

Mycobacterium-Infected Dendritic Cells Disseminate Granulomatous Inflammation.

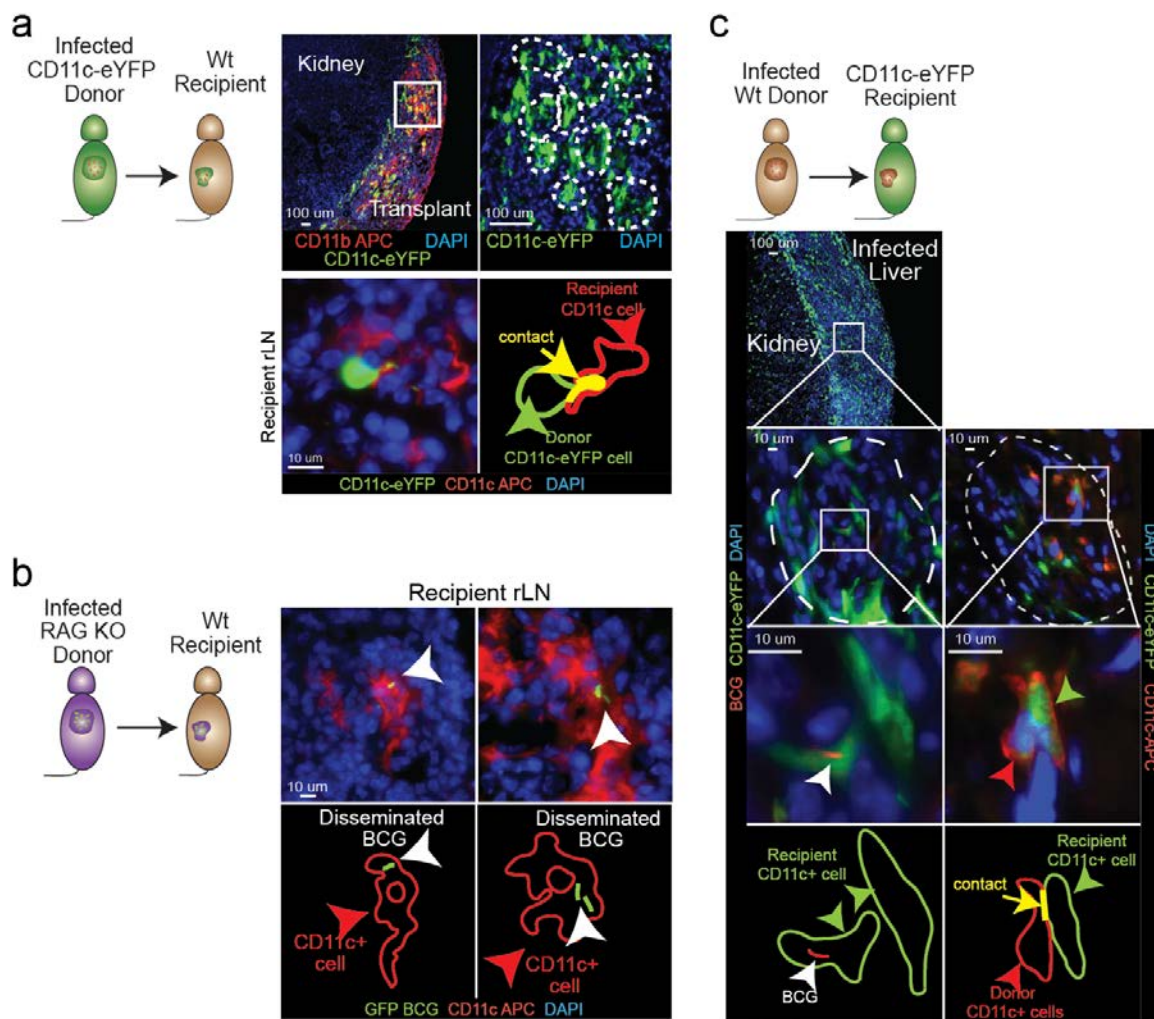
Supplementary Information

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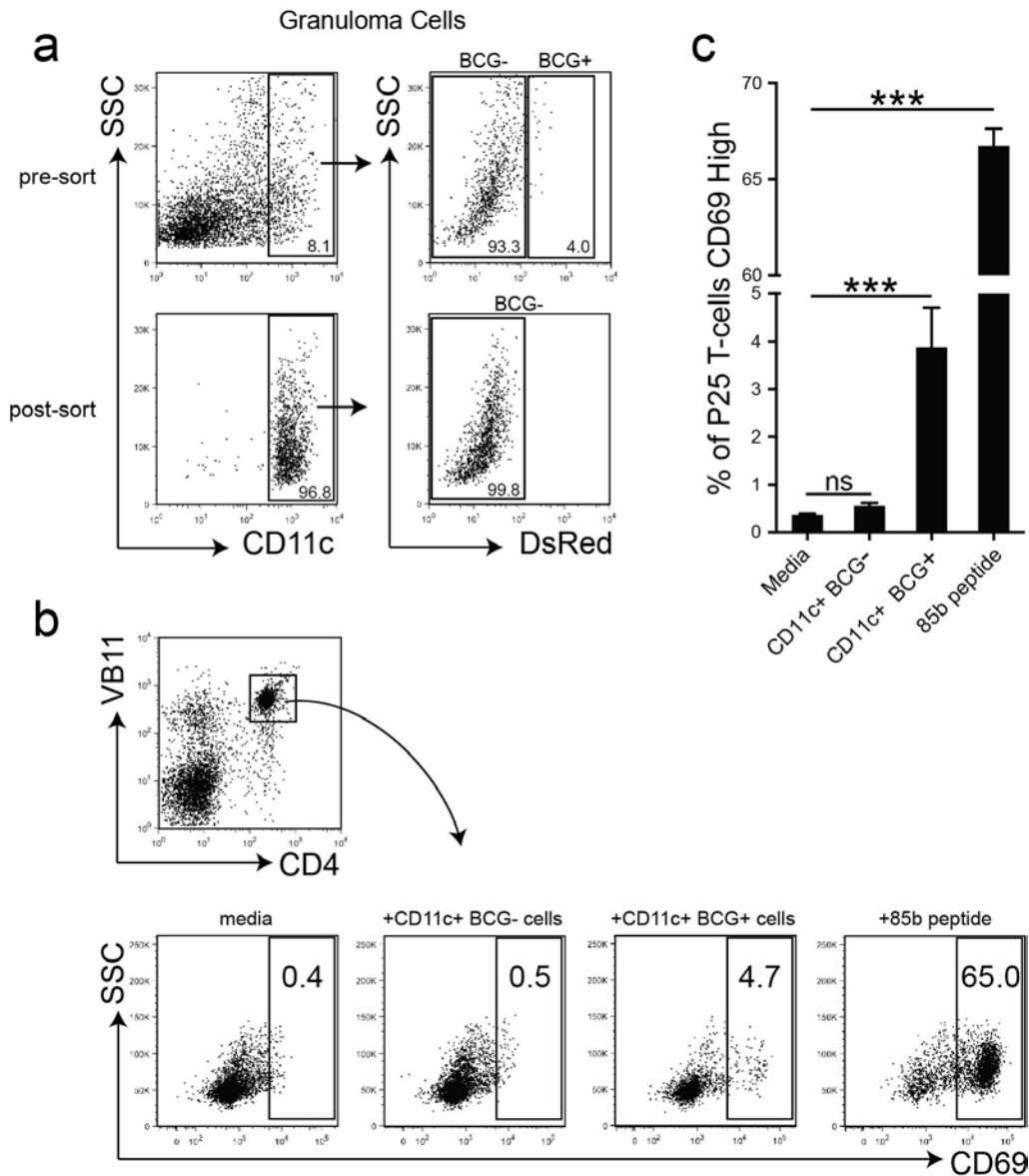
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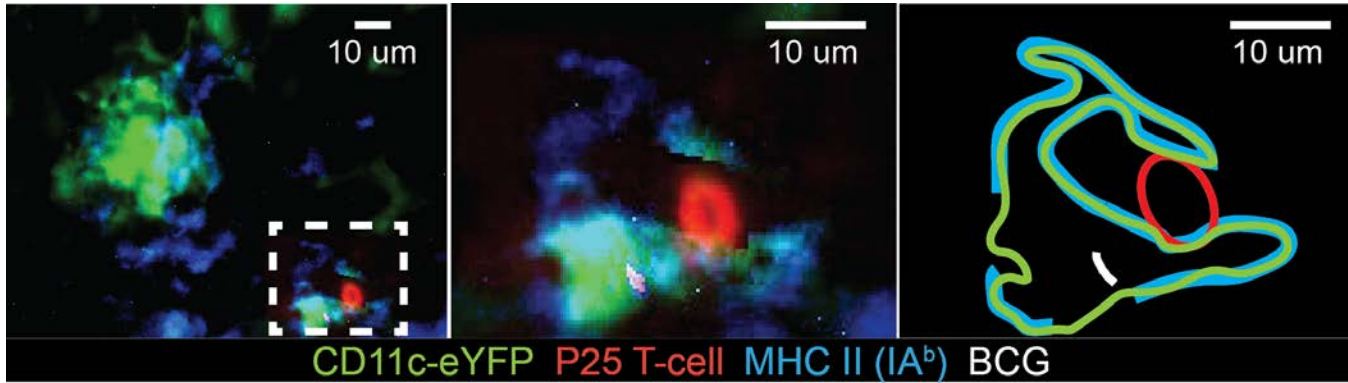
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Supplemental Figure 1: Contact between peripheral and granuloma CD11c+ cells. **A.** 3wk-infected (IP with BCG) liver from CD11c-eYFP mice was transplanted underneath the kidney capsule of Wt recipients and left for 7 days. *Top row:* micrographs of cross section of transplanted liver underneath the kidney capsule showing CD11b+ and CD11c+ cell-containing granulomas 7 days after transplant. Right images show magnification of representative area outlined in white. *Bottom Row:* high magnification micrograph of the renal lymph node (rLN) in Wt recipients after transplant of BCG-containing liver from CD11c-eYFP mice, showing contact between donor-derived CD11c-eYFP and recipient CD11c+ cell. **B.** Liver from RAG KO mice were acutely infected with GFP-BCG and transplanted into Wt recipients. Recipient rLN were harvested 7 days later. Red arrows indicate CD11c+ cells, white arrows show BCG. **C.** 3wk-infected (IP with BCG) liver from Wt mice was transplanted underneath the kidney capsule of CD11c-eYFP recipients and left for 7 days. *Top row:* Low magnification micrograph showing extensive infiltration of recipient CD11c-eYFP cells that migrated into the granuloma-containing liver. Individual granulomas (outlined by white dotted lines) that contain infiltrating CD11c-eYFP cells from the recipient (*second row, left and right*), can also be found infected BCG (*third and fourth row, left*. BCG indicated by white arrow). Co-staining with CD11c-APC antibody shows that some infiltrating CD11c-eYFP cells (green arrow in *third and fourth row, right*) make contact with donor CD11c-eYFP cells (red arrow in *third and fourth row, right*). Point of contact between donor CD11c cells (CD11c-APC antibody only) and recipient CD11c cells (colocalization with CD11c-APC antibody and CD11c-eYFP signal) shown with yellow arrow (*fourth row, right*).



Supplemental Figure 2: Incubation of P25 T-cells with uninfected and infected CD11c⁺ granuloma cells. **A.** Granuloma cells from mice infected with DsRed BCG were isolated and stained with CD11c. Uninfected and infected CD11c⁺ cells can be identified based on colocalization with DsRed fluorescence (pre-sorted, top panels). Granuloma isolates were sorted by flow cytometry into CD11c^{high}BCG⁻ and CD11c^{high}BCG⁺ populations (post-sort, bottom plots). Post-sort plot showing purified CD11c^{high}BCG⁺ cells is unavailable because all cells were needed for the proceeding assay (>99% purity of CD11c^{high}BCG⁺ was validated during sorting). **B.** Sorted CD11c^{high}BCG⁺ and CD11c^{high}BCG⁻ cells from **A** were incubated with cultures containing P25 T-cells for 24 hours. $\nu\beta 11$ vs. CD4 plot shows P25 T-cells from transgenic from mice identified in co-cultures with sorted CD11c^{high} cells. Activation of P25 T-cells in co-cultures was determined by CD69 expression. Controls included addition of media only (no CD11c^{high} cells), and addition of 1 μ g/ml 85b peptide only. **C.** Quantification of data from **B**, based on 3-5 replicate measurements. Error bars are mean \pm s.e.m. One-way ANOVA used to determine statistical significance. *P<0.05; **P<0.01; ***P<0.001.



Supplemental Figure 2: BCG-containing DCs outside the granuloma in contact with P-25 T-cells are MHC II positive. Micrographs from 50μm-thick mouse liver section 3 weeks after IP infection with 1×10^7 CFU BCG. *Left image:* lower magnification area showing a mature/larger granuloma and a nearby extra-granuloma CD11c⁺ cell that contains BCG bacilli (white dotted box). The CD11c⁺ cell is in apparent contact with a mycobacterial-specific, DsRed-expressing P25 T-cell. *Middle Image:* Enlargement of area within white dotted box showing the colocalization of endogenous CD11c-eYFP and MHC II (IA^b) antibody fluorescent signal. *Right Image:* outlines showing the cell boundaries of CD11c⁺ and P25 T-cells, as well as BCG bacilli inside the CD11c⁺ cell.