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Quantitative Assessment of Immediate Cutaneous Hypersensitivity in a Model of Genetic Predisposition to Atopy

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Key Words

Immediate cutaneous hypersensitivity
Candidate genes
Inbred strains
Segregation analysis
Bonitur method

Abstract

Genetic predisposition and environmental factors modulate the expression of allergic phenotypes. The frequent allergic phenotype 'immediate cutaneous hypersensitivity' was established in mice as a model for atopy. Genetic dissection of this trait requires a robust procedure to assess the allergic phenotype. To this end, different mouse strains were immunized with birch pollen extract. Immediate cutaneous hypersensitivity reactions were induced through intradermal allergen exposure. Wheel formation was quantitated and expressed as a hypersensitivity score according to the bonitur method. This procedure identified A/J and C57BL/6 mice as high- and low-responder strains, respectively. Crosses of A/J and C57BL/6 mice should allow the characterization of mendelian factors responsible for the two extreme phenotypes identified here.

Introduction

Allergies are multifactorial diseases; genetic predisposition and environmental factors determine the manifestation of an allergic phenotype [1]. In humans, identification of genetic factors predisposing to allergic diseases is notoriously difficult because of differences in diagnostic criteria for the definition of allergic phenotypes, variable onset of the disease, etiological heterogeneity, heterogeneity of environmental factors and different ethnic backgrounds [2].

Human and murine immune systems are similar in all important mechanisms and functions concerning the development of allergic immune responses. As in humans allergic phenotypes in mice are T and B cell-dependent [3]. Furthermore, the same effector populations (mast cells and eosinophils) are necessary for the expression of an allergic phenotype [4]. In addition to the overall similarity of immunological mechanisms involved in allergic reactions, three features make the mouse a particularly attractive model

for studying underlying genetic factors: (1) an allergic phenotype can be created under controlled environmental conditions in a defined genetic background, (2) there is a large variety of homozygous inbred mouse strains with homozygous inbred progeny-like recombinant inbred mice, congenic mice and recombinant congenic mice, and (3) experimental crosses can be performed to enable segregation analyses of polygenic traits.

Allergic hypersensitivity reactions frequently involve the skin after allergic sensitization through the airways, the gut or the skin itself. Therefore the skin is routinely used in humans to test for immediate hypersensitivity reactions. In mice, comparable immediate cutaneous hypersensitivity (ICHS) reactions can be induced [5, 6]. We established ICHS upon immunization with birch pollen extract (BPE) and describe the quantitative assessment of ICHS, a reproducible and robust method. In addition, we identify a high-responder and a low-responder mouse strain.

Materials and Methods

Animals

Female A/J, AKR and C57BL/6 mice, aged 6–8 weeks, were purchased from Harlan Winkelmann (Borchen, Germany). The animals were held under standard conditions.

Birch Pollen Allergen

BPE from *Betula verrucosa* was obtained from ALK-Abello (Hørsholm, Denmark). The extract is a mixture of birch pollen proteins mainly consisting of Bet v 1, which is known to be an immunodominant peptide. BPE was dissolved in PBS and sterile-filtered through a 0.2- μ m membrane prior to injection.

Immunization Procedure

Mice were immunized with a mixture of 20 μ g BPE and 150 μ g aluminum hydroxide by intraperitoneal injections. Mice received allergen on day 1, 14 and 21. ICHS of 39 A/J and 32 C57BL/6 mice was established on day 28. Peripheral blood from tail veins of 9 A/J and 12 C57BL/6 mice was also taken on day 28.

Immediate Cutaneous Hypersensitivity

The skin of the belly was carefully shaved with an electric clipper. To improve visibility of the intradermal immune reaction, 100 μ l of a 0.5% Evans blue solution were injected intravenously 15 min prior to allergen challenge. Allergen (without adjuvant) was applied intracutaneously. To allow quantification of the immune response, four different concentrations of BPE were injected (100, 250, 500 and 750 ng). The mast cell-degranulating dye compound 48/80 (Sigma Chemicals, Deisenhofen, Germany) was used as a positive control, PBS served as a negative control. Injection volumes were 50 μ l. 15 min after the local challenge, skin reactions were analyzed. For this purpose, mice were sacrificed and the subcutis was dissected. Due to extravasation of blue-stained plasma proteins wheel sizes could be evaluated in almost all cases.

Bonitur Method

As about half of the wheel formations were asymmetric and therefore did not allow precise area measurement, quantification was performed by the bonitur method according to Kendall [7]. With this method complex traits are quantitated via estimation through raters. Skin reactions per animal were evaluated if the 48/80 dye wheel was positive (blue) and the PBS wheel was negative (colorless). The 48/80 dye wheel (maximal response) scored 3 and the PBS area (negative control) 0. The Bet v wheels were assessed by comparison to the maximal and minimal scores. The four dose-dependent Bet v scores were added up to yield a hypersensitivity score ranging from 0 to 12 points. The ICHS were evaluated by three independent investigators (raters). Kendall's coefficient of concordance, which is a measure of agreement among raters, was found to be $W = 0.928$. Calculation was performed with SPSS for Windows, release 7.0.

Quantification of Total and Bet v 1-Specific IgE

Sandwich ELISAs were performed as described previously [8]. For the assessment of Bet v 1-specific immunoglobulins plates were coated with 100 ng recombinant Bet v 1 (Bio-May, Austria) per well. Bet v 1-specific antibodies were detected with a sheep-antimouse Ig antibody (1.6 μ g/well; Biogenesis, New Field, UK). For standardization a pool serum from BPE-sensitized A/J mice was used with an assigned value of 10,000 arbitrary laboratory units/ml.

Results

Induction and Assessment of ICHS

In an initial set of experiments, several inbred mouse strains were tested for the severity of ICHS after sensitization to birch pollen allergen. Mice were immunized intraperitoneally with 20 μ g BPE in the presence of aluminum hydroxide as an adjuvant to polarize the immune response towards a Th2 response. After two booster injections, the development of allergic sensitization was investigated through the appearance of ICHS. Four different amounts of BPE (100, 250, 500 and 750 ng) were injected intracutaneously. Of the different strains tested, A/J mice responded to all four concentrations of BPE, whereas C57BL/6 did not react to any of these concentrations. Therefore these two mouse strains can be regarded as high-responder and low-responder phenotypes, respectively. Immunization protocols using less than 20 μ g of BPE did not result in maximal ICHS responses in any mouse strain tested (data not shown). Immunizations using more than 20 μ g BPE per injection maintained the phenotypic differences between the A/J, AKR and C57BL/6 strains (data not shown). Therefore BPE concentrations of 20 μ g per immunization were regarded as appropriate.

Quantification of ICHS with Bonitur Method

Skin reactions of about half of the high-responder mice were not sufficiently circumscribed to allow exact measurement of the intracutaneous edema. Therefore, skin reactions were quantified with the bonitur method which allows the quantification of subjective assessment of phenotypes. Three investigators (raters) independently evaluated the wheel areas after allergen exposure in comparison to reference substances inducing a maximal and a minimal wheel formation. These reference areas were scored with three and zero points, respectively. The allergen-induced areas were evaluated as follows: an area as large as the maximum = 3 points, not as large as the maximum, but still quite large = 2 points, small, but clearly larger than the minimum = 1 point, and same size as the minimum = 0 points. A high-responder mouse challenged with four concentrations of the allergen therefore yields 12 points, whereas a nonresponder mouse scores 0 points. The evaluation procedure is rather robust, as Kendall's coefficient of concordance for three raters valued $W = 0.928$.

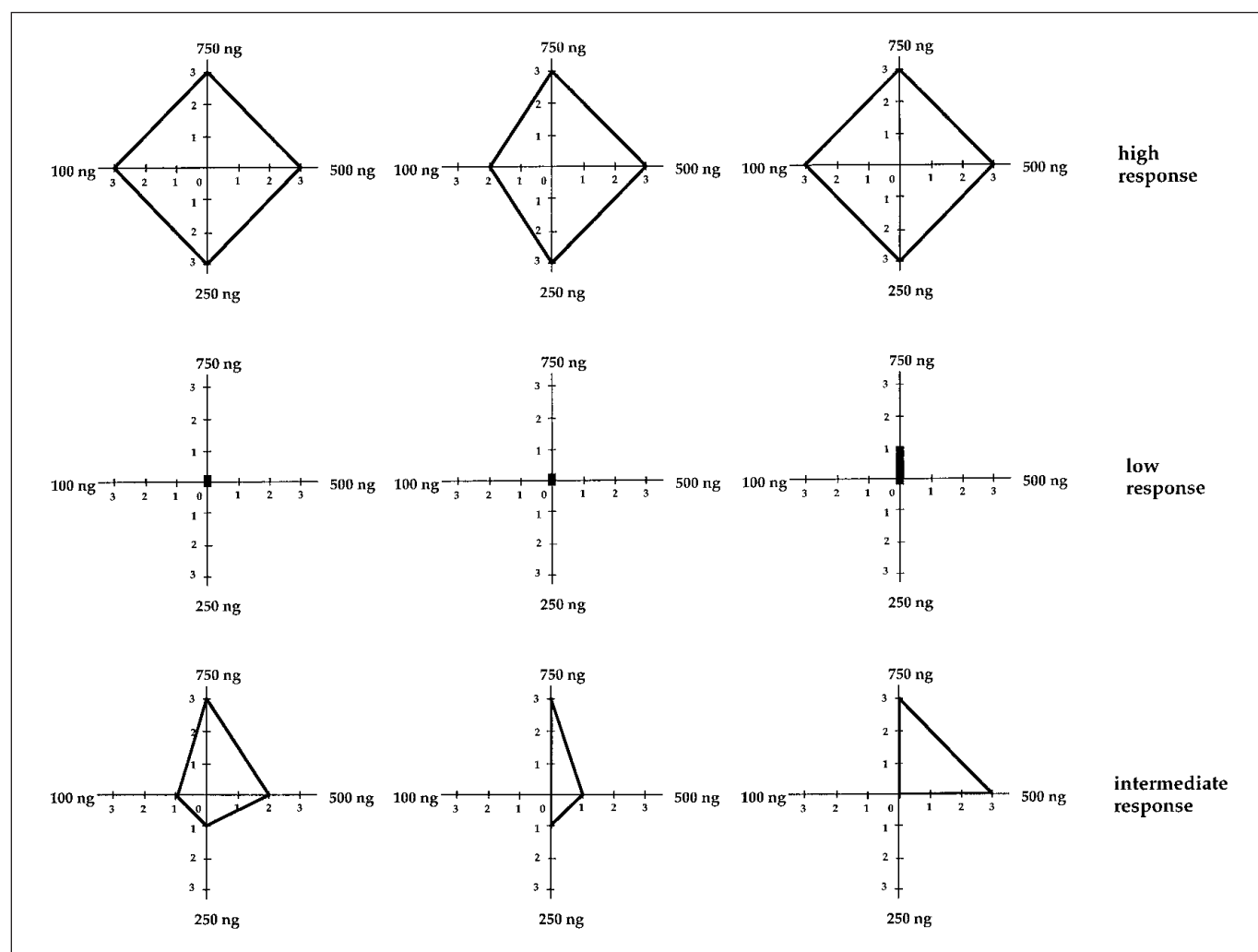
Figure 1 illustrates the visual appearance of skin edemas (fig. 1a) and the score sheets for high, intermediate and low responders (fig. 1b). Using this scheme, A/J mice were confirmed to be high responders, whereas C57BL/6 mice showed very little response to BPE (fig. 2). AKR mice dis-

Fig. 1. a Visual appearance of skin edemas. Compound 48/80 and PBS were used as reference substances for maximal and minimal skin reaction and injected intracutaneously. To visualize plasma extravasation due to mast cell degranulation 0.5% Evans blue solution was given intravenously 15 min prior to intracutaneous injection of the dyes. **b** Score sheets for 3 high, 3 low and 3 intermediate responder mice. For each concentration of BPE an axis was drawn and scaled from 0 to 3. Connecting the scores revealed areas which clearly distinguished between high responses (first row), low responses (second row) and intermediate responses (third row).



Compound 48/80 **PBS**

a



b

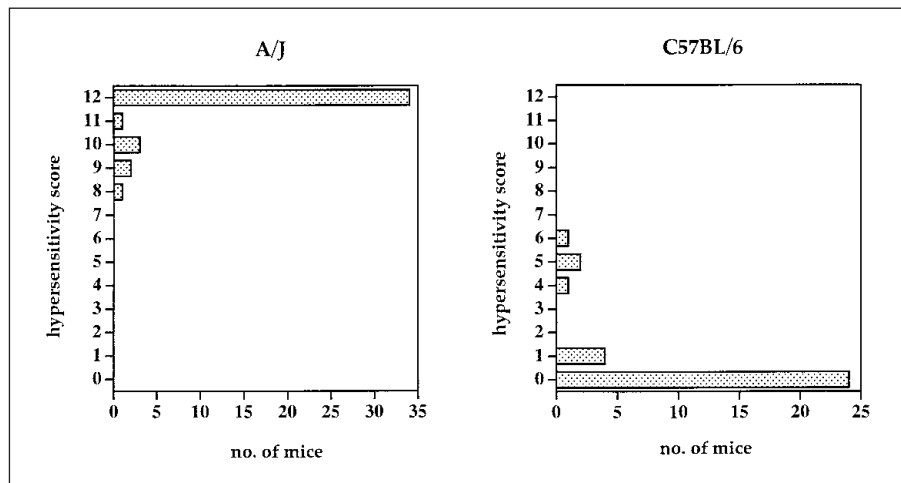


Fig. 2. Hypersensitivity scores of 39 A/J and 32 C57BL/6 mice.

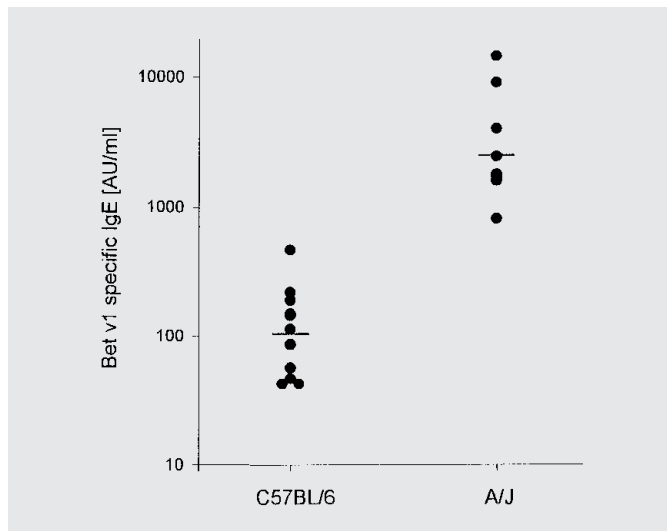


Fig. 3. Bet v 1-specific IgE with mean values of 9 A/J and 12 C57BL/6 mice. Concentrations are given as arbitrary units per milliliter.

played an intermediate phenotype (data not shown). These results were reflected in the amounts of Bet v 1 immunoglobulins in peripheral blood: in A/J mice high levels of Bet v 1-specific IgE (fig. 3), IgG1 and IgG2a (data not shown) could be detected whereas in C57BL/6 mice only small amounts of Bet v 1-specific IgE (fig. 3), IgG1 and IgG2a (data not shown) were detectable.

Discussion

ICHS equals the allergic phenotype 'positive skin prick test' in humans. The underlying mechanism in both cases is allergic sensitization due to IgE production and binding to mast cells and basophils [5, 9, 10]. Local allergen challenge induces the release of vasoactive substances followed by vascular leakage and extravasation of plasma proteins. The size of the resulting wheel thus correlates with the severity of allergic sensitization.

BPE contains allergens of high clinical relevance. Here we show that ICHS can be induced in susceptible mice by intraperitoneal immunization with BPE suspended in aluminum hydroxide. This route of application was chosen to ensure that a low response was not due to an insufficient immunization strategy but to a low response of the immune system towards the allergen. We chose the lowest dose of allergen sufficient to induce a full response in the high-responder strain, to avoid tolerance induction [11].

Allergies are regarded as polygenic traits and it is thought that more than two genes are responsible for the expression of an allergic phenotype [1]. If this were the case, the progeny of a high- and a low-responder strain will not only express the extreme parental phenotypes but also intermediate responses. Therefore it was important to apply a read-out system that would discriminate well between the extreme phenotypes. ICHS and its quantification by the bonitur method fulfill these criteria.

From our present data, we also conclude that MHC is not the dominating gene cluster for the expression of ICHS since AKR mice do not show maximal responses although they are sharing identical MHC class II alleles with A/J mice.

In summary, a quantitative allergic phenotype in response to BPE was established in mice A/J and C57BL/6 mice were defined as high- and low-responder strains, respectively. Large informative crosses between these two strains should make possible the characterization of mendelian factors responsible for the two extreme phenotypes identified here.

Acknowledgements

We thank Avrion Mitchison for continuous fruitful discussions. This work was supported by the Frauenförderung of the FU Berlin and the Fritz Thyssen Stiftung (AZ 1995-1079).

References

- 1 Casolaro V, Georas SN, Song Z, Ono SJ: Biology and genetics of atopic disease. *Curr Opin Immunol* 1996;8:796.
- 2 Ruffilli A, Bonini S: Susceptibility genes for allergy and asthma. *Allergy* 1997;52:256.
- 3 Mombaerts P, Mizoguchi E, Ljunggren HG, Iacomini J, Ishikawa H, Wang L, Grusby MJ, Glimcher LH, Winn HJ, Bhan AK, Tonegawa S: Peripheral lymphoid development and function in TCR mutant mice. *Int Immunol* 1994; 6:1061.
- 4 Nagai H, Yamaguchi S, Maeda Y, Tanaka H: Role of mast cells, eosinophils and IL-5 in the development of airway hyperresponsiveness in sensitized mice. *Clin Exp Allergy J* 1996;26: 642.
- 5 Saloga J, Renz H, Lack G, Bradley KL, Greenstein JL, Larsen G, Gelfand EW: Development and transfer of immediate cutaneous hypersensitivity in mice exposed to aerosolized antigen. *J Clin Invest* 1993;91:133.
- 6 Herz U, Lumpp U, Casimiro da Palma J, Enssle KH, Takatsu K, et al: The relevance of murine animal models to study the development of allergic bronchial asthma. *Immunol Cell Biol* 1996;74:209.
- 7 Kendall MG: Further contributions to the theory of paired comparison. *Biometrics* 1955;11:43.
- 8 Herz U, Braun A, Rückert R, Renz H: Various immunological phenotypes are associated with increased airway responsiveness. *Clin Exp Allergy* 1988;28:625.
- 9 Watanabe N, Ovary Z: Antigen and antibody detection by in vivo methods; a reevaluation of passive cutaneous anaphylactic reactions. *J Immunol Methods* 1977;14:381.
- 10 Daser A, Meissner N, Herz U, Renz H: Role and modulation of T-cell cytokines in allergy. *Curr Opin Immunol* 1995;7:762.
- 11 Levine BB, Vaz NM: Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. A potential mouse model for immune aspects of human atopic allergy. *Int Arch Allergy* 1970;39:156.

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