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Transplant induced food sensitization without allergy – mechanisms of tolerance

Thanh-Thao (Adriana) Le, MBBS, Jie Shen Fok, MBBS, Sherin Vareeckal Joseph, Bpharm, MPharm, PhD, Preethi Eldi, MBBS, MMolBiol, PhD, Timothy Chataway, BSc(Hons), PhD, William Smith, MBBS, PhD, Pravin Hissaria, MBBS, MD, DM



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- 1 Title page
- 2 **Transplant induced food sensitization without allergy – mechanisms of tolerance**
- 3 Author names, academic degrees and institutional affiliation:
- 4 Thanh-Thao (Adriana) Le, MBBS
- 5 Consultant Immunologist, Royal Adelaide Hospital, Adelaide, Australia.
- 6 +61 421 846 421; Thanh-Thao.Le@sa.gov.au
- 7 Jie Shen Fok, MBBS
- 8 Consultant Immunologist, Department of Respiratory Medicine, Box Hill Hospital,
- 9 Eastern Health, Melbourne, Victoria, Australia; Faculty of Medicine, Nursing and
- 10 Health Sciences, Monash University, Melbourne, Victoria, Australia.
- 11 Sherin Vareeckal Joseph, Bpharm, MPharm, PhD
- 12 Research Scientist, School of Pharmacy and Medical Sciences, University of South
- 13 Australia, Adelaide, Australia
- 14 Preethi Eldi, MBBS, MMolBiol, PhD
- 15 Post-Doctoral Research Fellow, Experimental Therapeutics Laboratory, Hanson
- 16 Institute and Sansom Institute, School of Pharmacy and Medical Science, University
- 17 of South Australia, Adelaide, Australia

18 Timothy Chataway, BSc(Hons), PhD

19 Proteomics Facility Manager and Senior Research Scientist, College of Medicine and

20 Public Health, Flinders University, Bedford Park, Adelaide, Australia

21 William Smith, MBBS, PhD

22 Head of Unit, Consultant Immunologist, Royal Adelaide Hospital, Adelaide, Australia

23 Pravin Hissaria, MBBS, MD, DM (corresponding author)

24 Consultant Immunologist, Clinical Immunology/Allergy Department, 9G, Port Rd,

25 Royal Adelaide Hospital, Adelaide, South Australia, Australia 5000

26 Consultant Immunopathologist, Human Immunology, Level 3, Frome Rd, SA

27 Pathology, Adelaide, South Australia, Australia 5000

28 +61 0432 003 558 (M) / +61 08 7074 2880 (W) ; Pravin.Hissaria@sa.gov.au

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38 Clinical implications

39 Bone marrow transplantation may transfer the production of food-specific IgE to the
40 recipient, but this may not be sufficient to cause allergy; we undertook detailed
41 immunological work-up in a strongly sensitized but non-allergic hematopoietic stem
42 cell transplant recipient to elucidate the potential mechanisms of tolerance.

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48 Transplant induced food sensitization without allergy – lessons to be learnt

49 *To the Editor:*

50 The development of food allergy in recipients of haematopoietic and solid organ
51 transplants is recognized but remains enigmatic¹. However, food allergen sensitization
52 following transplantation with maintenance of clinical tolerance has not been
53 described, and may shed light on the puzzle of immunological reactivity without
54 clinical allergy. We present the case of a 58-year-old man who underwent HLA-
55 matched allogeneic hematopoietic stem cell transplant (HSCT) from his 60-year-old
56 brother for management of acute myeloid leukaemia, and subsequently became
57 sensitized to peanut but did not react to peanut challenge. The donor had a history of
58 anaphylaxis to peanuts, dating back to childhood with clinical features including rash,
59 vomiting and difficulty breathing. Inadvertent recent exposures had still been
60 associated with immediate hypersensitivity symptoms. The patient had no prior
61 history of food allergy or atopic disease, and pre-transplant peanut specific IgE (sIgE)
62 was <0.35kU/L . Six months after his transplant, the recipient's peanut sIgE (table 1)
63 and skin prick testing (6mm wheal to commercial peanut extract) were positive.
64 However, in-hospital oral challenge to peanut (to 10g) was tolerated. Eighteen months
65 later, the patient's peanut sIgE level had increased (table 1) but he remained clinically
66 tolerant (to 50g). Donor chimerism studies demonstrated 100% engraftment.

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68 We undertook a series of further investigations in both the patient and his brother, to
69 explore the underlying reasons for our patient's clinical tolerance despite his new
70 sensitisation, with relevant ethics approval (HREC/17/RAH/283). Details regarding
71 methodology are provided in Online Repository materials.

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Recipient and donor serum sIgE levels to recombinant peanut allergens, peanut BAT, peanut specific IgG4 (sIgG4) levels and T cell immuno-phenotyping results are summarised in Table 1. Both the recipient and donor demonstrated dominant sensitization to rArah2. Despite this, the recipient had negative BAT to rArah2 extract. In contrast, the donor's BAT was positive for both whole peanut and rArah2. While the recipient had a positive BAT to whole peanut, the threshold of reaction was higher and degree of maximal basophil activation lower, compared to the donor. The recipient had lower peanut-specific IgG4 by EIA (Immunocap) but Western blot studies [Online Repository Figure E1] demonstrated stronger IgG4 bands to Ara h 2 and 6, while the donor had a more diverse IgG4 repertoire to recombinant peanut allergens.

Recipient and donor PBMCs were cultured in media containing peanut extract. Proliferating peanut-specific T cells from the allergic donor expressed significantly higher IL4 levels and Th2-skewing compared to recipient [Online Repository Figure E2b]. Furthermore, the recipient had fewer peanut-specific T cells [Figure E2a], which were predominantly of the regulatory phenotype [Figure E2c].

A modified passive cutaneous anaphylaxis (PCA) experiment was performed in which the allergic donor's serum was injected intradermally at serial dilutions into the recipient's arm, and his own serum injected in the other arm. One day later he underwent oral peanut challenge, without any wheal and flare reaction at any of the injection sites [Figure 1a]. A PCA experiment using the donor and recipient's sera was subsequently performed on two healthy non peanut-sensitized subjects [Figure 1b and 1c]. This resulted in a wheal and flare reaction at the sites of intradermal injection

of both donor and recipient serum. The reaction was more rapid and wheal size was larger at the sites of donor serum injection [Figure 1 and Table E1].

We present the intriguing case of a previously non-sensitized patient who reconstituted peanut-specific IgE post HSCT. However, despite high total peanut IgE and dominant sensitization to rArah2 and 6, both strongly associated with peanut anaphylaxis², the recipient remained clinically tolerant. The transfer of peanut allergy following both solid organ³ and bone marrow⁴ transplant has previously been described. While the suspected transfer of ‘latent atopy’ with asymptomatic aeroallergen sensitization has been reported following HSCT from a donor sibling, de novo sensitization was not confirmed with pre-transplant allergy testing.⁵

The phenomenon of post-transplant IgE sensitization is unlikely to be attributable to the passive transfer of IgE. Free IgE has a half-life of a few days, although bound IgE has a half-life of up to a few months¹. Our recipient patient’s peanut sIgE peaked at 24 months, while the median time to presentation with transplant-related food allergy has been reported to be 6 months.⁶ The time course in our patient suggests not only transfer of donor peanut-specific lymphocytes in the transplant (which may persist for several months⁷), but an ongoing peanut specific B-cell response.

While the recipient had detectable peanut-specific T lymphocytes and high peanut sIgE, he never developed symptoms of clinical allergy, even when his rArah2 sIgE reached a level higher than the donor’s at 24 months. The mechanisms for this tolerance may be multifactorial as demonstrated by the recipient’s 1) lack of basophil activation to rArah2; 2) development of limited but highly specific peanut-specific

IgG4 response (predominantly against rArah2/6); and 3) relative abundance of allergen specific T regulatory cells and reduced Th2/Th1 ratio. The recipient's tolerance may represent a quantitative rather than a qualitative phenomenon, given the donor had a consistently higher level of peanut sIgE compared to the recipient, as well as lower threshold of basophil reactivity and higher maximal basophil activation when stimulated with whole peanut.

Remarkably, the recipient did not demonstrate PCA after injection of allergic donor serum after peanut challenge. PCA is a proven in-vivo model of anaphylaxis in human allergic conditions and in a recent study⁸, all recipients reacted at sites of injection of serum with peanut sIgE level greater than 6kU/L. In our study, two non-sensitized non-allergic subjects developed wheal and flare reactions after peanut challenge at both donor and recipient serum injection sites. Hence, the recipient's peanut-specific IgE is capable of eliciting an allergic reaction, and other serum factors in the recipient (ie. sIgG4 to rArah2 and rArah6) are not sufficient to block the reaction. However, it is notable that in both control subjects, the intradermal reaction was more rapid and wheal size larger at the donor injection sites, suggesting that quantitative differences in specific IgE may also be relevant.

Specific IgE is necessary but not sufficient for the development of food allergy. The level of sIgE, allergen specific T regulatory cells, basophil reactivity, and sIgG4 repertoire may all have a role in determining whether an individual will be clinically allergic or tolerant. Allergen-specific IgE epitope diversity and promiscuity of IgE binding may also be important.

The recipient has been advised to eat peanut regularly to maintain tolerance – somewhat analogous to the situation where early introduction of regular dietary intake of peanut to children who are peanut sensitized but not yet allergic, has been shown to significantly reduce the subsequent risk of developing peanut allergy.⁹ This case provided a novel model to study the development of allergy and the window of opportunity where it might be possible to intervene in those who have high specific IgE but have not yet developed clinical allergy.

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190 **Figure legends**

191 *Figure 1* – Passive cutaneous anaphylaxis (PCA) experiments showing intradermal

192 test (IDT) sites >24h post injection, and shortly following oral peanut challenge.

193 Right arm: recipient serum IDTs; left arm: donor serum IDTs. T^{reac} = time to first

194 erythema, post oral peanut challenge. $\text{Max}^{\text{W/F}}$ = maximum wheal/flare size.

195 1(a) – HSCT recipient

196 1(b) – Non-sensitized non-allergic subject 1

197 1(c) – Non-sensitized non-allergic subject 2

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Time		6 months post-transplant	24 months post-transplant						
Test	sIgE (kU/L)			Whole peanut BAT (% CD63 expression)		rArah 2 peanut BAT (% CD63 expression)		Peanut specific T cell studies	
Recipient	Peanut	1.5	28	Positive control	32.4	Positive control	32.4	% IFN γ producing cells	24
	rArah 1	<0.35	1.5	Negative control	0.5	Negative control	0.5	% IL-4 producing cells	2
	rArah 2	1.5	34	Peanut 0.001mcg/ml	0.3	rArah 2 0.001mcg/ml	0.3	Th1/Th2 ratio	2.5
	rArah 3	<0.35	<0.35	Peanut 0.01mcg/ml	41.1	rArah 2 0.01mcg/ml	0.3	% Regulatory T cells	61
	rArah 8	<0.35	<0.35	Peanut 0.1mcg/ml	62.8*	rArah 2 0.1mcg/ml	0.1		
	rArah 9	<0.35	<0.35	Peanut 1mcg/ml		rArah 2 1mcg/ml	0.4		
	sIgG4(mgA/l)		0.93	EC50 (mcg/ml)	0.008	EC50	N/A		
Donor	Peanut	60	58	Positive control	60	Positive control	60	% IFN γ producing cells	20
	rArah 1	32	31	Negative control	0.1	Negative control	0.1	% IL-4 producing cells	8
	rArah 2	18	17	Peanut 0.001mcg/ml	18.5	rArah 2 0.001mcg/ml	0.5	Th1/Th2 ratio	10
	rArah 3	5.1	5	Peanut 0.01mcg/ml	90.2*	rArah 2 0.01mcg/ml	5.7	% Regulatory T cells	24.3
	rArah 8	<0.35	<0.35	Peanut 0.1mcg/ml	88.2	rArah 2 0.1mcg/ml	11.7		
	rArah 9	<0.35	<0.35	Peanut 1mcg/ml		rArah 2 1mcg/ml	41.2		
	sIgG4(mgA/l)		5.38	EC50 (mcg/ml)	0.0055	EC50 (mcg/ml)	0.379		

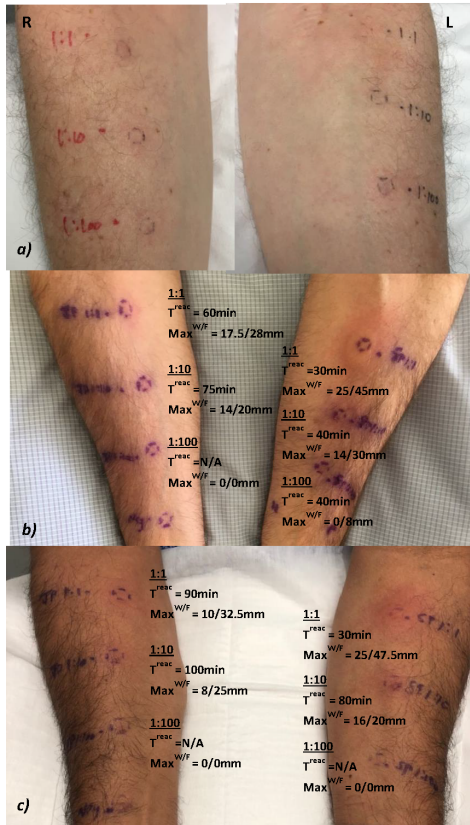
Table 1 – summary of peanut-specific serology, basophil activation testing (BAT) and T cell immunophenotyping results in bone marrow transplant donor and recipient.

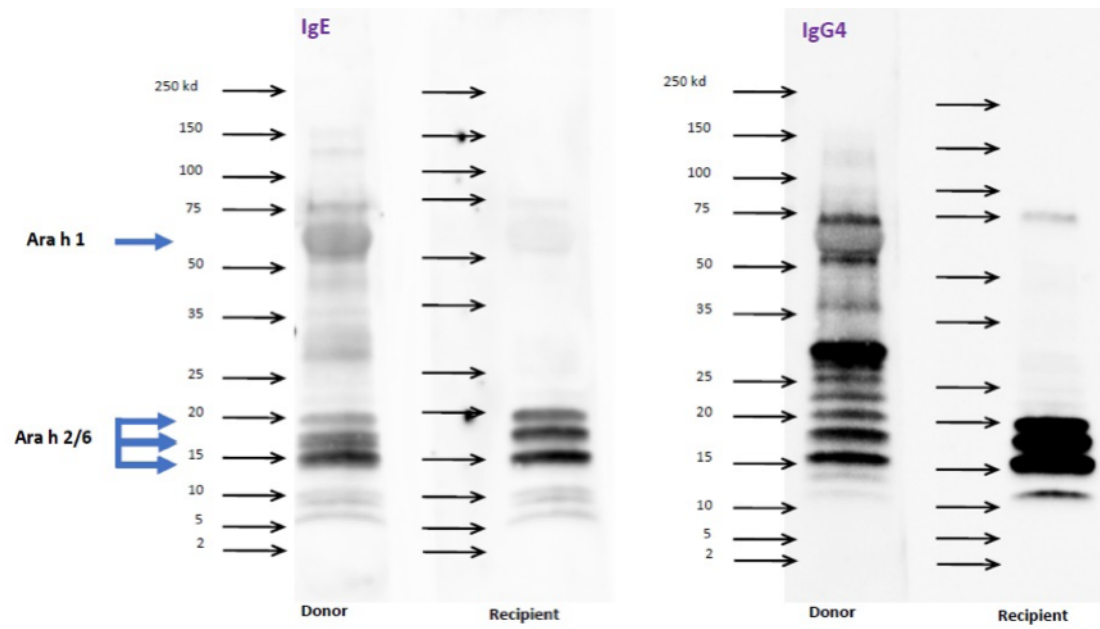
* Maximal basophil degranulation (basophil reactivity); EC50 = half maximal effective concentration (basophil sensitivity)

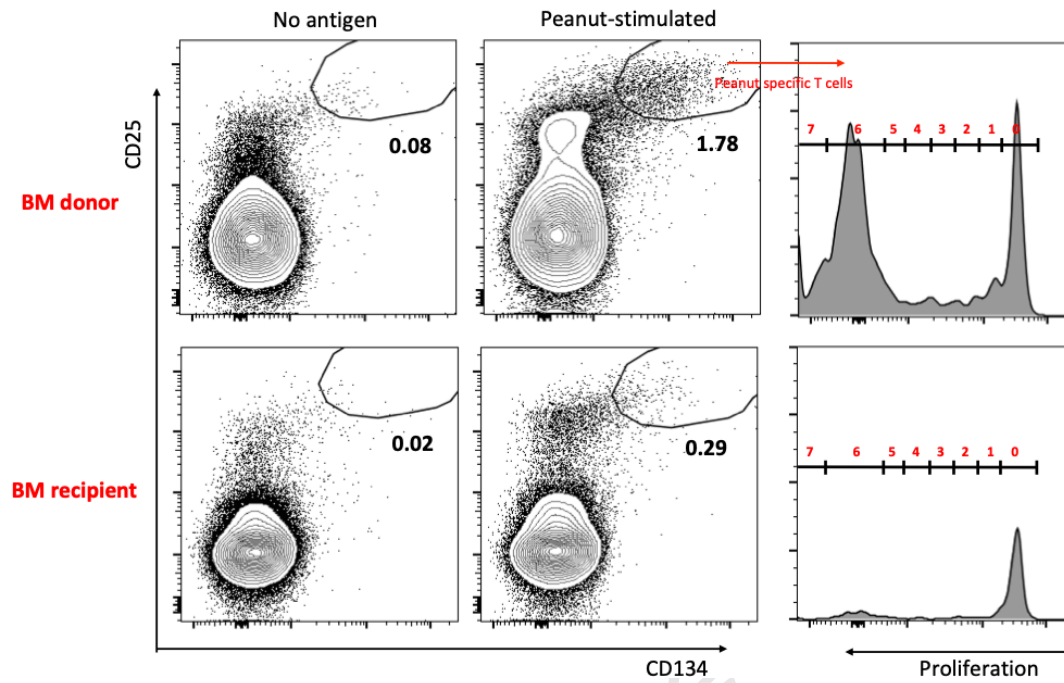
Table E1. Passive cutaneous anaphylaxis experiment results showing intradermal test (IDT) outcomes 24 hours post injection and following oral peanut challenge

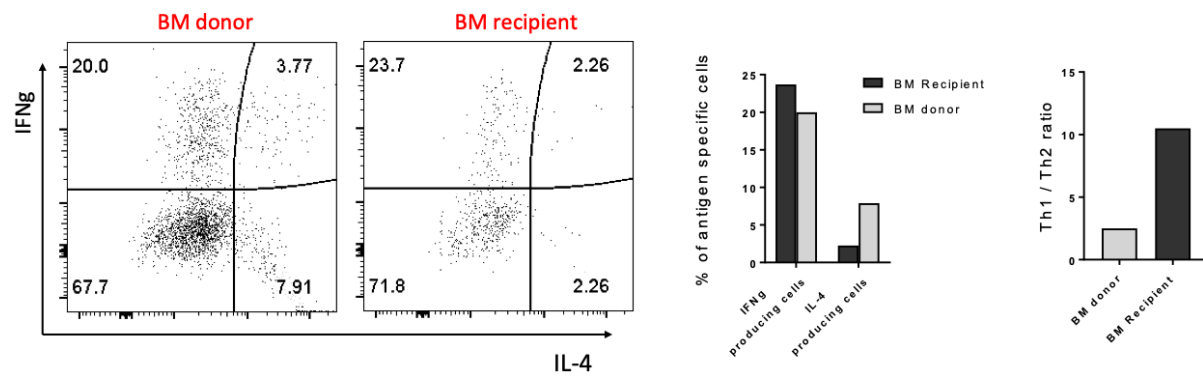
IDT serum + dilution	Bone marrow transplant recipient		Non-sensitized non-allergic subject 1		Non-sensitized non-allergic subject 2	
Recipient serum	T^{reac} (minutes)	$M^{\text{W/F}}$ (mm)	T^{reac} (minutes)	$M^{\text{W/F}}$ (mm)	T^{reac} (minutes)	$M^{\text{W/F}}$ (mm)
1:1	No reaction	No reaction	60	17.5/28	90	10/32.5
1:10	No reaction	No reaction	75	14/20	100	8/25
1:100	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Donor serum						
1:1	No reaction	No reaction	30	25/45	30	25/47.5
1:10	No reaction	No reaction	40	14/30	80	16/20
1:100	No reaction	No reaction	40	0/8mm	No reaction	No reaction

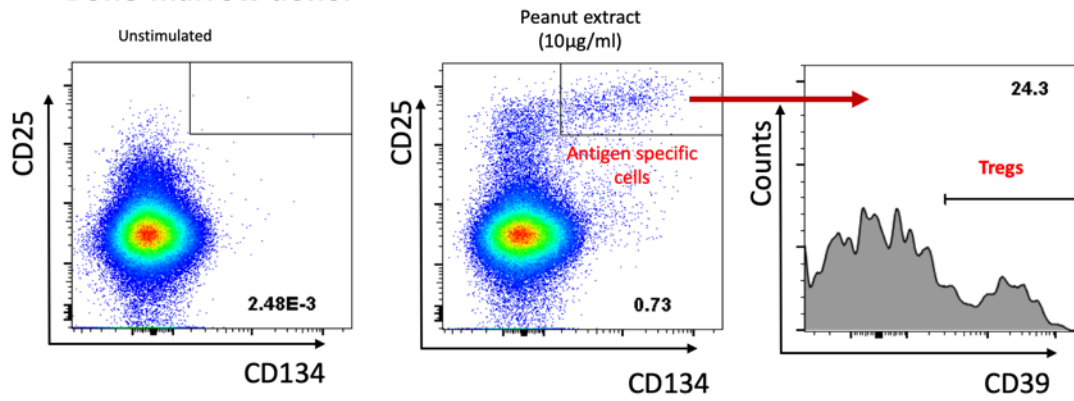
T^{reac} = time to first erythema, $M^{\text{W/F}}$ = maximum wheal/flare size.









Bone-marrow donor**Bone-marrow recipient**