Supplementary Material for:

"Targeting Effector Memory T Cells with the Small Molecule Kv1.3 Suppresses Allergic Contact Dermatitis"

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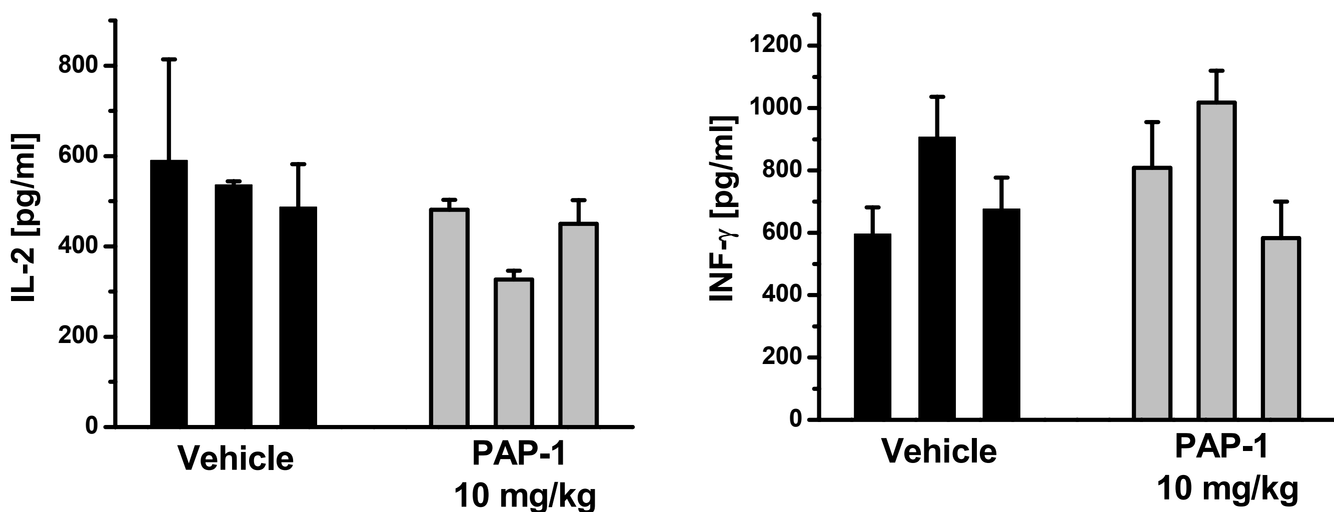
Primers for real-time PCR:

The sequences of the primers used were: CD4 (forward) 5'-ACGCACAGCCTAAGGATTCAG-3' and (reverse) 5'-CGCAAAGCCCAGCACTG-3', for CD8-β (forward) 5'-CCAGAAAGGCTTGACATGTGG-3' and (reverse) 5'-TGAGGGATACCAGCAGAACCA-3', for IL-17 (forward) 5'-GGAAGTTGGACCACCACATGA-3' and (reverse) 5'-CTCCCTCTTCAGGACCAGGATC-3', for Kv1.3 (forward) 5'-TTACTCTGGGCACTGAGCTGG-3' and (reverse) 5'-CTCAGGATGGCCAGTGACATAG-3', for TNF-α (forward) 5'-TGGGCTCCCTCTCATCAGTT-3' and (reverse) 5'-TGGGCTACGGGCTTGTCA-3'. HPRT1 (Mm00446968_m1), IL-2 (Rn00587673_m1) and IFN-γ (Rn00594078_m1) were measured with Applied Biosystem kits.

Fig. S1:

***In vivo* PAP-1 treatment does not affect ex-vivo cytokine production by splenic $CD8^+$ T cells.** Female Lewis rats were treated with vehicle (n = 3) or PAP-1 at 10 mg/kg (n = 3) at 8-hour intervals for 48 hours. Spleens were removed 2 hours after the last PAP-1 application and single cell suspensions prepared by mechanical disaggregation through a 100 µm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ). Red blood cells were lysed with NH_4Cl solution and the resulting mononuclear cells washed twice with PBS. $CD8^+$ cells were enriched by negative selection using the StemSep® rat $CD8^+$ T cell enrichment kit (StemCell Technologies, Vancouver) and found to be 98.5% $CD8^+$ by flow cytometry with PerCP-conjugated anti-rat CD8α mAb (BD Pharmingen). The $CD8^+$ cells were seeded at $2x10^5$ cells/well into a flat-bottom 96-well plate in complete RPMI medium. ConA at 5 µg/mL and $1x10^5$ irradiated MNCs (2500 rad) were then added and the cells were incubated for 48 hours before supernatants were collected and analyzed for IFN-γ, IL-2 and IL-4 by ELISA (Invitrogen-Biosource, Camarillo, CA).

There were no significant differences in the amount of IL-2 (p = 0.56) or INF-γ (p = 0.13) produced by $CD8^+$ T cells from PAP-1 treated animals compared to vehicle treated animals. IL-4 levels were low (5 pg/mL) and did not change with PAP-1 treatment (data not shown). Similar results for all three cytokines were obtained after simulation of $CD8^+$ cells with PMA plus ionomycin (data not shown).



Supplemental Table 1: 28-Day Toxicity Test of PAP-1.

Lewis rat were injected i.p. daily for 28 days with either 10 mg/kg of PAP-1 (n = 6, 3 male and 3 female rats) or with the vehicle Cremophor®EL/PBS (n = 6). At the end of the trial, rats were sacrificed and complete blood chemistry, hematology and necropsy performed by the Comparative Pathology Laboratory of University of California, Davis.

	Vehicle (n = 6; 3 male, 3 female)	**PAP-1** (n = 6; 3 male, 3 female)
Hematology		
White cells (K/μl)	10.8 ± 2.8[1]	8.4 ± 1.6; p = 0.14
Red cells (M/μl)	7.5 ± 0.6	7.6 ± 0.4; p = 0.78
Hemoglobin (g/dl)	14.9 ± 0.7	14.8 ± 0.4; p = 0.48
Hematocrit (%)	45.9 ± 3.5	45.7 ± 2.5; p = 0.91
Mean cell volume (fl)	59.7 ± 1.6	58.3 ± 1.5; p = 0.07
Mean cell hemoglobin (pg)	20.5 ± 1.3	19.6 ± 1.0; p = 0.51
Mean cell hemoglobin concentration (g/dl)	32.7 ± 2.4	32.7 ± 1.6; p = 0.98
Platelets (K/μl)	672 ± 171	815 ± 88; p = 0.15
Neutrophil (%)	33.6 ± 4.5	30 ± 4.0; p = 0.20
Lymphocyte (%)	56.7 ± 5.5	62.5 ± 4.5; p = 0.15
Monocyte (%)	8.0 ± 2.6	6.5 ± 1.1; p = 0.28
Eosinophil (%)	1.3 ± 1.3	0.8 ± 0.4; p = 0.41
Basophil (%)	0	0
Blood chemistry		
Albumin (g/dl)	4.4 ± 0.4	4.4 ± 0.5; p = 0.81
Alkaline phosphatase (U/l)	226 ± 70	184 ± 83; p = 0.08
Blood Urea nitrogen (mg/dl)	16.5 ± 1.6	15.0 ± 1.9; p = 0.26
Calcium (mg/dl)	11.5 ± 0.3	11.2 ± 0.4; p = 0.12
Cholesterol (mg/dl)	98 ± 6	95 ± 15; p = 0.36
Glucose (mg/dl)	121 ± 6	135 ± 24; p = 0.26
Phosphorus (mg/dl)	8.4 ± 0.5	8.2 ± 1.6; p = 0.73
Total protein (g/dl)	5.8 ± 0.3	5.8 ± 0.3; p = 0.41
Aspartate Aminotransferase (U/l)	123 ± 46	220 ± 166; p = 0.27
Alanine Aminotransferase (U/l)	67 ± 41	84 ± 67; p = 0.56
Triglyceride (mg/dl)	98 ± 28	107 ± 49; p = 0.71
Necropsy		
Gastrointestinal tract	Nothing significant	Nothing significant
Pancreas	Nothing significant	Nothing significant
Mesenteric lymph node	Nothing significant	Nothing significant
Heart	Nothing significant	Nothing significant

Thymus	Nothing significant	Nothing significant
Lung	Nothing significant	Nothing significant
Spleen	No pathological findings but see below[2]	No pathological findings but see below[2]
Liver	Nothing significant	Nothing significant
Kidney	Nothing significant (n = 4); unilateral hydronephrosis (n = 2)[3]	Nothing significant (n = 4); unilateral hydronephrosis (n = 2)[3]
Adrenal glands	Nothing significant	Nothing significant
Urinary bladder	Nothing significant	Nothing significant
Genital tract (Females: uterus and ovaries; males: testes and accessory sex glands)	Nothing significant	Nothing significant
Brain	Nothing significant	Nothing significant
Bone marrow	Nothing significant	Nothing significant

[1]mean ± SD
[2]All animals showed extra-medullary hematopoiesis in the red pulp and prominent marginal zones in the white pulp. This is a normal finding in adult rats.
[3]The unilateral hydronephrosis in 2 female control and 2 female PAP-1 treated rats is most likely congenital in origin and has been seen previously in female rats from the same vendor.

Supplemental Table 2: 15-Day *in vivo* cutaneous irritation test of PAP-1.

PAP-1 was tested for induction of cutaneous irritation *in vivo.* PAP-1 was injected subcutaneously at 10 mg/kg in 500 μl of either Cremophor®EL/PBS (n = 3) or peanut oil (n = 3) for 15 days daily into the same area on the right flank of the animals. Control rats (n = 3 per group) were treated with equal volumes of the two vehicles. At the end of the trial the rats were sacrificed and the injection area analyzed for skin irritation by histology by a trained pathologist at the Comparative Pathology Laboratory of University of California, Davis.

	Injection site skin Histopathology	Comments
Peanut Oil (n = 3)	The epidermis, dermis, hypodermis and adnexal structures are unremarkable. There are focally extensive round to oval clear spaces. The adjacent connective tissue surrounding these spaces contains fibroblasts, scattered lymphocytes and macrophages.	The clear spaces are consistent with the deposited peanut oil. The adjacent inflammation and dilated lymphatics are in response to the peanut oil and or associated trauma. No changes are present in tissue away from the clear spaces.
PAP-1 in Peanut Oil (n = 3)	No significant differences compared to vehicle	No drug associated changes noted
Cremophor/ PBS (n = 3)	The epidermis, dermis and adnexal structures are unremarkable. The hypodermis, and panniculus adipocytes have been replaced by diffuse loosely arranged swollen cells with vacuolated to basophilic granular cytoplasm. Several areas contain atrophic adipocytes surrounded by aggregates of the described cells. These cells are presumed to be macrophages. These cells are also present between the Panniculus Carnosus muscle bundles.	Presumed areas of Cremophor deposition have loss of normal adipocytes. The described swollen cells are presumed macrophages that have phago-cytized lipid released from the adipose tissue. However, these may also be atrophic adipocytes that have lost their lipid cytoplasm due to the detergent character of the Cremophor.
PAP-1 in Cremophor/ PBS (n = 3)	No significant differences compared to vehicle	No drug associated changes noted