

SUPPLEMENTAL MATERIALS

Figure S1. Comparison of parallelism for cytokine assays demonstrates ability to quantify cytokines across three species.

Figure S2. The tissue microenvironment is dynamic, and the ability to regenerate is associated with a specific immune response to injury.

Table S1. Summary of animals used in this study.

Table S2. One-way ANOVA analyses of log-transformed uninjured serum data.

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Table S5. One-way ANOVA analysis of tissue cytokine data to test for an effect of group at D1, 2, 10, 20.

Table S6. List of T cell phenotyping antibodies tried using flow cytometry in *Acomys cahirinus*.

Data File S1 contains summary tables of post-hoc multiple comparison tests used in Figure 2, Figure 3 and Figure S2.

Data File S2. Comparisons of amino acid sequence alignments for antigens in study.

SUPPLEMENTAL FIGURES

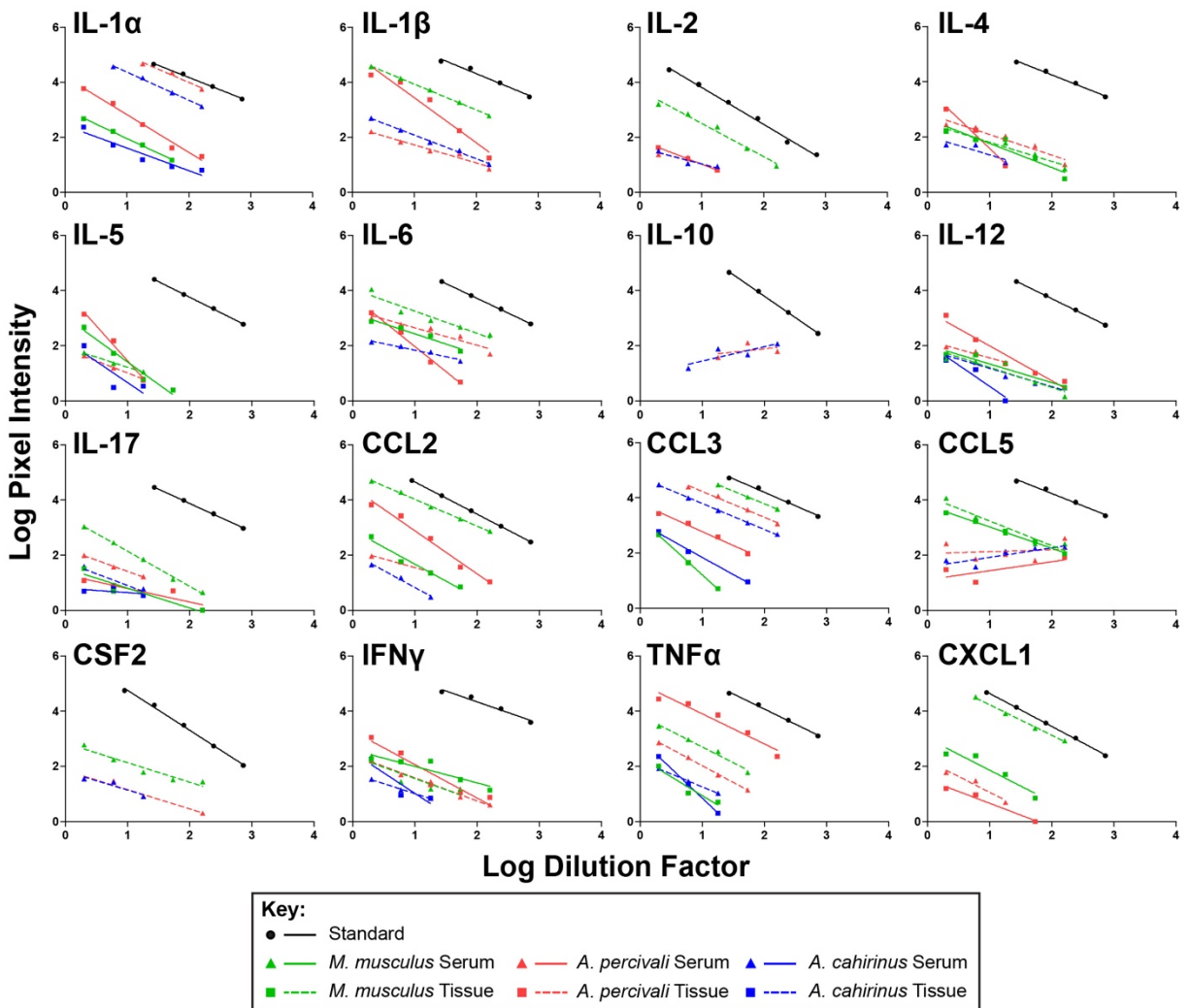
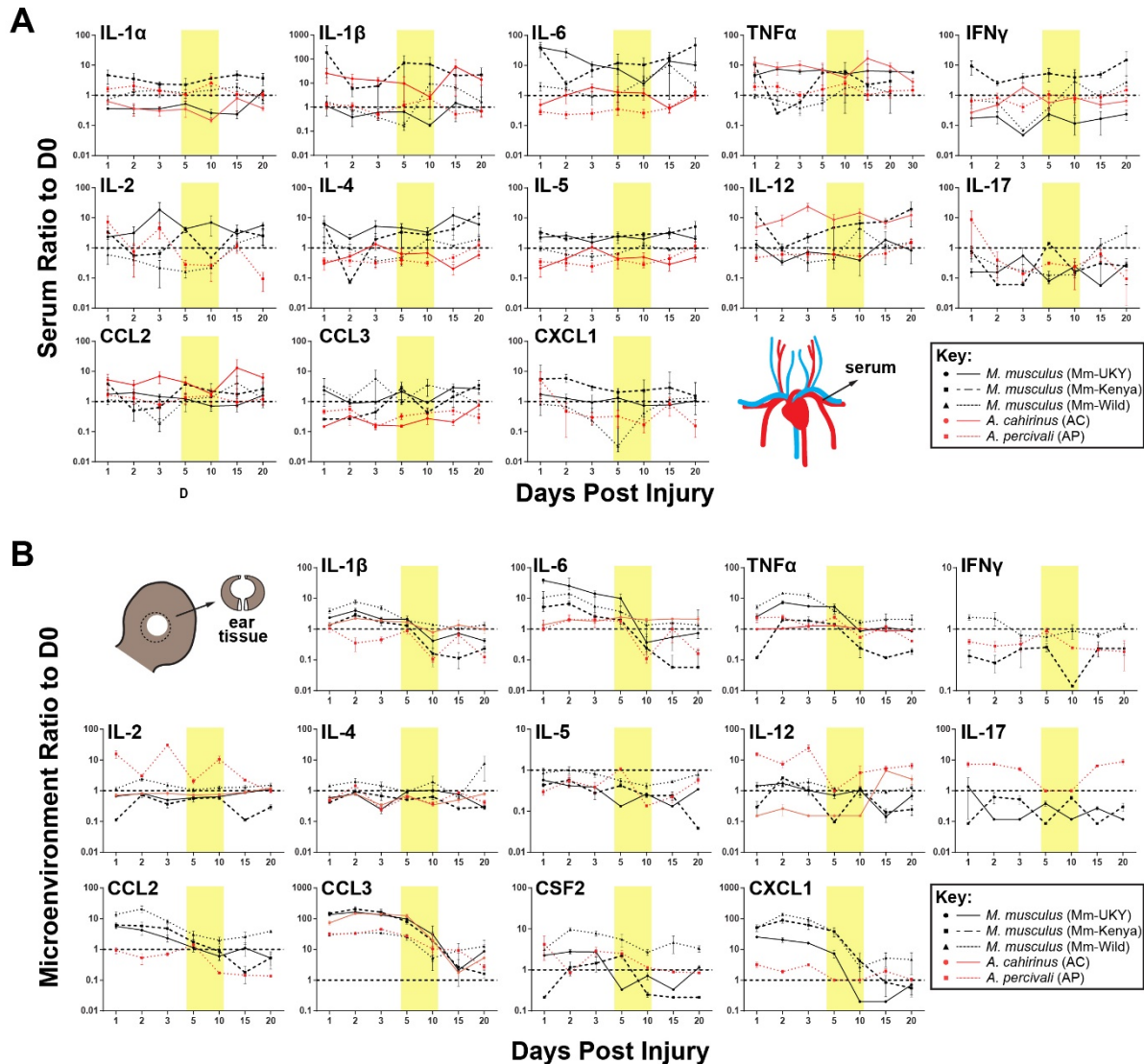


Figure S1. Comparison of parallelism for cytokine assays demonstrates ability to quantify cytokines across three species. Serial dilution of serum (triangles, solid lines) and tissue lysate (squares, dashed lines) shows similar negative slopes for *M. musculus* (green), *A. percivali* (red) and *A. cahirinus* (blue) compared to the standard (black, circles, solid line). Data represent the mean for duplicates and lines are linear regressions calculated from all data that was above the lower limit of detection.



SUPPLEMENTAL TABLES

Table S1: Summary of animals used in this study.

	<i>M. musculus</i>	<i>M. musculus</i>	<i>M. musculus</i>	<i>A. cahirinus</i>	<i>A. percivali</i>
Abbreviation:	Mm-UKY	Mm-Kenya	Mm-Wild	Ac	Ap
Strain:	ND4	“Swiss”	Wild-caught	UKY Colony	Wild-caught
Location:	Kentucky	Kenya	Kentucky	Kentucky	Kenya
Life history:	Lab-reared	Local breeder	Wild	Lab-reared	Wild
Regenerates?	No	No	No	Yes	Yes
Immune status:	Naïve	Primed	Primed	Naïve	Primed
Animal numbers by experiment in this study:					
Parallelism	3 (Female)	n/a	n/a	3 (Female)	3 (Female)
(In addition to pooled samples)	3 (Male)			3 (Male)	3 (Male)
Cytokine analysis time course					
Per timepoint:	3 to 5 (F) 3 to 5 (M)	5 to 9 (F)	1 to 3 (F) 2 to 4 (M)	3 to 5 (F) 3 to 5 (M)	5 to 7 (F) 3 to 5 (M)
Totals:	28 (F) 30 (M)	59 (F)	16 (F) 23 (M)	30 (F) 30 (M)	41 (F) 28 (M)
T cell analysis					
Flow:	22 (F)	n/a	n/a	21 (F)	n/a
Histo. / Western:	28 (F)	n/a	n/a	28 (F)	n/a
COX-2 inhibition					
D10 Histology:	10 (F)	n/a	n/a	n/a	n/a
Time course:	20 (F)	n/a	n/a	n/a	n/a

Table S2: One-way ANOVA analyses of log-transformed uninjured serum data. Groups listed in comparison column represent groups for which data was quantified and could be compared. *P*-values indicate where at least one group is significantly different from another group. Tukey-Kramer HSD post-hoc tests were used for pairwise comparisons are summarized in Figure 1A (See Data File S1).

Antigen	Comparison	N	F	<i>P</i> -value
IL-1 α	All groups	5	2.69	0.051
IL-1 β	All groups	5	11.15	<0.001*
IL-2	All groups	5	10.27	<0.001*
IL-4	All groups	5	6.00	0.001*
IL-5	All groups	5	2.63	0.055
IL-6	All groups	5	6.31	0.001*
IL-12	All groups	5	5.21	0.003*
IL-17	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	4	1.15	0.349
CSF2	None – all undetectable	n/a	n/a	n/a
CCL2	All groups	5	16.03	<0.001*
CCL3	All groups	5	2.59	0.058
IFN γ	All groups	5	3.25	0.026*
TNF α	All groups	5	23.80	<0.001*
CXCL1	All groups	5	4.07	0.010*

Table S3: Two-way ANOVA analyses of log-transformed serum time series data. Groups listed in comparison column represent groups for which data was quantified throughout the time series. Tukey-Kramer HSD post-hoc tests were used for pairwise comparisons and the results are summarized in Figure 1B (See Data File S1).

			Group		Day		Group*Day	
Antigen	Comparison	N	F	P-value	F	P-value	F	P-value
IL-1 α	All groups	255	32.51	<0.001*	0.93	0.471	1.62	0.039*
IL-1 β	Mm-UKY, Mm-Wild, Ap	157	3.02	0.052	1.06	0.391	1.08	0.386
IL-2	All groups	255	27.82	<0.001*	2.46	0.025*	1.96	0.006*
IL-4	All groups	255	34.54	<0.001*	1.47	0.188	1.55	0.053
IL-5	All groups	255	63.22	<0.001*	0.94	0.469	0.82	0.706
IL-6	All groups	255	64.61	<0.001*	2.29	0.037*	2.63	<0.001*
IL-12	All groups	255	33.25	<0.001*	2.21	0.660	0.91	0.583
IL-17	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	205	7.64	<0.001*	2.78	0.013*	2.60	<0.001*
CSF2	None – all undetectable	0	n/a	n/a	n/a	n/a	n/a	n/a
CCL2	All groups	255	5.72	<0.001*	1.46	0.193	1.16	0.278
CCL3	All groups	255	38.75	<0.001*	0.91	0.486	1.47	0.079
IFN γ	All groups	255	37.81	<0.001*	1.22	0.296	1.77	0.018*
TNF α	All groups	255	31.88	<0.001*	0.85	0.535	0.91	0.583
CXCL1	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	205	69.43	<0.001*	4.33	<0.001*	1.47	0.104

Table S4: Two-way ANOVA analyses of log-transformed tissue lysate time series data. Groups listed in comparison column represent groups for which data was quantified throughout the time series. *P*-values indicate where at least one group is significantly different from another group. Tukey-Kramer HSD post-hoc tests were used for pairwise comparisons and the results are summarized in Figure 2 (See Data File S1).

Antigen	Comparison	N	Group		Day		Group*Day	
			F	<i>P</i> -value	F	<i>P</i> -value	F	<i>P</i> -value
IL-1 α	None – all above limit of detection	0	n/a	n/a	n/a	n/a	n/a	n/a
IL-1 β	All groups	261	77.88	<0.001*	39.47	<0.001*	5.33	<0.001*
IL-2	All groups	261	190.93	<0.001*	6.68	<0.001*	13.59	<0.001*
IL-4	All groups	261	29.09	<0.001*	11.24	<0.001*	6.76	<0.001*
IL-5	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	211	22.73	<0.001*	10.03	<0.001*	10.70	<0.001*
IL-6	All groups	261	76.89	<0.001*	104.11	<0.001*	15.06	<0.001*
IL-12	All groups	261	123.20	<0.001*	10.42	<0.001*	18.28	<0.001*
IL-17	Mm-UKY, Mm-Kenya, Ap	177	598.63	<0.001*	4.42	<0.001*	17.55	<0.001*
CSF2	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	211	104.44	<0.001*	21.36	<0.001*	8.51	<0.001*
CCL2	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	211	72.96	<0.001*	38.71	<0.001*	5.23	<0.001*
CCL3	All groups	261	8.30	<0.001*	160.11	<0.001*	6.24	<0.001*
IFN γ	Mm-Kenya, Mm-Wild, Ap	161	40.47	<0.001*	2.42	0.030*	3.00	<0.001*
TNF α	All groups	261	149.87	<0.001*	81.32	<0.001*	11.84	<0.001*
CXCL1	Mm-UKY, Mm-Kenya, Mm-Wild, Ap	211	137.76	<0.001*	201.75	<0.001*	26.64	<0.001*

Table S5: One-way ANOVA analysis of tissue cytokine data to test for an effect of group at D1, 2, 10 and 20. *P*-values indicate where at least one group is significantly different from another group. Tukey-Kramer HSD post-hoc tests were used for pairwise comparisons and are summarized in Figure 3 (See Data File S1).

Antigen	Comparison	N	Day	F-Ratio	<i>P</i> -value
IL-1 β	All groups		1	5.39	0.002*
			2	32.52	<0.001*
			10	20.70	<0.001*
			20	19.05	<0.001*
IL-4	All groups		1	9.71	<0.001*
			2	4.8	0.004*
			10	5.23	0.003*
			20	18.89	<0.001*
IL-6	All groups		1	14.56	<0.001*
			2	47.24	<0.001*
			10	23.30	<0.001*
			20	21.50	<0.001*
IL-12	All groups		1	57.33	<0.001*
			2	14.22	<0.001*
			10	7.66	<0.001*
			20	26.33	<0.001*
IL-17	Mm-UKY, Mm-Kenya and Ap		1	45.87	<0.001*
			2	93.60	<0.001*
			10	441.78	<0.001*
			20	116.93	<0.001*
CCL2	Mm-UKY, Mm-Kenya, Mm-Wild, Ap		1	21.55	<0.001*
			2	33.43	<0.001*
			10	7.04	0.002*
			20	11.68	<0.001*
TNF α	All groups		1	39.61	<0.001*
			2	54.97	<0.001*
			10	27.95	<0.001*
			20	67.67	<0.001*
CXCL1	All groups		1	60.99	<0.001*
			2	210.02	<0.001*
			10	15.23	<0.001*
			20	9.54	<0.001*

Table S6: List of T cell phenotyping antibodies tried using flow cytometry in *Acomys cahirinus*.

Antigen	Clone	Supplier	Catalog#	Status
CD45	OX-1	BioLegend	202207	No – no separation
	30-F11	BioLegend	103106	No – no separation
	I3/2.3	Molecular Probes	A15395	No – no separation
CD3	17A2	BioLegend	100236	Yes – good population
TCRab	H57-597	BioLegend	109207	No – poor separation
	R73	BioLegend	201107	No – no separation
TCRgd	GL3	BioLegend	118107	No – poor separation
	VC7-13D5	BioLegend	107507	No – poor separation
CD4	GK1.5	BioLegend	100433	No – no separation
	RM4-4	BioLegend	116011	No – poor separation
CD8	53-6.7	BioLegend	100726	No – no separation
CD25	P61	BioLegend	102015	No – poor separation
	3C7	BioLegend	101915	No – no separation
CD69	H1.2F3	BioLegend	104511	No – poor separation
CD196	29-2L17	BioLegend	129813	No – poor separation
	140706	BD Pharmingen	561753	Yes – good population
CD206	C068C2	BioLegend	141705	Yes – good population
CD49b	HMa2	BioLegend	103515	Yes – good population
	DX5	BioLegend	108909	No – poor separation

Supplemental Files

Data File S1 contains summary tables of post-hoc multiple comparison tests used in Figure 2, Figure 3 and Figure S2.

Data File S2. Comparisons of amino acid sequence alignments for antigens in study. Predicted amino acid sequence was translated from mRNA (*A. cahirinus*) or obtained from NCBI (*H. sapiens*, *M. musculus*, *R. rattus*) and aligned together using MAFFT. Figures were created using BOXSHADE where solid black shading is equivalent, gray shading is similar and no shading is dissimilar.