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A distinct microbiota composition is associated with protection from food allergy in an oral mouse immunization model

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ABSTRACT

In our mouse model, gastric acid-suppression is associated with antigen-specific IgE and anaphylaxis development. We repeatedly observed non-responder animals protected from food allergy. Here, we aimed to analyse reasons for this protection. Ten out of 64 mice, subjected to oral ovalbumin (OVA) immunizations under gastric acid-suppression, were non-responders without OVA-specific IgE or IgG1 elevation, indicating protection from allergy. In these non-responders, allergen challenges confirmed reduced antigen uptake and lack of anaphylactic symptoms, while in allergic mice high levels of mouse mast-cell protease-1 and a body temperature reduction, indicative for anaphylaxis, were determined. Upon OVA stimulation, significantly lower IL-4, IL-5, IL-10 and IL-13 levels were detected in non-responders, while IL-22 was significantly higher. Comparison of fecal microbiota revealed differences of bacterial communities on single bacterial Operational-Taxonomic-Unit level between the groups, indicating protection from food allergy being associated with a distinct microbiota composition in a non-responding phenotype in this mouse model.

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1. Introduction

Severity and unpredictability of clinical reactions in context with food allergy are major challenges for patients, caretakers and health care personnel. The observed clinical response might differ between food allergic patients ranging from mild local symptoms like the oral allergy syndrome to severe systemic reactions such as anaphylaxis [1,2]. Actually, food allergy is among the main causes for potentially life-threatening anaphylaxis accounting for 41% of fatal reactions as reported to an European anaphylaxis registry [3]. For an efficient definition of allergy prevention measures, a profound mechanistic knowledge on sensitizing events is fundamental.

During the past years, we have investigated the association between anti-ulcer drug intake and food allergy development [4–10]. In first human studies in adult patients, a 3 months treatment with anti-ulcer drugs led to an increase of pre-existing food-specific IgE titers in 10% of patients, and to de novo sensitization against common dietary compounds in 15% of patients [8]. Among them, in 60% of patients with hazelnut-specific IgE clinically relevant food allergy was diagnosed by double-blind placebo controlled food challenges [10]. Further studies

Abbreviations: OVA, ovalbumin; i.v., intravenous; PPI, protonpump inhibitor; OC, oral challenge; mMCP-1, mouse mast cell protease-1; i.g., intragastric; TMB, tetramethylbenzidine; HE, haematoxylin/eosin; PAS, periodic acid-Schiff reagent; CAE, chloracetate-esterase; OTU, operational taxonomic unit.

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indicated an influence of either maternal gastric acid-suppression during pregnancy or anti-ulcer drug treatment of pediatric patients on the development of food allergy also in children [7,11–14].

Based on these murine and human data, a mouse model of oral sensitization under concomitant acid-suppression was developed being associated with induction of allergen-specific IgE, elevated Th2 cytokines and positive skin tests [5]. This immunization protocol induced severe clinical responses evidenced by positive mucosal testing, a drop of body temperature after provocations and a sustained mediator release [5,6,9].

However, in both, human and experimental studies, a certain percentage of individuals is protected from food allergy development during intake of anti-ulcer medication. This heterogeneity of reactivity especially in experimental studies with inbred mouse strains has been a matter of debate. To gain novel mechanistic insights, the overall aim of the current study was to phenotype those mice being protected from food allergy development (non-responders) in comparison with animals revealing marked systemic food allergic symptoms after immunizations based on our experimental food allergy protocol.

2. Material and methods

2.1. Animals and immunization regimen

Sixty-four female BALB/cAnNCrl mice (aged 6–8 weeks, 15–20 g) were purchased from Charles River Laboratory (Charles River Laboratory, Sulzfeld, Germany). Mice were kept in polycarbonate Makrolon cages (Ehret GmbH, Emmendingen, Germany) with filter tops and espen wood bedding (Ehret GmbH, Emmendingen, Germany) and housed under conventional conditions (12 h light/dark cycle at 22 °C). The animals were kept on an ovalbumin (OVA) free diet (Ssniff, Soest, Germany) with ad libitum access to food and water. Treatment of the animals was performed by trained staff in the morning in an animal experimentation room. Animals were treated according to European Union guidelines of animal care and with permission of the ethical board of the Medical University of Vienna and the Austrian Federal Ministry of Science and Research (permission number GZ BMWF-66.009/0051-II/10b/2008). All animals were subjected to our previously established food allergy protocol [5] with modification. On days 1 to 3, animals were treated intravenously (i.v.) with the proton pump inhibitor (PPI; Losec® Astra Zeneca GmbH, Wedel, Germany; 116 μg omeprazole in 100 μL sterile sodium chloride) 2 times within 1 h. On days 2 and 3, mice were fed 0.2 mg OVA (Sigma Aldrich, Vienna, Austria, #A5503) in combination with sucralfate (2 mg; Ulcogant®, Merck, Vienna, Austria) 15 min after the second PPI i.v. injection. This immunization cycle was repeated for 7 times (Fig. 1A). Out of the total of 64 animals undergoing the immunization protocol, we defined 10 animals of interest based on their IgE and IgG1 antibody titers after the last immunization step. These ten mice revealed antibody levels below the detection limit and were classified as antibody non-responder group (group N, n = 10/64; Fig. 1B). They were compared to 10 control animals with an OVA-specific IgE antibody response above 15 ng/mL classified as highly sensitized (allergic) group (group A; n = 10/64). This cut-off level was chosen based on our numerous previous immunization studies investigating clinical response upon oral immunizations under gastric acid suppression [5,6,9] and own unpublished data. All other sensitized animals with IgE responses below 15 ng/mL and above background values as well as OVA-specific IgG1 responses (n = 44) were excluded from this study. Four weeks after the last immunization, mice were subjected to an oral PBS challenge for control purposes to exclude unspecific changes during provocation and 10 days later to an oral OVA provocation (50 mg per mouse; oral challenge 1 (OC1)). Mice were fasted overnight before oral challenges with access to water only. One hour after each challenge, blood was collected for measurements of mouse mast cell protease-1 (mMCP-1) as well as OVA uptake. Four days thereafter, animals were re-challenged with OVA i.g. (OC2) to induce a strong local intestinal allergic response. One hour later, mice were challenged i.v. (50 μg OVA in 50 μL 0.9% sodium chloride) to trigger a systemic anaphylactic response. Mice were sacrificed 15 min thereafter.

Blood samples were taken prior to the first immunization step and 2 weeks after the last immunization, 1 h after the PBS challenge as well as after the first OVA challenge (OC1).

2.2. Antibody measurements

Mouse sera were collected before the first and 2 weeks after the last immunization step and screened for OVA-specific IgE, IgG1, IgG2a and IgA in ELISA, as described recently [5] using rat anti-mouse IgG1, IgG2a, IgA and IgE (0.1 μg per well, BD Biosciences, Heidelberg, Germany) and peroxidase-labeled goat anti-rat IgG (1:1000, Amersham, Buckinghamshire, UK). After sacrifice, mouse intestines were removed and flushed with 2 mL extraction buffer (Complete Mini, Roche) for detection of mucosal total and OVA-specific IgA levels. For total IgA determination, microtiter plates were coated with a rat anti-mouse IgA (0.1 μg per well; BD Biosciences) overnight at 4 °C. After washing, wells were blocked with 1% bovine serum albumin in TBS containing 0.05% Tween for 2 h. Thereafter, standard dilution series or mucosal lavage fluid (diluted 1:1000) were added for 30 min. After repeated washing, a biotin-labeled anti-mouse IgA antibody (0.1 μg

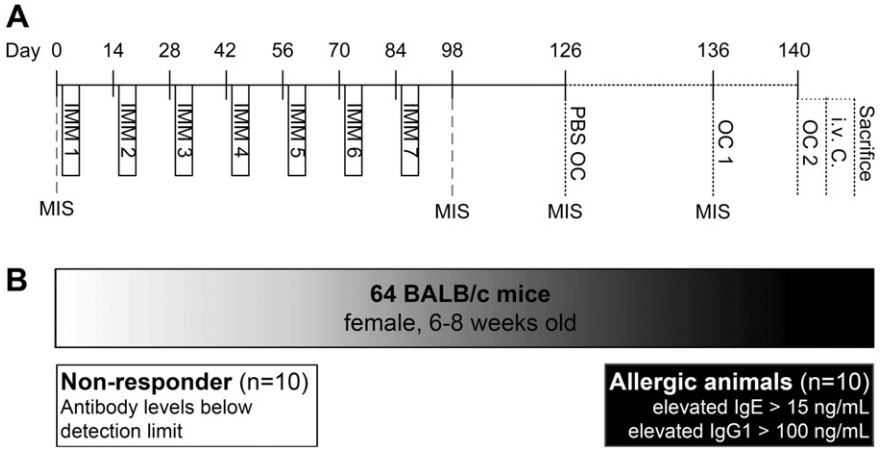

Fig. 1. Immunization scheme and selection of animals. Sixty-four BALB/c mice were immunized (panel A) according to the protocol described in the methods section. Based on the lack of OVA-specific IgE and IgG1 antibodies, 10 animals were selected as the group of interest (non-responder, group N; panel B). They were compared to highly sensitized animals, which were characterized as being anaphylactic during the study evaluations (group A). IMM, immunization; MIS, mouse immune serum; OC, oral OVA challenge; i.v. C., intravenous challenge.

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