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ORIGINAL ARTICLE

Increase of natural killer cells in children with liver transplantation-acquired food allergy

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Abstract
Background: Transplantation-acquired food allergies (TAFA) are frequently reported and considered to be caused by immunosuppressive therapy.

The aim of this study was to investigate the allergic and immunologic responses in children who had liver or kidney transplantations.

Methods: Twelve children receiving liver transplantations and 10 children receiving kidney transplantations were investigated. All children underwent the allergy work-up and in most of them, lymphocyte screening and serum cytokine measurements were also performed.

Results: TAFA were found in 7/12 (58%) children with liver transplantations and in none of the 10 children with kidney transplantations. The mean age at transplantation was significantly lower in children who underwent liver transplantations ($p < 0.001$). The immunosuppressive therapy administered to children with liver transplantation was tacrolimus in 11 patients and cyclosporine in one patient, while all 10 children with kidney transplantation received tacrolimus plus mycophenolate. The most common antigenic food was egg. The natural killer (NK) cell numbers were significantly higher in liver-transplant children than in kidney-transplant children. No significant differences were found in the serum cytokine levels.

Conclusions: This study confirms that liver-transplant children treated with tacrolimus alone have a higher risk of developing TAFA than kidney-transplant children treated with tacrolimus plus mycophenolate. NK cells might be involved in this difference.

Abbreviations: TAFA, transplantation-acquired food allergy; SPT, skin prick test; s-IgE, specific IgE.

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Introduction

Food allergies after transplantation are frequently reported in literature as transplantation-acquired food allergy (TAFA). The reported prevalence of TAFA in paediatric patients is between 6% and 57%.[1] Tacrolimus-based maintenance immunosuppression, frequently used in paediatric solid-organ transplantation, seems to play a role by inducing a shift towards T-helper 2 cells.[1,2]

The occurrence of new-onset food allergy has increasingly been reported after paediatric liver transplantation. In addition, new-onset food allergy appears to be more common after liver transplantation compared with other solid-organ transplantation in patients who receive similar immunosuppressive therapy.[3–5]

This finding suggests that tacrolimus is not the only predisposing factor in the development of food allergy. To date, the underlying physio-pathological mechanism has not been fully understood,[6] but a T-cell imbalance is considered the most probable effect.[7]

In this study, we investigated allergic and immunologic responses in children who had undergone liver or kidney transplantations.

Methods

Patients

From 1992 to 2012, we enrolled 22 patients (12 liver-transplant and 10 kidney-transplant) who were followed at the Anna Meyer Children's University Hospital.

Informed consent was given by the parents and/or patients, and after obtaining written approval, the data were collected from the medical records, including the following items: sex, age, indication for transplantation, age at the time of transplantation, and immunosuppressive drugs administered. For each patient, we also collected the clinical history related to any allergic reactions occurring before or after the transplantation, focusing on the presence of atopy in first-degree relatives as well. In patients who had immediate cutaneous, respiratory, and/or gastrointestinal symptoms, or anaphylactic reactions suspected of deriving from food allergy, a detailed medical history was obtained in order to identify potential allergens.

The diagnosis of food allergy was based on the presence of a positive skin prick test (SPT) or specific IgE (s-IgE), and convincing symptoms following specific food exposure. In some doubtful cases (negative SPT and/or s-IgE, but a positive clinical history), an oral provocation test was also performed. Symptoms were considered severe in case of anaphylactic reactions, and persistent if recurrent during the previous six months.

During the period the children suffered from allergy, lymphocyte screening was performed, and serum cytokine levels were determined in most of the patients.

Skin prick tests

SPTs were performed and read after 10–15 min by the same investigator to avoid operator-related variability. We tested all children with commercial extracts at a 0.1 mg/mL concentration (Alk Abellò, Milan, Italy) of common inhalants (pollens, mites, moulds, cat and dog epithelia, grass, olive, *Cupressus arizonica*, *Betula pendula*, *Artemisia vulgaris*, *Carpinus betulus*, and *Parietaria* mix) and foods (milk, albumen, soy, wheat, cod fish, peanut). Patients suspected of being allergic to other specific foods were tested with the culprit food using the skin prick or prick-to-prick methods. Skin prick tests were performed on the volar surface of the forearm using a standard 1-mm tip lancet, according to the recommendations of the European Academy of Allergy and Clinical Immunology. Positive controls for the prick and prick-to-prick tests were performed with histamine (Alk-Abellò, Milan, Italy: 10 mg/mL concentration). Normal saline was used as a negative control for the prick and prick-to-prick tests. The SPT results were considered positive if the difference between the mean diameter of the wheal and the negative control was at least 3 mm. The children had been off antihistamines and oral corticosteroids for 10 days before the skin testing.

In vitro tests

Serum IgE detection

Total IgE levels were detected in most of the children enrolled (12 liver-transplant and six kidney-transplant patients) using a radioimmunosorbent test (kU/L), and s-IgE to the antigenic food were measured in all children with a history of immediate reactions (Immunocap RAST, Uppsala, Sweden). A positive result was obtained if the level of s-IgE to the food was >0.35 kU/L.

Cell analysis

Using flow cytometry (BD Multitest™ 6-colour TBNK), we measured the CD4/CD8 ratios and the numbers and percentages of the following peripheral cell types in 12 liver-transplant and seven kidney-transplant patients: T cells (CD3+), B cells (CD19+), T helper lymphocytes (CD3+CD4+), cytotoxic T lymphocytes (CD3+CD8+), and natural killer (NK) cells (CD3−CD16+CD56+).

Cytokine detection

Serum cytokine levels (pg/mL) were measured in nine liver-transplant children, seven kidney-transplant children and eight healthy controls (seven females and one male; mean age: 97 months [20–228 months]). The cytokines detected were IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A (BD™ Cytometric Bead Array).

Statistical analysis

The frequencies, percentages, and means were calculated using descriptive statistics. The means were compared using the Student's *t*-test, and the frequencies were compared using a χ^2 test. A *p*-value of 0.05 or less was considered statistically significant.

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