

Long term outcome of acquired food allergy in pediatric liver recipients: a single center experience

Antigoni Mavroudi,¹ Ioannis Xinias,¹
Aristidis Deligiannidis,²
Efthimia Parapanissiou,² George Imvrios³

¹Third Pediatric Department,
²Immunology Department, Regional
Tissue Typing Lab, ³Organ
Transplantation Unit, Hippokratio
Hospital, Aristotle University
of Thessaloniki, Greece

Abstract

Food induced sensitization has been reported in pediatric liver recipients. However long term follow up has not been established so far.

We report here our experience regarding 3 pediatric patients who developed acquired food allergy after liver transplantation. The first patient suffered from persistent diarrhea and eczema. The second one presented with abdominal pain with no signs of rejection, abdominal discomfort, vomiting when ingesting milk proteins and responded well to the elimination diet. The third patient presented with facial angioedema and hoarseness of voice. She had multiple food allergies and reacted to milk, egg and sesame. All the patients had elevated total Immunoglobulin E (*IgE*) and elevated specific *IgE* antibodies to the implicated food allergens. The first patient presented clinical manifestations of allergy when she was 19 months old. The second patient became allergic at the age of 16 and the third patient at the age of 3. The symptoms of food allergy persisted for 8 years in the first case and for 2 years in the other two cases. Low levels of specific *IgE* antibodies to the implicated food allergens and an enhanced T-helper 1 cell immune response toward interferon-gamma production were markers of tolerance acquisition. The long term prognosis in our cases was excellent. Food allergy resolved in all the patients. The long term prognosis of acquired food allergy after liver transplantation is currently obscure. More studies would be needed including greater number of patients to determine whether acquired food allergy is transient in pediatric liver recipients.

Introduction

Food induced sensitization to various food allergens has been reported in pediatric liver

recipients after orthotopic liver transplantation. The increased prevalence of food allergy was noted in tacrolimus-immunosuppressed pediatric liver recipients ranging from 10% to 17%.¹ Post-Transplant Food Allergy (PTFA) although ascribed to tacrolimus treatment by various authors tacrolimus treatment alone cannot account for the development of food allergy after liver transplantation. In adults the only cases of post-transplant sensitization to food allergens have occurred via passive transfer from food allergic donor.²

The phenomenon is consistent with previous findings of allergy transfer via bone marrow transplantation and the finding that donor derived stem cells present in a liver graft can sustain long-term hematopoiesis in a recipient.^{3,4} However, the development of a new food allergy after liver transplantation has been reported in children receiving liver grafts from donors with no known food allergy.²

We present three out of twenty pediatric liver recipients followed in our center who developed food allergy after receiving liver transplants from donors with no documented history of food allergy. Possible mechanisms underlying the development of food allergy after transplantation are discussed. Long term follow up in all three cases has provided evidence on the prognosis of PTFA.

Case #1

The case involves a 10-year-old girl who underwent orthotopic liver transplantation (OLT) for extrahepatic biliary atresia at 7 months of age. The child has a positive family history of atopy as her mother has asthma. The transplant was from a deceased donor with no history of atopy. Her post operative growth improved satisfactorily. The child's weight ascended from the 9th percentile before operation to the 25th percentile, while her height remained on the 25th percentile. Methylprednisolone, azathioprine and tacrolimus immunosuppression were initially used. Tacrolimus therapy was continued at a dose of 0.5-3mg/Kg/day achieving a serum level of 2-10 ng/mL.

Prior to transplantation the child followed an unrestricted diet with no history of adverse food reaction. Twelve months after OLT, she presented mucoid bloody stools. Her laboratory investigation revealed leukocyte count of $9.3 \times 10^3/\text{mm}^3$, with 41% neutrophils, 42% lymphocytes, 7.5% monocytes and 5% eosinophils (normal <6%). The ESR was 5 mm/h. The total *IgE* was 29 UI/mL (normal range:0-50). Her platelet count was normal. The liver enzymes were not elevated and the prothrombin time (PT) and the Activated Partial Thromboplastin Time (PTT) were not prolonged. The liver biop-

Correspondence: Antigoni Mavroudi, Third Pediatric Department, Hippokratio Hospital, Aristotle University of Thessaloniki, 66 Spirou Loui St., 54352 Pilea - Konstantinopolitika, Thessaloniki, Greece.
Tel. +30.2310.909632 - Fax: +30.2310.992981.
E-mail: antigonemavroudi@gmail.com

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sy showed perivenular drop out but no rejection. Between the 12th till the 18th months after liver transplantation the child received monthly hospital admissions due to persistent symptoms. Each time the child was not febrile and the investigation for viral enteropathogens, such as rotavirus, enteric adenovirus, cytomegalovirus and herpes simplex virus, was negative. Stool cultures for bacterial and parasitic enteropathogens were also negative. Management was with oral rehydration and a 7 day course of Trimethoprim/Sulfamethoxazole (TMP/SMX) per os 6 mg/Kg BW/24h divided in two daily doses as prophylactic treatment, due to immunosuppression. At the age of 26th months - 19th month after liver transplantation - on admission due to persistent symptoms, her blood count revealed 6.3×10^3 leukocytes with 50% neutrophils, 29% lymphocytes and 12% eosinophils (756/mm³ absolute count). On clinical examination eczema was noted. She underwent allergological investigation which showed elevated total serum levels of Immunoglobulin E (*IgE*) of 99 UI/mL (normal range 0-50) and specific *IgE* antibodies to alpha-lactalbumin and beta-lactoglobulin of 3.58 UI/mL and 16.34 UI/mL respectively (negative <0.35). Specific *IgE* to egg, soy, gluten, fish, house dust mites, grasses and moulds were absent. The results of the skin prick tests were parallel to those of specific *IgE*. The mother was then instructed to start a diet free of cow's milk protein and substitute milk with an extensively hydrolyzed milk formula. On follow-up her eczema deteriorated and the mother reported sporadic episodes of diarrhea. Laboratory findings included a leukocyte count of 7.4×10^3 with 24% eosinophils (normal <6%), normal liver function tests, and tacrolimus blood levels of 6.9 ng/mL. The

patient was closely monitored for milk protein avoidance and was started on an elementary milk substitute formula with marked and rapid improvement of symptoms. The following four days, eosinophils decreased to normal values. Careful interview showed that the child's mother hadn't introduced a strict milk protein avoidance diet and the child had ingested several milk containing foods and dairy products.

At the age of 9.5 years cytokine assessment revealed a cytokine production toward T-helper 2 cell (*Th2*) immune response characterized by elevated levels of interleukin 4 (*IL-4*) and (interleukin 13) *IL-13*, normal (interleukin 5) *IL-5* and slightly elevated interferon-gamma (*IFN-γ*). Determination of specific *IgE* antibodies to milk proteins showed that they were still moderately elevated. The regulatory cytokines (interleukin 10) *IL-10* and the transforming growth factor-beta (*TGF-β*) were also elevated (Table 1). Six months later reassessment of specific *IgE* antibodies to milk proteins showed that the titers were negative. She received hospital admission and she underwent a milk challenge test which was negative. She has not been avoiding milk and dairy products ever since.

Case #2

Is an 18-year-old boy who received a liver graft at 6 months of age due to extra-hepatic biliary atresia. The deceased donor had no known history of atopy. The patient had been receiving orally an immunosuppressive regimen of cyclosporin 2-6 mg/Kg/day and methylprednisolone at 0.1 mg/Kg/day before he was switched to tacrolimus at 0.15-0.3 mg/Kg/day achieving a serum level of 3 ng/mL - 9.2 ng/mL and methylprednisolone. The child and his family had no history of any allergic disease. After transplantation the patient followed an unrestricted diet without any signs of adverse reactions to foods. Patient's post-operative course was uneventful. On follow ups his liver enzymes were normal and liver biopsies performed every 3 years have not shown signs of rejection.

At the age of 16 years the patient complained of abdominal pain and discomfort and occasionally nausea and vomiting after ingesting milk and dairy products. On clinical examination no hives or eczema was present. His abdominal ultrasound showed a normal liver structure and the Doppler ultrasonography showed unrestricted blood flow in the hepatic artery and portal vein. Laboratory investigation showed not elevated liver enzymes and normal PT and PTT values. He revealed total serum *IgE* of 250 UI/mL (normal range 0-100) without eosinophilia, as the absolute count was 117/mm³ (normal <500/mm³) and specific *IgE*

Table1. Cytokine assessment in the 3 pediatric cases.

Cytokine concentration (pg/mL)	Case #1 (age 9.5)	Case #2 (age 16)	Case #3 (age 12)
<i>IL-2</i>	7.8 normal <46	9 normal <48	14 normal <46
<i>INF-γ</i>	18 ↑ normal <11	20 ↑ normal <16	17 ↑ normal (3-16)
<i>IL-4</i>	54 ↑ normal <13	100 ↑ normal <45	28 normal (0-45)
<i>IL-13</i>	17 normal <23	20 normal <60	7.7 normal <23
<i>IL-10</i>	14 ↑ normal <4	21 ↑ normal <10	9.1 ↑ normal <4
<i>TGF-β</i>	20 ↑ normal <13	19 normal <20	18 ↑ normal (0-10)

IL, interleukin; *INF-γ*, interferon-gamma; *TGF-β*, transforming growth factor-β.

antibodies to milk of 3.78 UI/mL (negative <0.35). He was placed on a milk elimination diet with complete resolution of his symptoms. Two years later at the age of 18 the level of specific *IgE* antibodies to cow's milk were 0.78 UI/mL. A moderate amount of cow's milk protein was reintroduced in the patient's diet without reoccurrence of his previous symptoms.

Assessment of cytokine production showed an enhanced T-helper 1 cell (*Th1*) immune response, as *IFN-γ* was moderately elevated without accompanying downregulation of *Th2* cytokines, as *IL-4* and *IL-13* were elevated at the time when cow's milk allergy diagnosis was established. The regulatory cytokine *TGF-β* was within the normal range (Table 1).

Case #3

The third case involves a 12-year-old girl who underwent OLT for extrahepatic biliary atresia at 8 months of age. The living donor was her mother who had no personal or family history of atopy. The patient has been on tacrolimus immunosuppression for the last eleven years achieving a serum level of 4 ng/mL. She experienced the first allergic reaction almost 2 years after transplantation when she was about 3 years old. When she ingested a piece of candy made of sesame seeds she presented with lip swelling, facial angioedema and hoarseness of voice. She was managed with intramuscular Adrenalin injection and corticosteroids. She has been avoiding sesame and no accidental exposures occurred ever since. The child presented similar symptoms when she was drinking milk or when ingesting dairy products, white fish and egg proteins. Detection of specific *IgE* antibodies to various foods showed by the age of 3 years elevated levels of specific *IgE* antibodies to milk, egg, white fish, pork and chicken.

At the age of 5 years the level of specific *IgE* antibodies to cow's milk decreased significantly at the level of 0.78 UI/mL and she has been able to tolerate milk ever since. At the age of 11 years she started to tolerate well cooked egg

but she was still unable to tolerate raw egg. Total serum *IgE* was at the level of 992 UI/mL and the absolute eosinophil count was 195/mm³ (normal <500 /mm³). In spite of the moderately elevated specific *IgE* antibodies to pork and chicken the patient has always been able to tolerate them and her diet in regards to pork and chicken has never been restricted.

Cytokine assessment showed increased levels of the regulatory cytokine *TGF-β*, an enhanced *Th1* immune response toward *IFN-γ* production and normal *Th2* cytokines (Table 1).

Conclusively, sensitization to milk protein in our cases occurred in children who received liver grafts from donors with no known history of food allergy. The long term follow up was 9.5 years in the first case, 17.5 years in the second case and 11 years in the third case. Food allergy diagnosis was established in all the patients by assessing the specific *IgE* antibodies to common food allergens and by evaluating the patient for resolution of the allergic symptoms. Eosinophilia was present in only one patient who had a persistent allergy to cow's milk protein which resolved after eight years. The onset of allergy became evident when the patient was 19 months old in the first case and 16 years old and 3 years old in the second and third case respectively. The duration of symptoms was 8 years in the first case and 2 years in the other two cases. All the patients had an enhanced *Th1* dominant immune response before achieving tolerance. High total serum *IgE*, elevated specific *IgE* antibodies to various food allergens and the use of tacrolimus as immunosuppressive regimen was a common feature in all our cases.

Discussion

Type I allergic reactions to common food allergens have increasingly been reported in transplant recipients, mainly in orthotopic liver transplanted children.^{2,5-11} The transfer of allergy from a food allergic liver donor to a previously non allergic liver transplant recipient was first reported in 1997.⁶ The only reports of acquired food allergy in adults after liver trans-

plantation have occurred via passive transfer from food allergic liver donor, whereas acquired food allergy after liver transplantation has been widely reported in children receiving liver transplants from donors with no known food allergy.

Tacrolimus mechanism of action involves the inhibition of a protein phosphatase, calcineurin, which results in repression of an early step of T cell activation. Although its administration usually produces a therapeutic immunosuppression, a paradoxical elevation of total serum *IgE* was observed in these patients. Another explanation could be that the transplanted liver retains sufficient allergen specific *IgE* in its large vascular pool to sensitize the recipient's mast cells. Although *IgE* circulates in the peripheral blood for only a few days, it remains bound to high affinity receptors on tissue mast cells for several months. However, the transplanted liver contains not just donor T and B lymphocytes, but also dendritic cells and pluripotential hematopoietic stem cells capable of surviving for several months after liver transplantation. These cells could possibly migrate to the recipient's lymphoid organs or other sites and there provoke recipient sensitization.¹²

Eosinophilia was present in one of the three patients receiving tacrolimus immunosuppression the other two patients had normal eosinophil count while peripheral blood eosinophilia has been frequently observed in patients with post-transplant food allergy. The high prevalence of eosinophilia in tacrolimus treated patients supports the hypothesis that the cases of new onset food allergy are a result of calcineurin inhibitor imbalance between *Th1* and *Th2* helper cells and not a result of passive transfer of sensitized donor lymphocytes in the transplanted organ.¹³ Tacrolimus binds to immunophilins (proteins that bind to immunosuppressive drugs) termed FKBP-FK binding proteins. This complex then binds to and inhibits the activity of calcineurin, a serine threonine phosphatase that plays a critical role in interleukin 2 (*IL-2*) promoter induction during T-cell activation.¹⁴

The immunological investigation by cytokine assessment showed that a *Th1* immune response toward *IFN- γ* was present in all patients. The first two patients showed a *Th2* cytokine dominance characterized by elevated *IL-4*, while the third patient revealed a normal serum *IL-4* level when she became tolerant to various food allergens. The regulatory cytokine *TGF- β* was elevated in the first case shortly before the patient became tolerant to cow's milk proteins and also in the third case when the patient outgrew her allergy (Table 1). The mechanism of tolerance to food is not completely understood. It is suspected that T-regulatory cells are involved in tolerance acquisition. The hypothesis for a key tolero-

genic role of regulatory T-cells has been reinforced by studies showing that children having outgrown food allergy with mostly gastroenterologic symptoms, have increased number of CD4+CD25+ T-regulatory cells in their gut mucosa. Regulatory T lymphocytes will secrete *IL-10* and *TGF- β* which may downregulate the *IgE*-driven reactions. Promoting the *IL-10* rich environments in the gut seems to be an option to prevent or even treat food allergy.¹⁵ The regulatory cytokine *TGF- β* was an early marker of tolerance acquisition in the two patients.

The first patient who had a positive family history of atopy and eosinophilia suffered from persistent food allergy, while the other two patients who had normal eosinophil count in the peripheral blood and a negative family history of atopy suffered from a more transient type of food allergy. Factors such as peripheral eosinophilia and a positive family history of atopy were related to persistent PTFA in our patients.

In the second case the onset of food allergy was delayed. The diagnosis in this case was based on the patient's gastrointestinal symptoms, the exclusion of other diagnosis and by taking into account the elevated level of the specific *IgE* antibodies to milk proteins. The diagnosis was supported by the resolution of the patient's symptoms when a milk protein elimination diet was introduced. Achievement of tolerance was determined by assessing the levels of specific *IgE* antibodies to milk proteins. Although a proper challenge has not been carried out, when the level of specific *IgE* antibodies to milk proteins became negative, the patient's diet relaxed with no adverse events. The level of the specific *IgE* antibodies to food proteins proved to be a useful marker for monitoring food allergy in all our cases. Low levels of specific *IgE* antibodies to the previously implicated food proteins were related to a successful reintroduction of the foods in the patient's diets.

Transplant acquired food allergies are not uncommon in pediatric liver recipients.^{2,10,13-14,16-20} Health providers should have increased suspicion of the complication in liver transplanted children in order to make an appropriate investigation and follow up. The long term prognosis of acquired food allergy in pediatric liver recipients is currently obscure. A letter to the editor of JACI 2008 by Frischmeyer-Guerrero P, *et al.*²¹ reports that 23 out of 25 children followed given diagnosis of food allergy after organ transplantation were able to follow unrestricted diets after a median duration of 2.4 years follow up in the clinic and 6.1 year by telephone. Maarof *et al.*²² in 2011 reported that *IgE*-mediated multiple food allergy after liver transplantation could be efficiently managed after switch from tacrolimus to cyclosporine immunosuppressive regimen with an elimination diet. The prognosis of

PTFA of the 3 patients followed in our clinic so far has been excellent. All our patients remained on tacrolimus immunosuppression. They were all placed on elimination diets of the offending food allergens and they all achieved tolerance after a long term follow up. The onset of allergic reactions to cow's milk protein was 19 months old, 16 years old and 3 years old in cases 1, 2 and 3 respectively. Cow's milk allergy persisted for 8 years in the first case and for 2 years in the other two cases.

Development of food tolerance in patients with acquired food allergies after a liver transplant remains an important issue. The patients themselves and health care providers often report that restricted diets have a significant impact on their quality of life. Families of transplanted patients with acquired food allergies always address questions to physicians who provide follow up in regards to food tolerance acquisition. The prognosis of PTFA has not been well established so far. Only limited data are available in regards to the prognosis of PTFA and more studies including greater number of patients are required in order to establish the long term outcome of PTFA in pediatric liver recipients.

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