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Research paper

Stratification of peanut allergic murine model into anaphylaxis severity risk groups using thermography

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A B S T R A C T

Murine models are readily used to investigate mechanisms potentially involved in anaphylaxis. Determining successful sensitization with current methods remain potentially lethal, invasive, expensive and/or cumbersome. Here we describe the use of thermography to read intradermal testing to detect peanut allergic sensitization in the murine model and as a first time sensitive tool for anaphylaxis stratification. The relative wheal size in the thermal image can be used to stratify anaphylaxis severity risk groups prior to a challenge. This screening method is nonlethal, inexpensive, minimally invasive and can be carried out expeditiously.

1. Introduction

Murine models are readily used to investigate mechanisms potentially involved in anaphylaxis (Liu et al., 2016; Bøgh et al., 2016). Determining successful sensitization prior to an allergic challenge has historically been problematic. Initially the use of cutaneous anaphylaxis (both immediate and passive) was employed. This method, although successful, involved multiple steps that often led to the euthanizing of the mouse in order to read the results (Ovary, 1958). The euthanizing step prohibits challenging the same mouse, making it undesirable for the anaphylaxis models. Immediate cutaneous hypersensitivity testing can be carried out in a non-lethal form but still requires multiple steps (Saloga et al., 1993). Serum specific immunoglobulin levels in the murine model are relatively expensive, blood draws are invasive, and it also requires multiple steps (Birmingham et al., 2003). None of methods mentioned used to determine allergic sensitizations have been able to predict the degree of an allergic reaction within a murine model (Liu et al., 2016; Bøgh et al., 2016). Thus, they cannot control for disease state severity which hampers mechanistic and therapeutic investigations.

In vivo murine model allergy testing traditionally relies on taking measurements at the end of (or late in) the cutaneous reactions. Yet the literature reports that allergic reactions to have at least 2 phases: early and late (Sampath et al., 2017). An exhaustive literature search could not find any correlation studies on *in vivo* allergy testing measurements taken early in the cutaneous reaction and anaphylaxis severity. We hypothesize that the use of thermal imaging can determine successful sensitization and that early cutaneous measurements will allow for the stratification of anaphylaxis severity risk groups in the peanut murine model prior to a challenge.

2. Materials and methods

All procedures and manipulations of animals have been approved by the Institutional Animal Care and Use Committee (IACUC) # 2015–0161 in accordance with the United States Public Health Service *Policy on the Humane Care and Use of Animals*, and the NIH *Guide for the Care and Use of Laboratory Animals*. The facilities at Case Western Reserve University also comply with regulations under the Federal Animal Welfare Act, the US Department of Agriculture, the Ohio State Department of Health, and Ohio State and City laws.

Twenty unshaven C3H/HeJ female mice were sensitized to peanuts with oral crude peanut extract and cholera toxin protocol previously described (Li et al., 2000; Jhaveri and Bonfield, 2015). All intradermal (ID) testing was performed 4 to 6 weeks after the last week of sensitization. Intradermal testing with crude peanut extract (CPE) was only attempted once per mouse. Intraperitoneal (IP) CPE challenges were conducted between 8 and 14 days after ID testing. The same CPE batch

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was used for sensitization, ID testing and IP challenge. Ten peanut naïve C3H/HeJ female mice were used as controls.

A United States dime (17.9 mm in diameter), 30 μL histamine (0.1 mg/1 mL, commercially obtained from GREER Laboratories), and 30 μL saline were used as controls. Crude peanut extract (25% protein by BCA) concentration used for ID testing was 1.67 μg of protein/μL in phosphate buffered solution (PBS). A total of 30 μL of solution containing 50 μg of protein was injected for each ID test. The control solutions and the CPE were allowed to reach room temperature prior to injecting.

Thermal images using a FLIR i3 were taken prior to ID injection, immediately following and at 10 min intervals up to 30 min. Thermal images were captured from 1 to 1.5 ft from the mouse. Special thermal chambers were not used. Mice were held inside an active hood, while the thermal imager was held outside the hood. The hood sash was raised so as not to interfere with the reading of the thermal imager. A distance of approximately 1 to 1.5 ft was chosen because it was the closest distance that could capture the full body of the mouse in one image while providing adequate contrast of the thermal topography of the ID testing. This optimal position in distance is specific to the type of thermal detector used. Three images were taken at each interval. The average longest wheal diameter was used for stratification calculations (Peppers et al., 2017). Within the 3 images, two of the images were always within 4% or less of each other. The diameter of the CPE was divided by the diameter of the US dime image within the same picture. If one image was poor or > 10% different than the other two images the average of the other two images was used. Measurements of thermal images were performed using the free version of FLIR software.

Intraperitoneal challenges were conducted with 185 μg of protein in 100 μL of PBS. Control mice were injected with 100 μL PBS. In one mouse the IP injection syringe needle became dislodged and the majority of the injection did not enter the mouse. The mouse was scratched from all rectal temperatures and clinical scores.

Core temperatures were measured rectally with a digital thermometer. The lowest core rectal temperature was recorded between 30 and 60 min in each mouse. Clinical scores 0–5 were taken every ten minutes and prior to rectal temperatures taken for the same time interval. The scoring scale was defined as 0: no symptoms 1: increased scratching, rubbing around the nose and head, and/or ear canal digging with hind legs 2: Puffiness around eyes and/or mouth, decreased activity with increased respiratory rate 3: Periods of motionless for > 1 min, lying prone on stomach 4: No response to whisker stimuli, reduced or no response to prodding, momentary tremor 5: Sustained tremors, convulsion or death.

Two investigators kept separate clinical scores and shared results only after the conclusion of each run. One investigator was blinded to ID predictions. The lowest blinded scores were used for statistical purposes.

Serum samples were collected from the C3H/HeJ mice sensitized to peanuts 3–5 days after intradermal testing and ~ 1-week prior to IP challenges by retro-orbital puncture and stored at −80 °C prior to testing. Total serum immunoglobulin IgE was run on 5 low risk mice and 4 high risk mice using affymetrix eBioscience Mouse IgE Elisa Ready-SET-Go!® kit on a Molecular Devices VERSAmax tunable microplate reader.

GraphPad Prism 7 was used to calculate unpaired, two tailed student *t*-tests and one-way Analysis of Variance (ANOVA) with 95% confidence levels. The relative % of CPE was determined by dividing the longest diameter of the CPE by the diameter of the dime in the same thermal image and then multiplying by 100 ((CPE/dime)*100). Stratification cut off determinations were theoretically derived prior to all challenges as described in the discussion section.

3. Theory and calculations

In vivo allergy testing historically has relied on taking measurements at the end (or late in) of the cutaneous reaction. Allergic reactions are reported to have at least 2 phases: early and late. Taking measurements early in order to ascertain differences in initial rates of allergic reactions may be an indicator of anaphylaxis severity, and to the best of our knowledge, has never been reported or explored in the murine model.

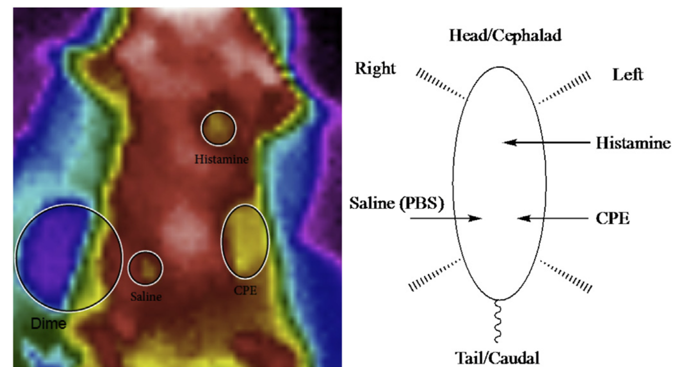

Fig. 1. Example of Thermal Image.
Picture depicts the ventral side of the mouse with the head towards the **top** and the tail at the bottom. Measurements were taken at ten minutes after intradermal injection.

4. Results

Testing and thermal images were carried out on the ventral side of the mouse as depicted in Fig. 1. The head is at the top and the tail is at the bottom of the picture. The histamine (top center) is outlined in a white circle, along with the saline (low abdominal on left). The (CPE) intradermal is on the right mid-abdomen outlined in the white circle as well. Wheal formation is seen in yellow. The histamine and CPE increased in size over time while the saline decreased in size (data not shown). The dime is depicted in purple to the left of the mouse.

Specific measurements from the thermal images at 10 min for the size of the peanut, saline, and two positive controls are listed in Table 1 for each mouse. Mouse 4 in Table 1 did not display a response at 10 min to peanut, however at 20 min a yellow thermal wheal was seen (data not shown).

An unpaired two tailed student *t*-test on lowest core temperature measured between control mice and sensitized mice during an intraperitoneal challenge afforded a significant $p < .0001$ (Fig. 2).

Fig. 3 shows the results of the two severity risk groups, which was determined by the relative size of thermal cutaneous edema of the CPE and dime at 10 min (> / = 60% high and < 60% low risk groups). This grouping method yielded a $p = .0003$ and the only fatalities during the challenge were in the high risk group (Fig. 3, Left). However, when stratification was determined using ID measurements at 20 min the correlation decreased to $p = .049$ (Fig. 3, Right).

The three anaphylaxis severity risk groups were based on the relative peanut ID thermal image sizes set at > / = 70, < 70 but > / = 50, and < 50% for high, medium and low risk respectively. An ANOVA of the three severity risk grouping yielded a $p < .0001$ (Fig. 4, Left).

If the ID measurements taken at 20 min were used to place the grouping there was a loss of statistical significance, $p = .3571$ with the 3 stratification method (Fig. 4, Right). Blinded clinical scores were also used and compared with risk groups yielding a $p = .0006$ with an unpaired two-tailed student *t*-test (Fig. 5).

Linear regression of the CPE/dime relative wheal size and degree of hypothermia yielded an R^2 of 0.77685 (Fig. 6). Elimination of the outlier mouse 4 with a "0" relative % size at ten minutes increased the R^2 to 0.8735. Linear regression calculations of the CPE/histamine relative wheal size and the degree of hypothermia yielded an R^2 of 0.23371 (data not shown). An ANOVA of the CPE/histamine relative % size yielded a $p = .0033$ in Fig. 7 (low risk = 0–100%, moderate >

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