

The mouse cholera toxin model for evaluation of allergenicity



Of mice and men....(Mestas, J Immunol 2004; 172:2731)

	Mouse	Human
Neutrophils in blood	10-25%	50-70%
Lymphocytes in blood	75-90%	30-50%
Predominant T cells in skin and gut	$\gamma\delta$ TCR	$\alpha\beta$ TCR
Fc α RI	absent	present
Fc γ RIIA	absent	present
IgG classes	IgA, IgD, IgE, IgG1 IgG2a/b/c, IgG3, IgM	IgA, IgA2, IgD, IgE, IgG1 IgG2, IgG3, IgG4, IgM
Th expression of IL-10	Th2	Th1 and Th2
IL-13 effect on B cells	none	IgE switch
Epithelial cells present Ag to CD4+	no	Yes
Immunological tissues investigated	Lung, gut, spleen, thymus lymph nodes, etc, etc..	Blood

Of mice and men

■ Mice and humans both have about 30,000 genes – and share 99% of them – but the mouse genome is shorter than that of humans (2.5 billion letters compared with 2.9 billion)

■ About 1,200 new genes have been discovered in the human because of mouse-human genome comparisons.

■ Mice have many more olfactory genes compared to the human. Smell matters for mice, especially for sex and mating; they also have more genes involved in reproduction (such as aphrodisin, which stimulates mating behaviour in males) and immunity.

■ It might be said that we are essentially mice without tails – but we even have the genes that could make a tail.

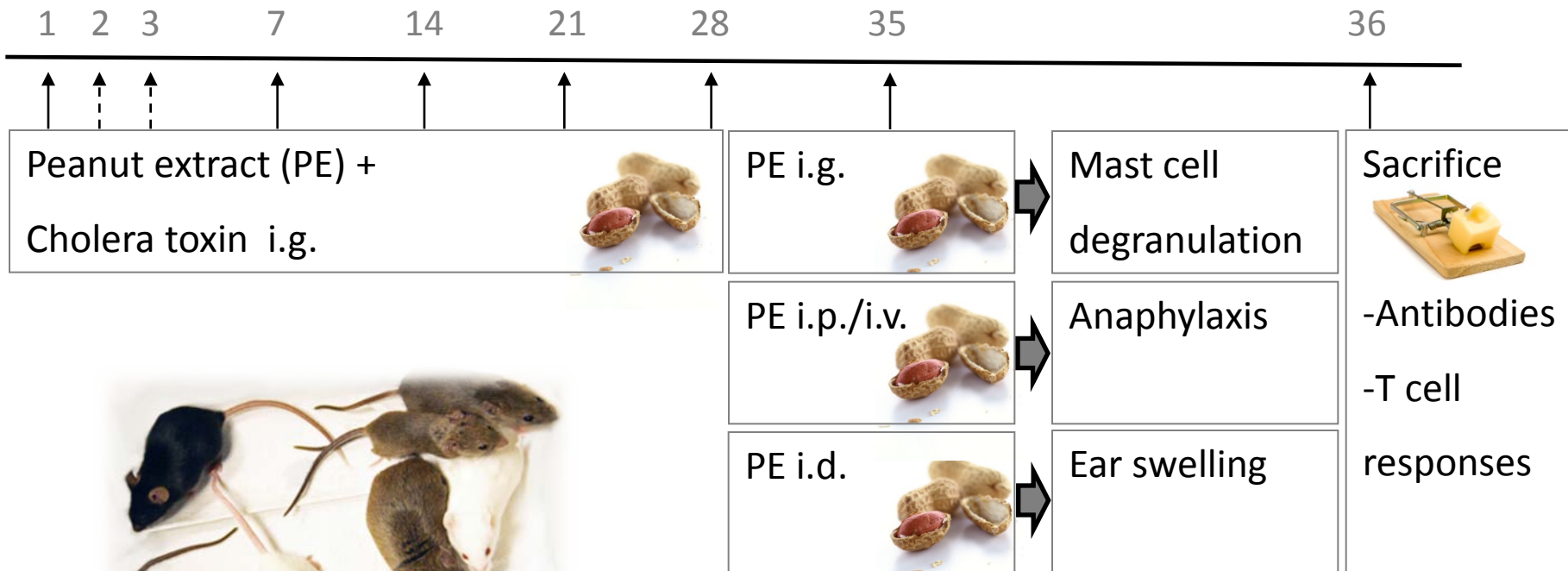
■ 90 percent of genes associated with disease are identical in the human and the mouse, supporting the use of mice as model organisms.



A peanut food allergy model

Sensitisation

Challenge



A peanut food allergy model: Parameters

- **Cytokine production** after restimulation of MLN and spleen cells
- **Flow cytometry** of isolated cell populations of MLN, Intestinal epithelial lymphocytes (IEL), Lamina Propria lymphocytes (LPL), Peyer's Patches (PP)
- **Antibody measurements** of allergen-specific IgG1, IgG2a, IgE, IgA, IgM
- **Histological analysis** of the intestine or lymphoid organs
- **Mast cell degranulation** in vivo (histamine, MMCP-I release)
- **Functional parameters:** Gut permeability, airway reactivity
- **Anaphylaxis:** temperature and anaphylaxis score
- **Ear swelling** after intra-dermal challenge



A peanut food allergy model: determinants

(dirty little secrets....)

- Allergen used (peanut, whey, β -lactoglobulin, etc..)
- Dose of allergen during sensitization and challenge
- Dose of CT (10, 15, 20 μ g)
- “Unofficial” adjuvants: Alcohol, NSAIDs, high protein dose (!)

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of the daily dose of PNA (200 mg) previously found to be effective in the murine PNA model. Control PNA mice received an equal amount of water (PNA/sham, N=8). Mice were challenged i.g. with PN (200mg/mouse) 24 h after completion of treatment (at week 14 after initial PN sensitization). To determine prolonged protection, mice were re-challenged 6 additional times at wks 18, 22, 28, 34, 40, and 50 after the 1st treatment course. To

Mice were sensitized as previously described,¹⁶ with slight modifications. Specifically, oral gavages were performed with intragastric feeding needles (191183; DELVO, Zurich, Switzerland). Sensitized mice were challenged with 3.75 mg of crude peanut extract (Greer Laboratories, Lenoir, NC) in 500 μ L of PBS either intraperitoneally or intravenously 1 to 2 weeks after the last gavage.

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- Matrix: Lipids, sugars, aggregated proteins
- Contamination of protein with endotoxin: e.g. β -lactoglobulin
- (Microbial) status of the mouse housing
- Mouse strain



A (mouse) model for food allergy

- **Mechanisms** of (food) allergy
- **Risk assessment** of (novel) proteins:
 - Allergenicity
 - Availability of proteins *in vivo*
- **Diagnostics** of allergy:
 - New biomarkers of disease
- New **therapeutic** opportunities:
 - Medicines
 - Immunotherapy (antibodies)
 - Allergen immunotherapy



A (mouse) model for food allergy

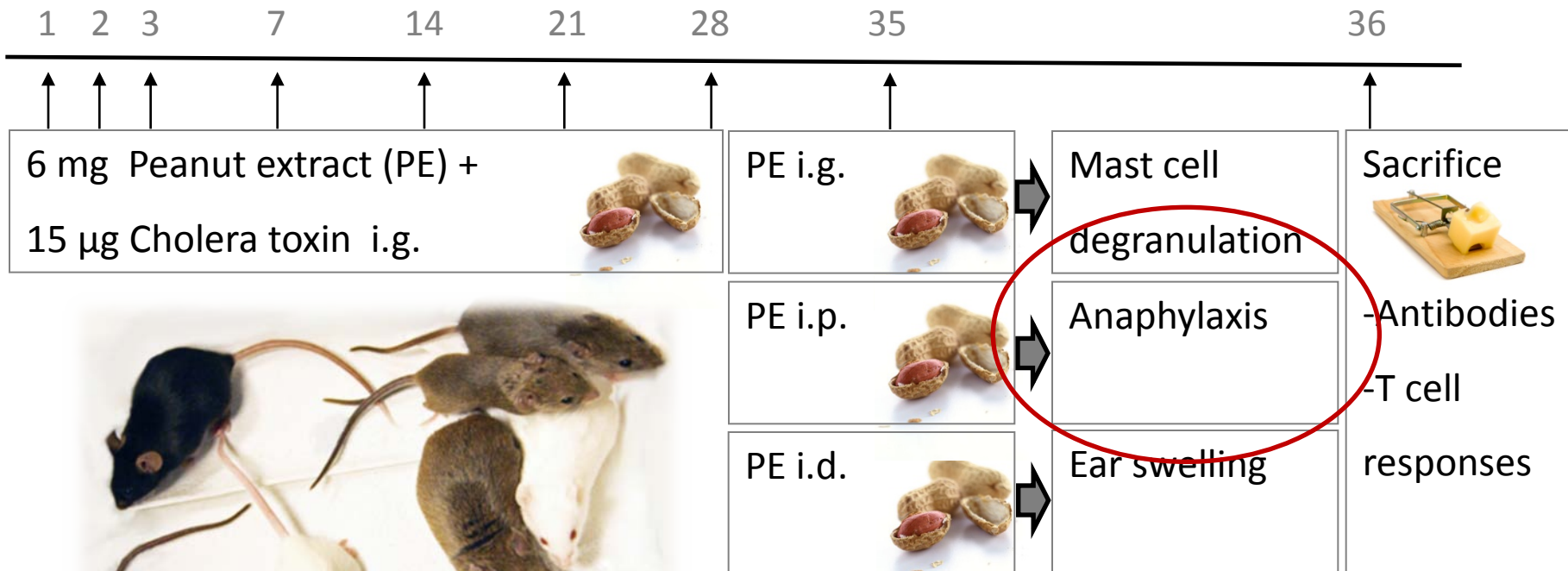
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A peanut food allergy model

Sensitisation

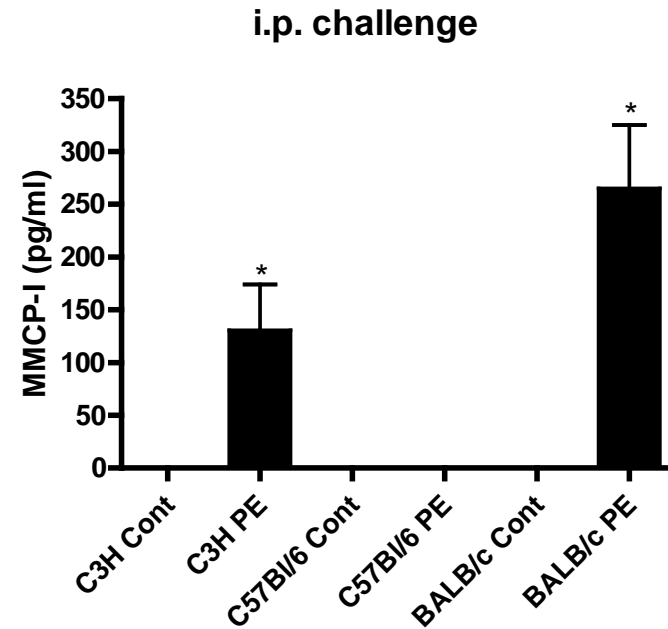
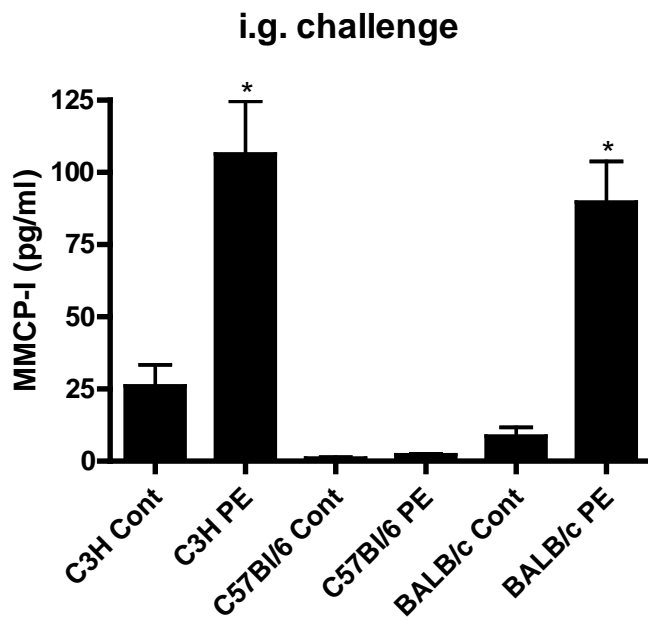
Challenge



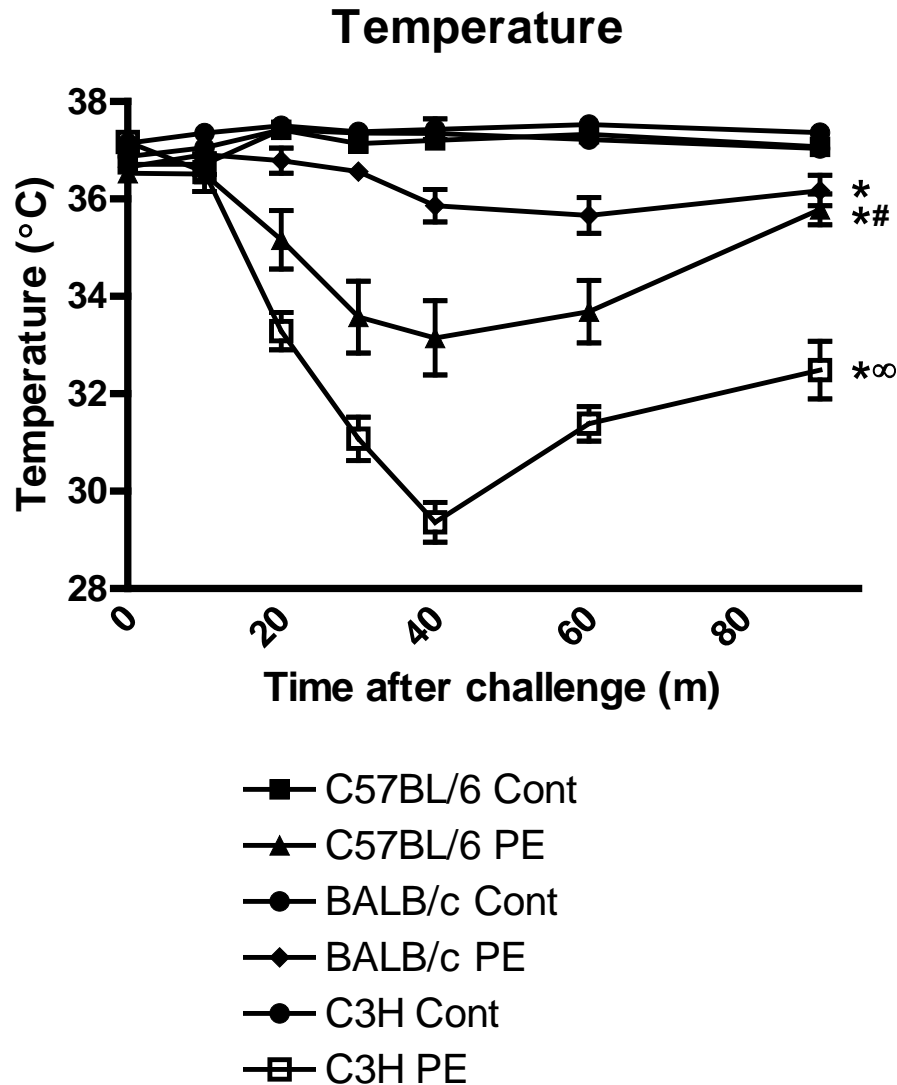
A peanut food allergy model in 3 mouse strains

	C3H/HeOuj	C57BL/6	BALB/c
Antibodies:			
IgE	+	++	+++
IgG1	+	++	+
IgG2a	++	IgG2c	-
Cytokines:			
Th1 (IFN γ)	++	+	++
Th2(IL-5, IL-13)	+/-	+	++

Mast cell degranulation



Peanut-induced systemic anaphylaxis

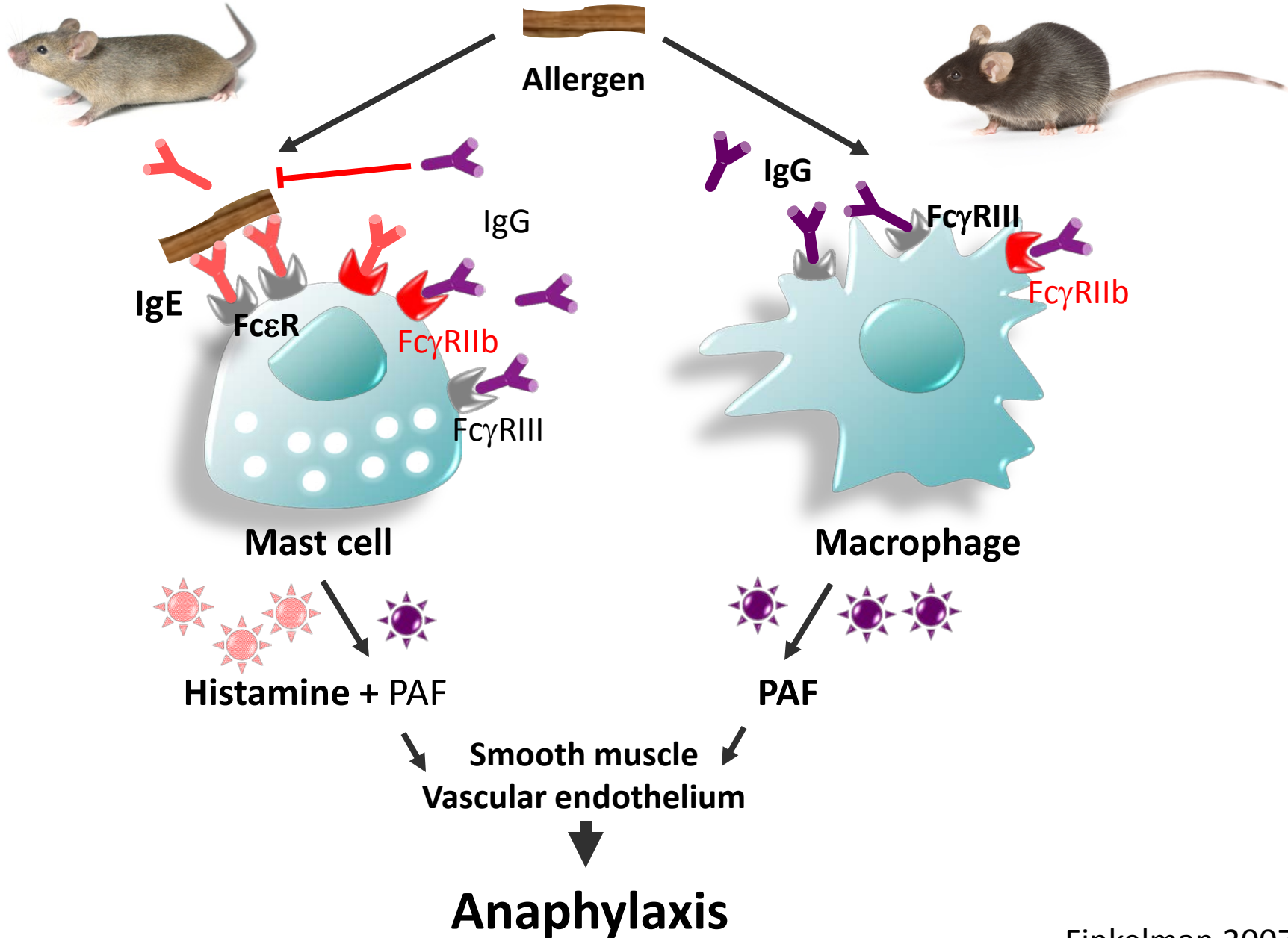


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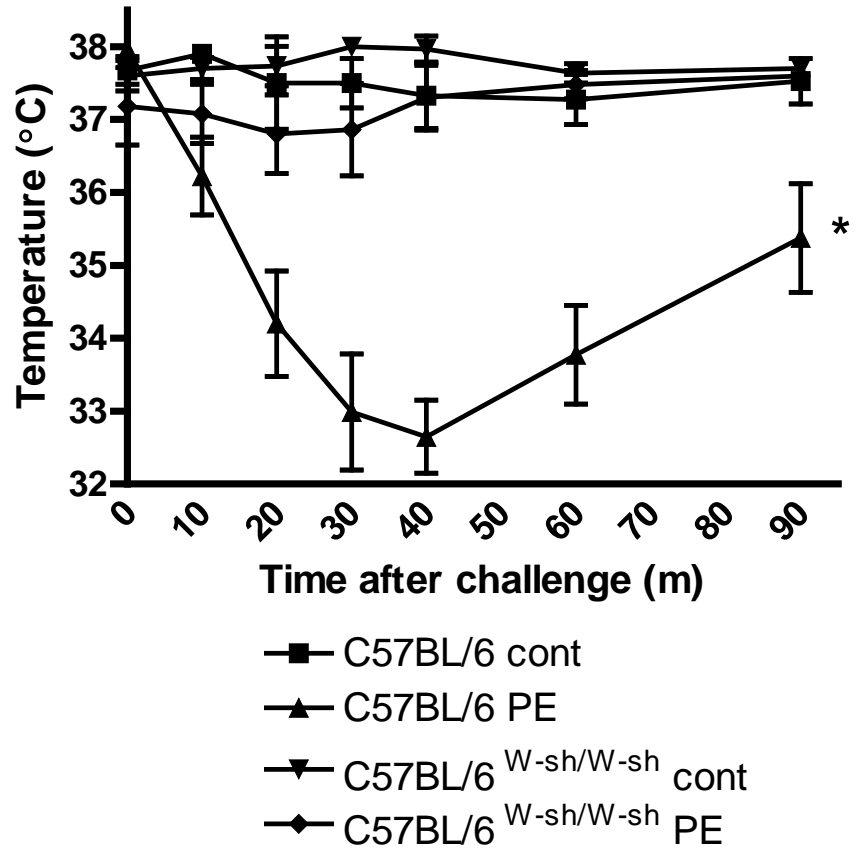
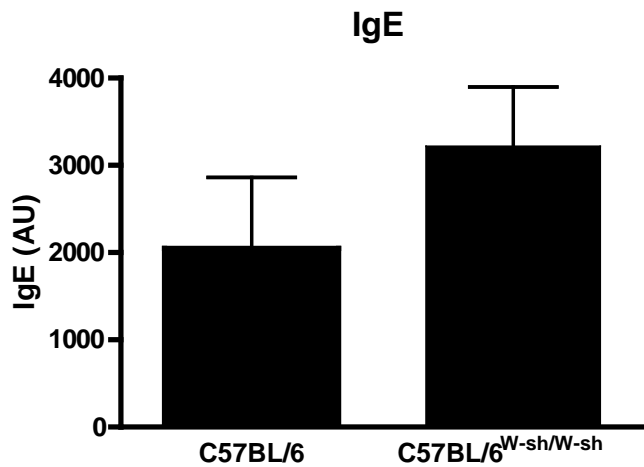
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Antibodies:			
IgE	+	++	+++
IgG1	+	++	+
IgG2a	++	IgG2c	-
Cytokines:			
Th2(IL-5, IL-13)	+/-	+	++
Mucosal mast cell degranulation	++	-	++
Anaphylaxis	+++	++	-
Ear swelling	++	+	+

Classic Pathway

Alternative Pathway



Mast cells and peanut-induced anaphylaxis



Food allergy, more than mast cells and IgE?

- Anaphylaxis can occur in the absence of mucosal mast cell degranulation (MMCP-I) in C57BL/6 mice

But.....

- Mast cells are essential for peanut-induced anaphylaxis in C57BL/6 mice, are these connective tissue mast cells ?
- Components of the alternative pathway such as macrophages and PAF do play a role in peanut induced anaphylaxis, but only in C57BL/6 mice (Smit JJ, Plos One, 2011)

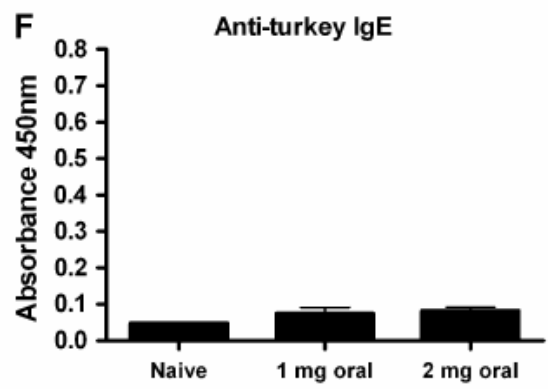
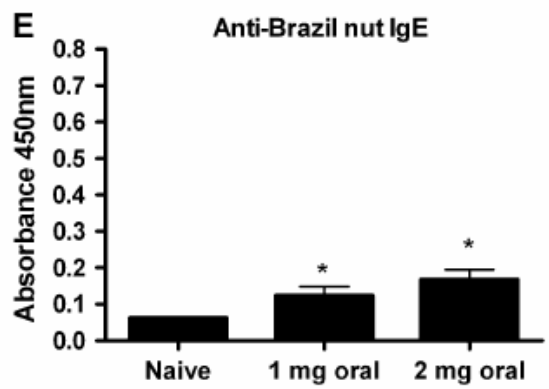
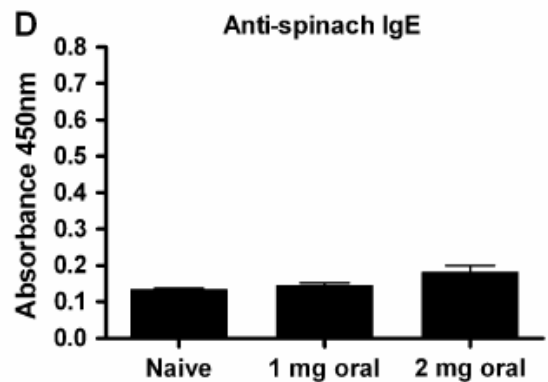
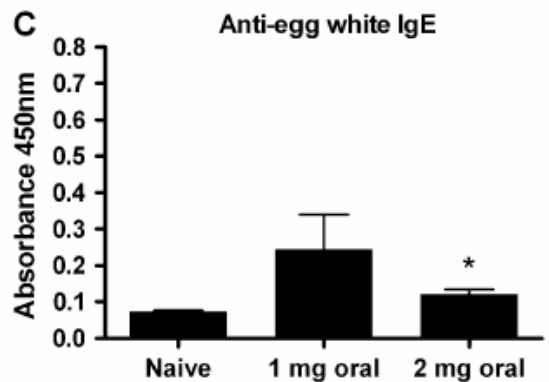
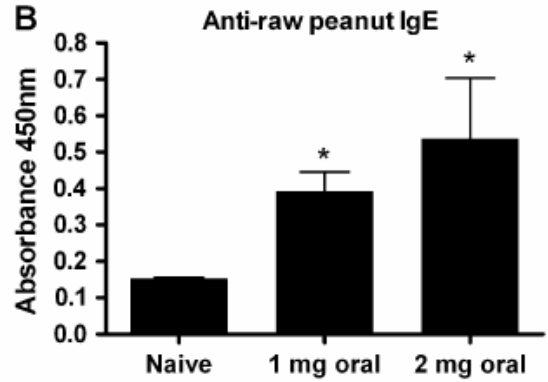
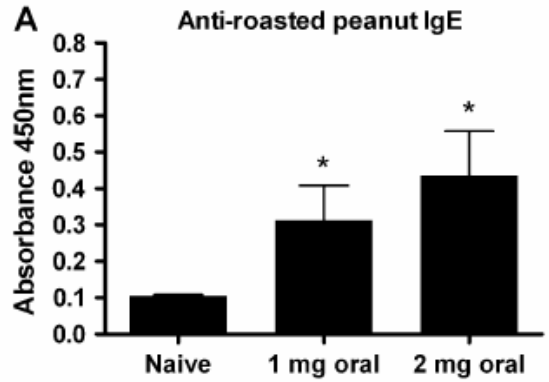


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IgE responses allergens in the CT model



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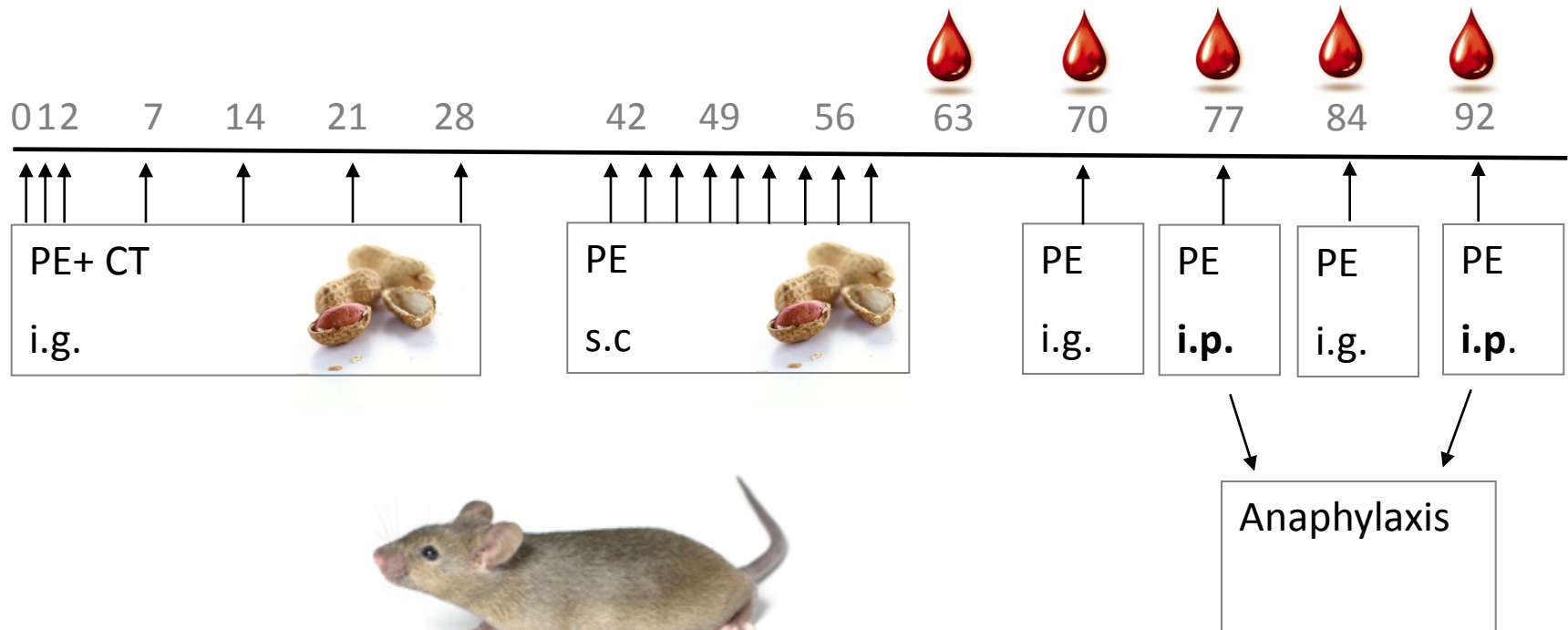
Immunotherapy in food allergy



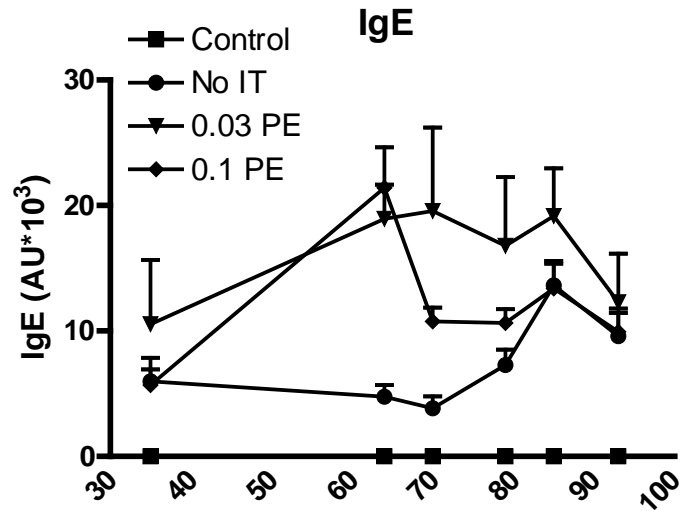
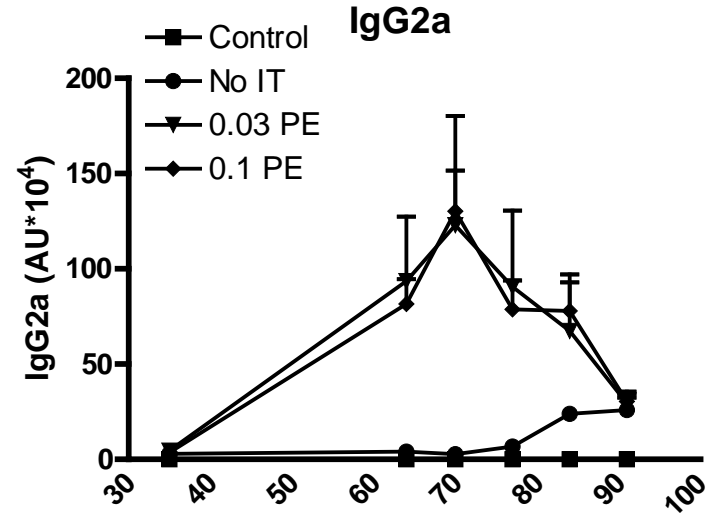
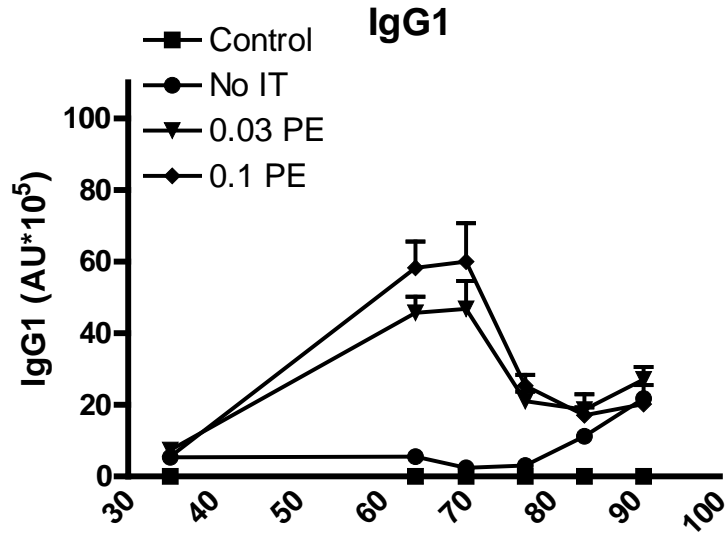
Sensitisation

S.c. therapy

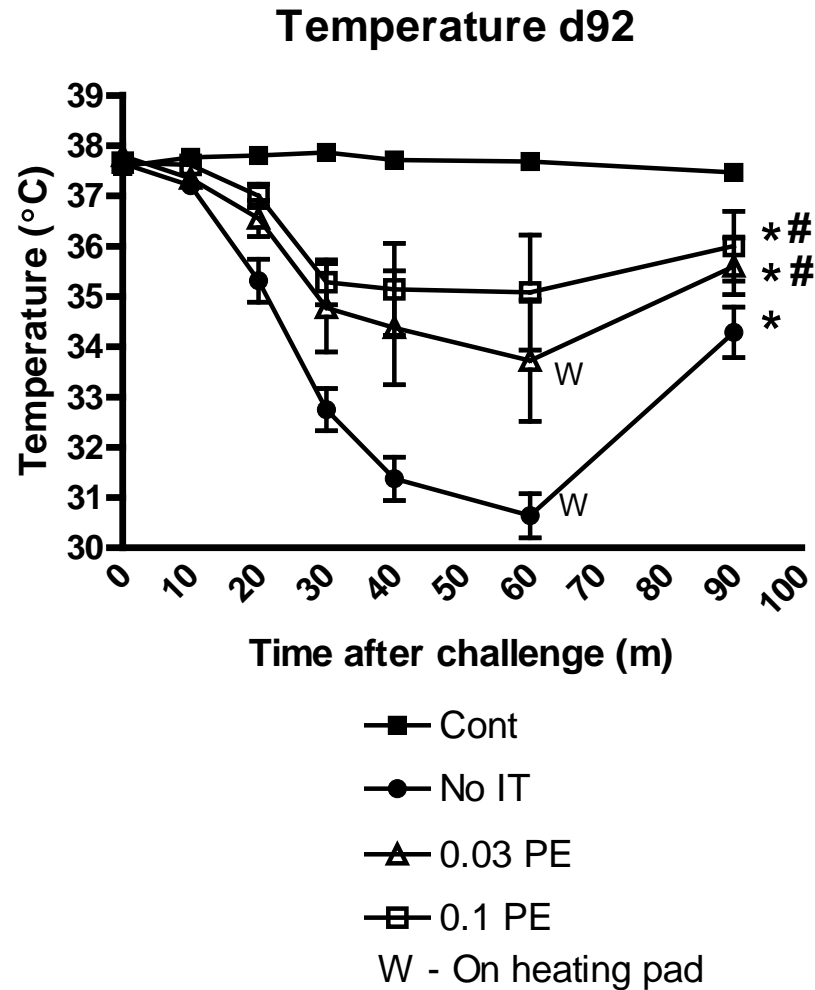
Challenge



Efficacy of immunotherapy



Efficacy of immunotherapy



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