

Supplementary material

Natural and Induced Tolerance to Hymenoptera Venom: A Single Mechanism?

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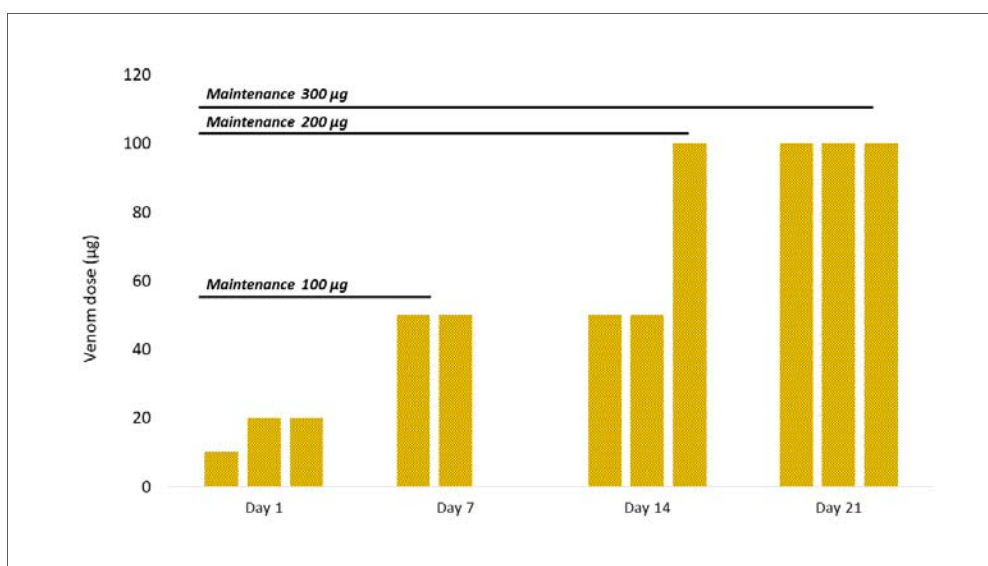


Figure S1. Dosing scheme for BVIT up dosing. Each column represents an individual dose, separated 30 minutes from the next dose of the same cluster. The AG subjects receiving 100 µg maintenance did the up dosing in two visits, the 200 µg subjects in three and the 300 µg subjects in four. At the end of each cluster, patients remained under observation for two hours.

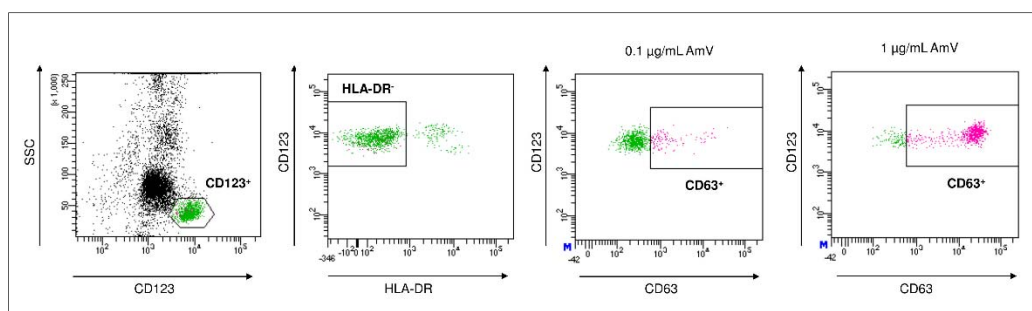


Figure S2. Basophil activation test gating strategy. SSC, Side scatter. The identification of basophils was performed according to the expression of CD123 and HLA-DR markers (CD123⁺HLA-DR⁻), while those degranulated (pink) were identified according to the expression of CD63 marker (CD63⁺). Results from total peripheral blood cells stimulated with 0.1 and 1 µg/mL of *Apis mellifera* venom (AmV) are displayed.

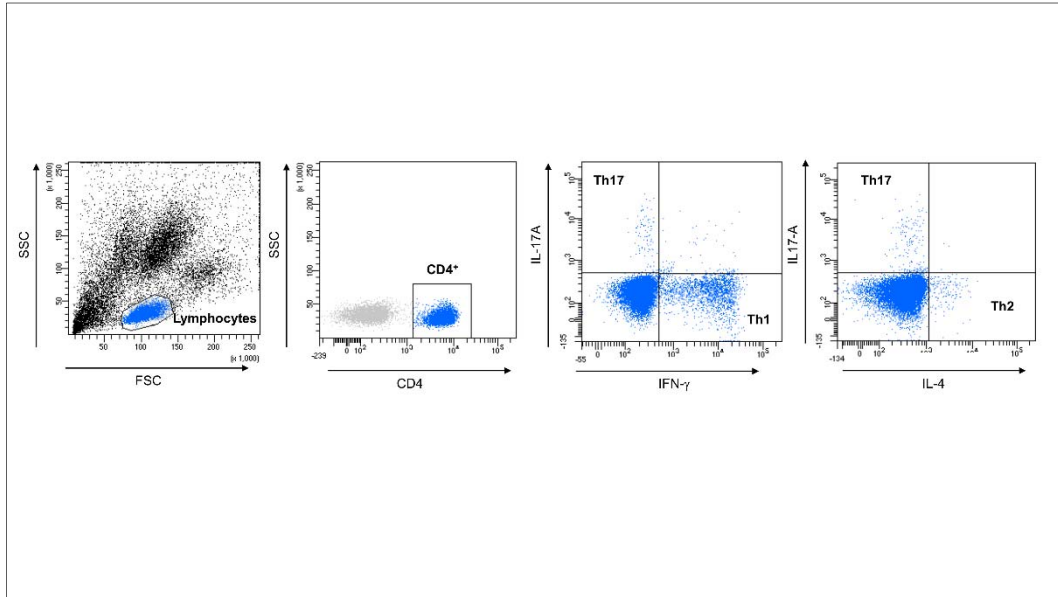


Figure S3. Th1/Th2/Th17 gating strategy. FSC, Forward scatter; SSC; Side scatter. The identification of Th1/Th2/Th17 subsets was performed according to the expression of CD4 (blue) and the production of IFN- γ , IL-4 and IL-17 cytokines. Results from total peripheral blood cells stimulated with phorbol 12-myristate 13-acetate at 50 ng/mL and ionomycin calcium salt at 1 μ g/mL are displayed.

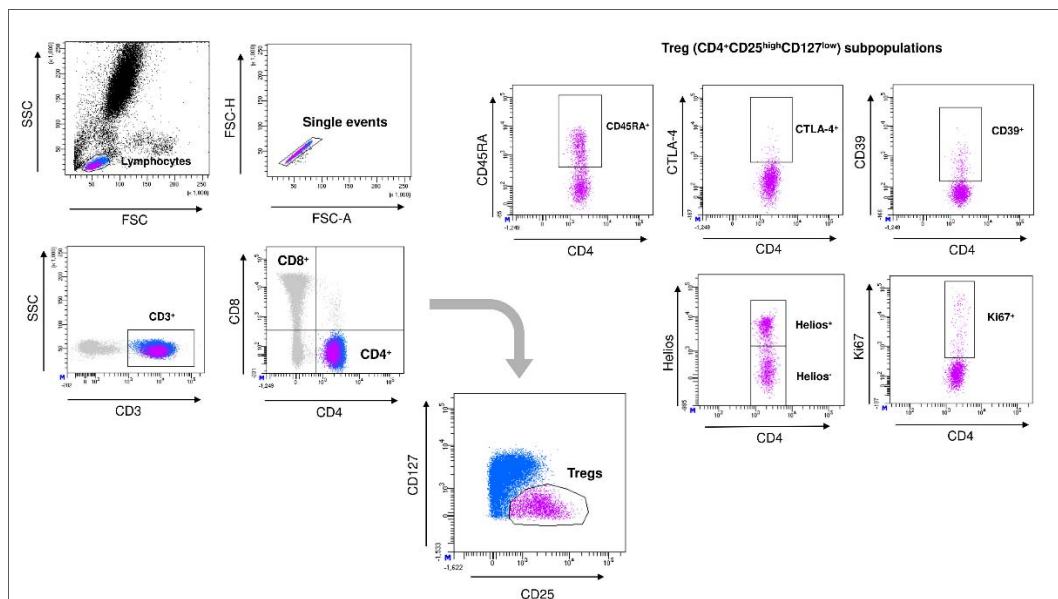


Figure S4. Treg gating strategy. FSC, forward scatter; SSC, Side scatter. The phenotype of Treg (CD4⁺CD25^{high}CD127^{low}) subpopulations from total peripheral blood was performed according the gating strategy here displayed.

Table S1. Mean value and standard deviation (in braquets) of every outcome for allergic patients (**AG**) at baseline (**T0**), after first cluster of BVIT (**T1**), after completing updosing (**T2**) and at the end of the first year of BVIT (**T3**); and for the tolerant beekeepers (**TG**) in a single time. T cell populations (both effector and regulatory) are expressed in %; basophil activation % after priming with two venom doses; interleukynes in pg/ml; kynurenine in ng/ml, sIgE in IU/ml and sIgG4 in µg/ml

		AG				TG
		T0	T1	T2	T3	
T effector cells	CD4 ⁺ Th1	12.08 (8.11)	10.82 (6.83)	12.55 (7.51)	14.59 (5.41)	6.85 (3.96)
	CD4 ⁺ Th2	0.82 (0.78)	0.93 (0.94)	0.90 (0.85)	1.39 (0.75)	0.34 (0.66)
T regulatory cells	CD4 ⁺ /CD25 ^{high} /CD127 ^{low}	8.56 (1.61)	8.73 (1.77)	8.43 (1.66)	7.35 (2.08)	8.28 (1.75)
	Treg CTLA-4 ⁺	12.25 (10.68)	10.13 (7.31)	12.25 (7.74)	4.51 (4.95)	26.82 (11.85)
	Treg Helios ⁺	69.90 (19.04)	73.58 (15.50)	69.38 (18.11)	81.81 (19.32)	86.48 (11.47)
Basophil Activation %	0.1 µg	5.27 (10.35)	7.21 (16.42)	6.72 (11.75)	2.64 (3.26)	0.66 (0.67)
	1 µg	54.71 (27.83)	49.80 (29.07)	50.79 (27.18)	22.36 (21.15)	15.62 (20.77)
Interleukynes	IL-4	393.35 (405.43)	515.80 (449.78)	732.08 (762.62)	1,634.38 (945.46)	266.98 (166.63)
	IL-10	21.96 (15.17)	25.32 (25.23)	53.82 (47.92)	26.11 (21.36)	56.20 (31.43)
	Kynurenine	0.61 (0.21)	0.54 (0.21)	4.78 (3.19)	6.98 (2.42)	0.70 (0.79)
Immunoglobulin E	sIgE AmV	23.10 (52.67)	31.74 (71.55)	37.52 (73.41)	11.09 (14.48)	1.32 (1.85)
	sIgE rApi m 1	10.19 (23.22)	13.62 (31.20)	23.16 (55.98)	3.61 (7.12)	0.27 (0.29)
	sIgE rApi m 2	0.57 (1.52)	0.53 (1.35)	7.81 (20.83)	0.95 (2.11)	0.42 (0.41)
	sIgE rApi m 3	1.05 (2.71)	1.00 (2.50)	6.51 (13.07)	0.51 (0.89)	0.04 (0.16)
	sIgE Api m 4	0.85 (1.63)	1.25 (2.07)	1.89 (2.70)	0.33 (0.58)	0.07 (0.11)
	sIgE rApi m 5	2.34 (4.73)	2.21 (4.27)	4.81 (6.90)	1.36 (2.00)	0.09 (0.10)
	sIgE rApi m 10	11.81 (28.85)	12.52 (27.80)	20.21 (44.56)	9.03 (19.74)	0.31 (0.47)
	sIgG4 AmV	0.62 (1.03)	0.80 (1.78)	5.68 (6.63)	5.57 (3.95)	66.99 (173.61)
Immunoglobulin G4	sIgG4 rApi m 1	0.47 (1.01)	0.44 (0.89)	4.12 (6.37)	3.44 (2.50)	45.13 (125.15)
	sIgG4 rApi m 2	0.01 (0.04)	0.02 (0.05)	0.60 (2.42)	0.55 (0.81)	6.71 (5.51)
	sIgG4 rApi m 3	0.04 (0.15)	0.03 (0.10)	0.21 (0.68)	0.07 (0.15)	1.22 (1.80)
	sIgG4 Api m 4	0.09 (0.17)	0.19 (0.45)	1.71 (2.79)	0.24 (0.44)	7.82 (7.37)
	sIgG4 rApi m 5	0.02 (0.02)	0.02 (0.03)	0.08 (0.12)	0.06 (0.09)	1.37 (1.73)
	sIgG4 rApi m 10	0.02 (0.03)	0.02 (0.03)	0.16 (0.29)	0.04 (0.07)	1.56 (2.80)