

Supplementary Material

BCG vaccination prevents reactivation of latent lymphatic murine tuberculosis independently of CD4⁺ T cells

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Supplementary Figures and Tables



Supplementary Figure 1: T cell numbers, serum cytokine and chemokine levels prior to *Mtb* infection. (A) Number of total CD3⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, CD3⁺CD4⁻CD8⁻ (DN) and CD3⁻ NKp46⁺ cells (left plots), as well as numbers of total CD44⁺, CD44⁺CD69⁺CD103⁺ cells amongst CD3⁺CD4⁺ and CD3⁺CD8⁺ cells (right plots) in spleen and inguinal LN 60 days after vaccination with BCG or BCG Δ BCG1419c. (B) Levels of serum cytokines and chemokines at 60 days after vaccination. Results are presented as pooled data means ± SEM from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: One-way ANOVA per analyte followed by Dunnett's multiple comparisons test; significant differences relative to unvaccinated mice are indicated by asterisks: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.001. Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; (Supplementary to Fig. 2)



Supplementary Figure 2: Serum cytokine and chemokine levels 120 days after *Mtb* **infection.** (A) Levels of serum cytokines and chemokines at 120 days after *Mtb* infection. Results are presented as pooled data means \pm SEM from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: One-way ANOVA per analyte followed by Dunnett's multiple comparisons test; significant differences relative to unvaccinated mice are indicated by asterisks: * p<0.05; b.d. below detection limit. Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; mAb, monoclonal antibody (Supplementary to Fig. 3)

Supplementary Figure 3







Supplementary Figure 3: CD8⁺ T cell profiling after *Mtb* infection. (A, B) Frequencies of CD3⁺CD4⁺ cells in LN (A) and lung (B) at 14, 28 and 50 days following *Mtb* infection. (C, D) Frequencies of CD3⁺CD8⁺ cells in LN (C) and lung (D) at 14, 28 and 50 days following *Mtb* infection. (E, F) Total numbers of CD3⁺CD8⁺ cells in LN and lung at 14, 28 and 50 days following *Mtb* infection. (G, H) Frequencies of CD3⁺CD8⁺CD44⁺ cells in LN and lung at 14, 28 and 50 days following *Mtb* infection. (I, J) Total number of CD3⁺CD8⁺CD44⁺ cells in LN and lung at 14, 28 and 50 days following *Mtb* infection. Results are presented as individual data points from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: One-way ANOVA per time point followed by Dunnett's multiple comparisons test; significant differences relative to unvaccinated mice are indicated by ted asterisks; statistical differences relative to unvaccinated anti-CD4-treated mice are indicated by red asterisks: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; LN, lymph node; mAb, monoclonal antibody (Supplementary to Fig. 4)



Supplementary Figure 4: Memory CD8⁺ T cell profiling after *Mtb* **infection.** (A) Frequencies of CD44⁺CD62L⁻, CD44⁺CD62L⁺ and CD44⁺CD69⁺CD103⁺ cells amongst CD8⁺ T cells. Results are presented as pooled data means from two pooled independent experiments (n = 8-10 mice per group). Statistical analyses: Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; LN, lymph node; mAb, monoclonal antibody (Supplementary to Fig. 4)

Supplementary Material

LN d14	unvacc.	anti CD4	BCG s.c.	BCG i.t.	∆1419c s.c.	Δ1419c i.t.	SPL d14	unv	vacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	υ	Lung d14	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.
unvacc.		0.758	0.9516	0.998	0.6265	0.9892	unvacc.			0.9928	0.9747	0.4905	0.8973	0.9999	u	unvacc.		0.9095	0.4945	0.8678	0.4945	0.9909
anti CD4			0.9969	0.9409	>0.9999	0.3756	anti CD4				0.7759	0.8309	0.5897	0.9996	a	anti CD4			0.9747	>0.9999	0.9747	0.9982
BCG s.c.				0.9978	0.9823	0.6718	BCG s.c.					0.1356	0.9996	0.9162	в	BCG s.c.				0.9873	>0.9999	0.8478
BCG i.t.					0.8663	0.8988	BCG i.t.						0.0682	0.6508	в	BCG i.t.					0.9873	0.9946
∆1419c s.c.						0.2637	∆1419c	.c.						0.7816	۵	51419c s.c.						0.8478
Δ1419c i.t.							∆1419c	t.							۵	51419c i.t.						
LN d28	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	SPL d28	unv	vacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	υ	Lung d28	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.
unvacc.		0.498	0.9355	0.6945	0.568	0.5537	unvacc.			0.0049	0.9614	0.4416	>0.9999	>0.9999	u	unvacc.		0.9038	0.6787	0.9749	0.9038	0.6787
anti CD4			0.0117	0.0026	0.0014	0.0013	anti CD4				0.0457	0.3687	0.0025	0.0031	a	anti CD4			0.9977	0.4853	>0.9999	0.9977
BCG s.c.				0.9952	0.979	0.976	BCG s.c.					0.9067	0.8959	0.9219	в	BCG s.c.				0.2429	0.9977	>0.9999
BCG i.t.					>0.9999	>0.9999	BCG i.t.						0.3107	0.3511	в	BCG i.t.					0.4853	0.2429
Δ1419c s.c.						>0.9999	∆1419c	.c.						>0.9999	۵	51419c s.c.						0.9977
Δ1419c i.t.							∆1419c	t.							۵	51419c i.t.						
LN d50	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	SPL d50	unv	vacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	υ	Lung d50	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.
unvacc.		0.0128	0.9986	0.9632	0.9812	0.945	unvacc.			0.0001	0.9993	0.1203	0.945	0.8257	u	unvacc.		0.0042	0.9995	0.9906	0.6839	0.6588
anti CD4			0.0559	0.0015	0.0894	0.115	anti CD4				0.0001	0.0417	0.0003	0.0006	а	anti CD4			0.003	0.0283	0.0001	0.0001
BCG s.c.				0.8532	0.9998	0.9976	BCG s.c.					0.0781	0.8427	0.6718	в	BCG s.c.				0.9495	0.8879	0.8774
BCG i.t.					0.68	0.544	BCG i.t.						0.5751	0.728	в	BCG i.t.					0.3468	0.3189
Δ1419c s.c.						>0.9999	Δ1419c	.c.						0.9997	۵	61419c s.c.						>0.9999
Δ1419c i.t.							Δ1419c	t.							۵	61419c i.t.						
LN d120	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	SPL d12	unv	vacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.	υ	Lung d120	unvacc.	anti CD4	BCG s.c.	BCG i.t.	Δ1419c s.c.	Δ1419c i.t.
unvacc.		0.0351	0.9548	0.8294	0.9902	0.2145	unvacc.			0.0001	0.3001	0.0902	0.9905	0.9373	u	unvacc.		0.0001	>0.9999	0.9839	0.9957	0.9974
anti CD4			0.0041	0.3843	0.1696	0.0001	anti CD4				0.0695	0.1481	0.001	0.0012	a	anti CD4			0.0001	0.0001	0.0001	0.0001
BCG s.c.				0.3297	0.7149	0.7568	BCG s.c.					0.9969	0.6876	0.8044	в	BCG s.c.				0.986	0.9978	0.8479
BCG i.t.					0.9935	0.009	BCG i.t.						0.3488	0.4524	в	BCG i.t.					0.8479	0.8529
Δ1419c s.c.						0.0669	∆1419c	.c.						0.9997	۵	61419c s.c.						>0.9999
Δ1419c i.t.							Δ1419c	t.								1419c i.t.						

Supplementary Table 1: P-values for CFU results presented in Figure 3A-C. A one-way ANOVA per time point followed by Tukey's multiple comparisons test was performed for CFU results shown in Figure 3A. The mean of each group was compared to the mean of every other group and p values for each combination and time point are shown. (Supplementary to Figure 3)