions.

An inbred strain of mice, El, has been regarded as a good genetic model for human epilepsy or complex partial seizures.^{2,3)} El convulsion can be induced by physical stresses such as rhythmic vestibular stimulation.^{4,5)} The major genes, *El-1* and *El-2*, responsible for this epileptic phenotype have been roughly mapped on chromosome 9 and chromosome 2, respectively.^{3,6)} Hamada has isolated a strain from an El colony that elicits no convulsion. Interestingly, this strain, named ASK, in contrast to El, has subsequently been shown to be highly sensitive to the anaphylactic shock induced by an antigen challenge.⁷⁾ However, the bases of this sensitivity in ASK mice have not been well analyzed. It has been shown that the susceptibility to local or systemic anaphylaxis is variable among various strains of mice.^{8,9)} However, the genetic basis which determines such different phenotypes remains to be elucidated. Thus, ASK and El mice, which share a common genetic background but show distinct susceptibility to anaphylactic shock, will be useful for elucidating the genetic and molecular bases which control the sensitivity to immediate allergic reactions.

In the present report, we characterized the susceptibility of ASK mice to anaphylactic shock in comparison with the

parental strain, El, and with some other mouse strains.

MATERIALS AND METHODS

Mice El and ASK mice (4–5 weeks of age) were purchased from SEAC Yoshitomi (Fukuoka, Japan). Male BALB/c, C57BL/6 and C3H/HeN mice were obtained from The Animal Breeding Facility of Okayama University Medical School (Okayama, Japan). They were used at 8–10 weeks of age.

Immunization and Challenge Mice were immunized with 10 µg of trinitrophenyl keyhole limpet hemocyanin (TNP-KLH) and 2 mg of alum. On day 10 post-immunization, mice were challenged intraperitoneally with 150 µg TNP-ovalbumin (OVA) in 0.2 ml saline. In some cases, mice were sensitized passively by an intraperitoneal injection of 50 µg of monoclonal anti-TNP IgG1 or anti-TNP IgE prepared as described elsewhere,¹⁰⁾ and were challenged with TNP-OVA 2 d later. The challenged mice were observed for the development of anaphylactic reactions up to 2 h after the challenge. A severe symptom is defined as repeated convulsion with tachypnea, and mild anaphylaxis as slight lethargy without convulsion. Anti-TNP IgG1 and IgE levels in the sera from immunized mice were determined by ELISA as described previously.^{10,11)}

Preparation of Cultured Mast Cells from Bone Marrow As described by Ogasawara *et al.*,¹²⁾ bone marrow cells from ASK or El mice were cultured at 5×10⁵ cells/ml in RPMI-1640 medium containing 10% fetal calf serum (FCS) in the presence of 5 ng/ml murine recombinant IL-3 (PeproTech Inc., NJ, U.S.A.) for 5 weeks. Approximately 90% of the resultant cells were confirmed to be granule-rich mast cells by toluidine blue-staining.

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Degranulation of Cultured Mast Cells Cultured mast cells were suspended at 5×10^5 cells/ml in RPMI-1640 medium containing 2% FCS (assay medium), and sensitized with $5 \mu\text{g/ml}$ anti-TNP IgE for 1 h. After washing the mixture with minimum essential medium (MEM) to remove the free IgE, 5×10^4 sensitized mast cells were incubated with varying concentrations of TNP-OVA or ionomycin in 0.2 ml assay medium for 20 min. All incubations were carried out at 37°C under 5% CO_2 and 95% air. After centrifugation, β -glucuronidase activity in the supernatants was assayed using *p*-nitrophenyl- β -D-glucuronide (Sigma, MO, U.S.A.) as described by Ogasawara *et al.*¹²⁾ The total enzyme activity was determined after lysing an equivalent number of mast cells with 0.085% Triton-X100. Data were presented as the mean of duplicate measurements. A representative result from three repeated experiments was shown.

Determination of Histamine The samples, plasma or cell lysate, were mixed with an equal volume of 0.8N perchloric acid followed by centrifugation at $1200 \times g$ for 15 min. The histamine content in the supernatants was determined fluorometrically by high performance liquid chromatography as described previously.^{13,14)}

Flow Cytometric Analysis of Type I Fc ϵ Receptor (Fc ϵ RI) Bone marrow-derived mast cells were incubated with $5 \mu\text{g/ml}$ biotinylated monoclonal anti-TNP IgE for 30 min on ice, then washed once with phosphate-buffered saline. After labeling them with $2 \mu\text{g/ml}$ fluorescein isothiocyanate (FITC)-avidin, the cells were analyzed for the expression of Fc ϵ RI by FACSCalibur (Beckton Dickinson).

RESULTS

Sensitivity of ASK Mice to Anaphylactic Shock As originally reported by Hamada,⁷⁾ we confirmed that ASK mice were far more sensitive to anaphylactic shock than the parental strain, El, as shown in Table 1. When TNP-KLH-primed ASK mice were challenged intraperitoneally with TNP-OVA on day 10 post-immunization, they developed anaphylactic symptoms such as lethargy, tachypnea and myoclonic jerk, and died within 1 h, while El mice did not show any such symptoms under the same conditions. The mortality of ASK mice was always more than 75% in several repeated experiments. Similar results were obtained with ASK and El mice that had been passively sensitized with anti-TNP IgE or IgG1. It was confirmed that BALB/c, C3H and C57BL/6 mice were resistant to anaphylaxis induced in the same manner (data not shown). These observations indicate that ASK mice are a highly sensitive strain to anaphylactic reactions. The sensitivity of ASK mice would not be due to their high IgE antibody responses, since ASK produced a slightly lower level of anti-TNP IgE and IgG1 than El when immunized with TNP-KLH and alum, as shown in Fig. 1.

Chemical Mediator Involved in the Anaphylaxis in ASK Mice To determine the major mediator responsible for inducing the anaphylactic shock in ASK mice, histamine and serotonin antagonists were examined for their suppressive effects. An H1 receptor antagonist, diphenhydramine, effectively blocked the anaphylactic death, as shown in Fig. 2. Ketanserin, a serotonin antagonist, showed weaker blocking effects than diphenhydramine, although the drug effectively prevented mice from dying. These data suggest that hista-

Table 1. ASK Mice: a Strain Sensitive to Anaphylactic Shock

	Mortality		
	Immunized ^{a)}	Anti-TNP IgE ^{b)}	Anti-TNP IgG1 ^{b)}
El	0/4	0/4	0/4
ASK	4/4	4/4	4/4

a) Mice were immunized with TNP-KLH/alum and challenged with TNP-OVA intraperitoneally on day 10 post-immunization. b) Mice were injected with monoclonal anti-TNP IgE or IgG1, then challenged with TNP-OVA after 2 d.

Fig. 1. Comparison of Anti-TNP Antibody Responses between ASK and El Mice

Male ASK, El and C57BL/6 mice were immunized with TNP-KLH/alum. Serum anti-TNP IgG1 (A) and anti-TNP IgE (B) levels were assayed on day 10 post-immunization. Each circle represents the value from an individual mouse tested. Horizontal lines indicate mean values.

Fig. 2. Diphenhydramine Effectively Blocked the Anaphylactic Shock in ASK Mice

TNP-KLH-primed ASK mice were challenged intraperitoneally with TNP-OVA on day 10 post-immunization. Diphenhydramine (20 mg/kg) or ketanserin (10 mg/kg) were administered subcutaneously 30 min prior to the antigen challenge. Each circle represents an individual mouse tested. Closed circles indicate dead animals.

mine is at least a major chemical mediator in inducing the acute anaphylactic reaction in ASK mice.

Plasma Histamine Levels after Antigen Challenge Systemic anaphylaxis in humans and rodents has been associated with a notable increase in plasma histamine levels.^{15,16} Thus, the enhancement of plasma histamine levels was compared between ASK and El mice when systemic anaphylaxis was induced by the antigen challenge in these mice. As shown in Fig. 3A, ASK showed about a 40-fold increase in plasma histamine level following the antigen challenge, while only a 2-fold increase was observed in El mice under the same conditions, thus suggesting that the distinct sensitivity of these two strains to the anaphylactic shock may be, at least in part, due to their differing increases in plasma histamine. The plasma histamine response of ASK is not considered anomalously high since it is comparable to that in C3H or C57BL/6 mice (data not shown).

As shown in Fig. 3B, (El $\times$ ASK) F₁ mice, either male or female, showed a similar level of enhancement of plasma histamine to ASK mice. Thus, it is suggested that El mice have some genetic defect(s) which leads to a reduced plasma histamine response, while this defect is thought to be recovered in ASK mice. Although detailed data are not presented here, the following two points were confirmed. (1) Lowered plasma histamine response in El may not be due to the enhanced degradation or excretion of released histamine since similar plasma histamine levels were attained when 5 or 10 mg/kg histamine was administered into ASK and El mice.

Fig. 3. Plasma Histamine Levels after the Antigen Challenge in Sensitized ASK and El Mice

(A) ASK and El mice were sensitized passively with an intraperitoneal injection of monoclonal anti-TNP IgE, and challenged with TNP-OVA after 48 h. These mice were bled 20 min after the challenge. Plasma histamine levels were assayed as indicated in Materials and Methods. (B) Female ASK ($n=3$), male El ($n=3$) and male ($n=6$) or female ($n=7$) F₁ derived from female ASK and male El were sensitized and challenged in the same manner. All animals were 8–9 weeks of age. Closed and open bars indicate histamine levels before and after the antigen challenge, respectively.

(2) Mast cell densities in peritoneal cells and in ear skin, and histamine contents in peritoneal cells in El mice were comparable to those in ASK mice. Thus, it is suggested that El mice are less potent in releasing histamine in response to antigenic stimuli. The following experiments were performed to confirm this possibility at the mast cell level.

Degranulation of Cultured Mast Cells from ASK and El Mice The efficiency of histamine release from mast cells was compared between ASK and El mice. Mast cells were propagated from their bone marrow cells by culture in the presence of interleukin 3 (IL-3) in order to obtain a sufficient number of cells. The cultured mast cells from each strain were sensitized with anti-TNP IgE followed by stimulation with TNP-OVA or a calcium ionophore, ionomycin. As shown in Fig. 4A, ASK-derived mast cells efficiently degranulated in response to either stimulus, as assessed by β -glucuronidase release, while El-derived mast cells released the enzyme much less efficiently under the same experimental conditions. Total β -glucuronidase levels and histamine contents were comparable between the two mast cell preparations. ASK mast cells underwent antigen-induced degranulation as efficiently as C57BL/6-derived mast cells (data not shown). Thus, it is suggested that the impairment of degranulation machinery in El mice is recovered in ASK mice. Lowered potency in IgE-mediated degranulation in El mast cells is not due to the reduced expression of Fc ϵ RI, as shown in Fig. 4B.

Sensitivity of ASK Mice to Histamine In addition to the recovered degranulation potency, we found that ASK mice were highly sensitive to histamine itself, as shown in

Fig. 4. Degranulation of Bone Marrow-Derived Cultured Mast Cells from ASK and El Mice

Cultured mast cells were prepared and sensitized with anti-TNP IgE as described in Materials and Methods. (A) Mast cells from each strain of mice were stimulated with the indicated concentrations of TNP-OVA or ionomycin. Degranulation was assessed by measuring the released vs. total β -glucuronidase activity. (B) Expression of Fc ϵ RI in ASK and El mast cells was estimated by flow cytometry. The curves indicated by arrows are negative controls.

Fig. 5. ASK and El Mice Are Sensitive to Histamine

(A) ASK, C3H, BALB/c mice were injected intraperitoneally with indicated amounts of histamine. Mortality and the degree of anaphylaxis were estimated up to 2 h after the injection. Open circles with an x within them indicate dead ASK mice. (B) Skin reaction induced by histamine injection in ASK, El and BALB/c mice. Mice were injected intradermally in the back with 2 μ g of histamine, followed by an intravenous injection of 0.2 ml of 0.5% Evans blue solution.

Fig. 5. They died at a high frequency when administered 100–200 mg/kg histamine, while all C3H and BALB/c mice tested were resistant to 600 mg/kg histamine. This was further confirmed by a histamine-induced skin reaction. When ASK, El and BALB/c mice were given histamine intradermally in the back, followed by intravenous injection of Evans blue dye, definite vascular leakage was observed in ASK and El, but was negligible in BALB/c mice (Fig. 5B). C3H and C57BL/6 did not give positive reactions either (data not shown). Thus, ASK, as well as El, are thought to be highly sensitive to histamine.

DISCUSSION

As has been reported by Hamada,⁷⁾ ASK mice were confirmed to be highly susceptible to systemic anaphylactic shock following an intraperitoneal challenge with the priming antigen, but the parental strain, El, was resistant to the same treatment. Recently, it has been reported that ICR mice, compared with 6 other murine strains including BALB/c and B6, died of anaphylactic shock at a high frequency by intravenous, but not intraperitoneal, antigen challenge.¹⁷⁾ Hamada has reported that ASK was more sensitive than the ICR strain. Anti-TNP IgG1, as well as anti-TNP IgE, was effective in inducing lethal anaphylactic shock in ASK mice, consistent with recent reports that anaphylactic shock can be in-

duced in mice under IgE-deficient conditions.^{17,18)}

Here, the comparative studies among ASK, El and their F_1 mice revealed the following points. (1) El mice have recessive mutation(s) that lead to impaired histamine release from mast cells, but this defect is recovered in ASK mice. (2) ASK as well as El mice are innately sensitive to histamine. These are thought to be the bases of the susceptibility to anaphylactic shock in ASK mice. This recessive phenotype of El is thought to be expressed at least in mast cells, since the mast cells derived from El bone marrow showed much lower potency to degranulate in response to the antigen than those from ASK.

In the rat basophilic leukemia cell line, it has been shown that the engagement of Fc ϵ RI leads to the activation of cytoplasmic protein tyrosine kinase Syk, which in turn phosphorylates phospholipase (PLC)- γ_1 and PLC- γ_2 , and finally results in the elevation of the intracellular Ca^{2+} level.¹⁹⁾ These signaling pathways leading to histamine release can be bypassed by stimulating the cells with a calcium ionophore, ionomycin. El mast cells showed reduced responsiveness to both the antigen and ionomycin, thus suggesting that a defect in El mast cells resides in at least the downstream of Ca^{2+} influx.

ASK mice were originally isolated from an El colony as a non-epileptic strain. An epileptic phenotype is thought to be due to a recessive mutation since (El $\times$ ASK) F_1 showed no convulsion when it received physical stresses.⁷⁾ Here, we showed that plasma histamine increased normally after the antigen challenge in the F_1 mice, in contrast to the El mice. The correlation between histamine and epilepsy has not been well investigated, but some interesting observations have been made implicating their relevance.^{20,21)} The genetically epilepsy-prone Krushinski-Molodkina (KM) rat showed lower histamine levels in certain brain regions, including the striatum, hippocampus, amygdala, midbrain, thalamus and hypothalamus, than the epilepsy-resistant Wistar rat. Further, it has been shown that audiogenic seizures of KM rats were suppressed by metoprine, which can markedly increase brain histamine.²²⁾

We confirmed that not only ASK but also El mice show innate sensitivity to histamine. It has been generally considered that mice are relatively insensitive to histamine compared with guinea pigs.²³⁾ Thus, it is expected that ASK and El mice will also be useful for analyzing the basis for histamine sensitivity.

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