**CCR4 Expression on Host T cells is a Driver for Alloreactive Responses and Lung Rejection**

**Authors**

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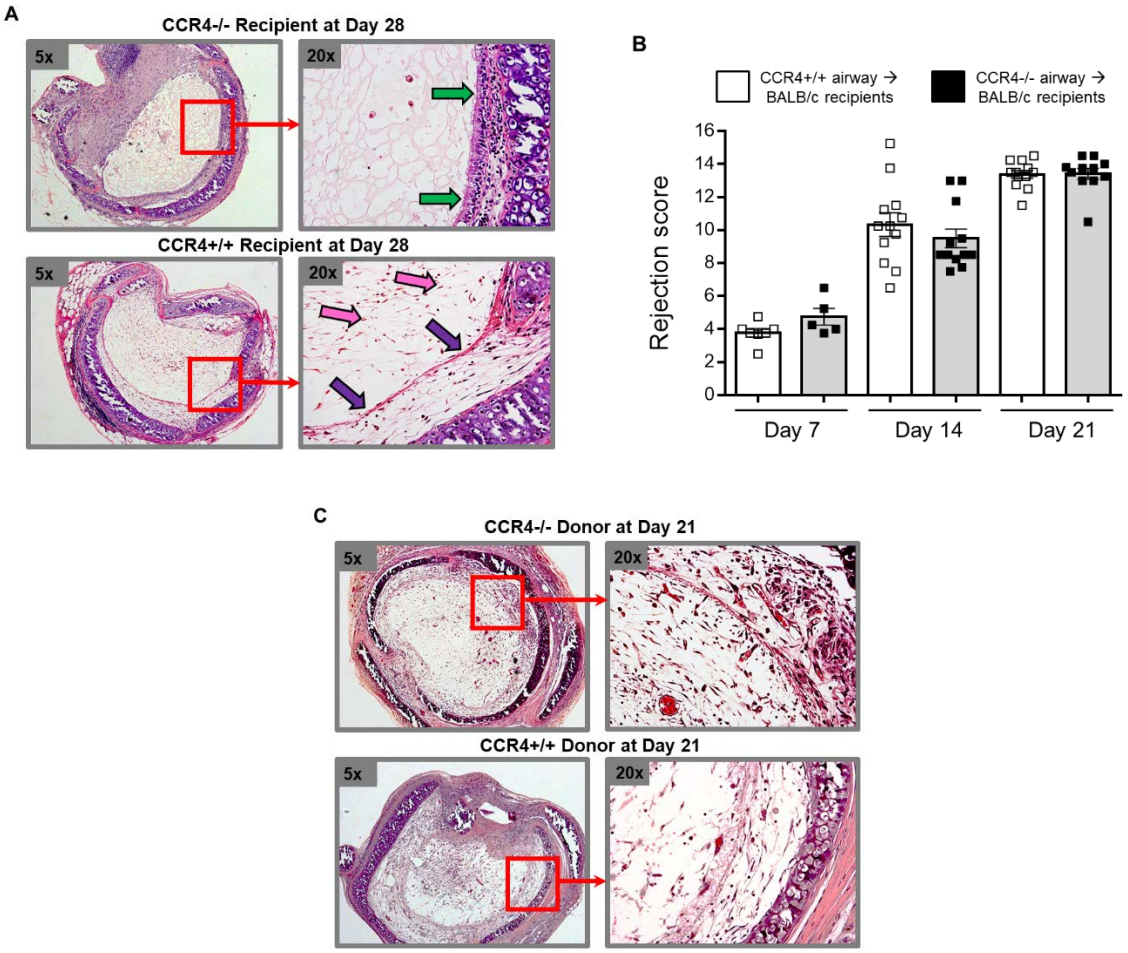
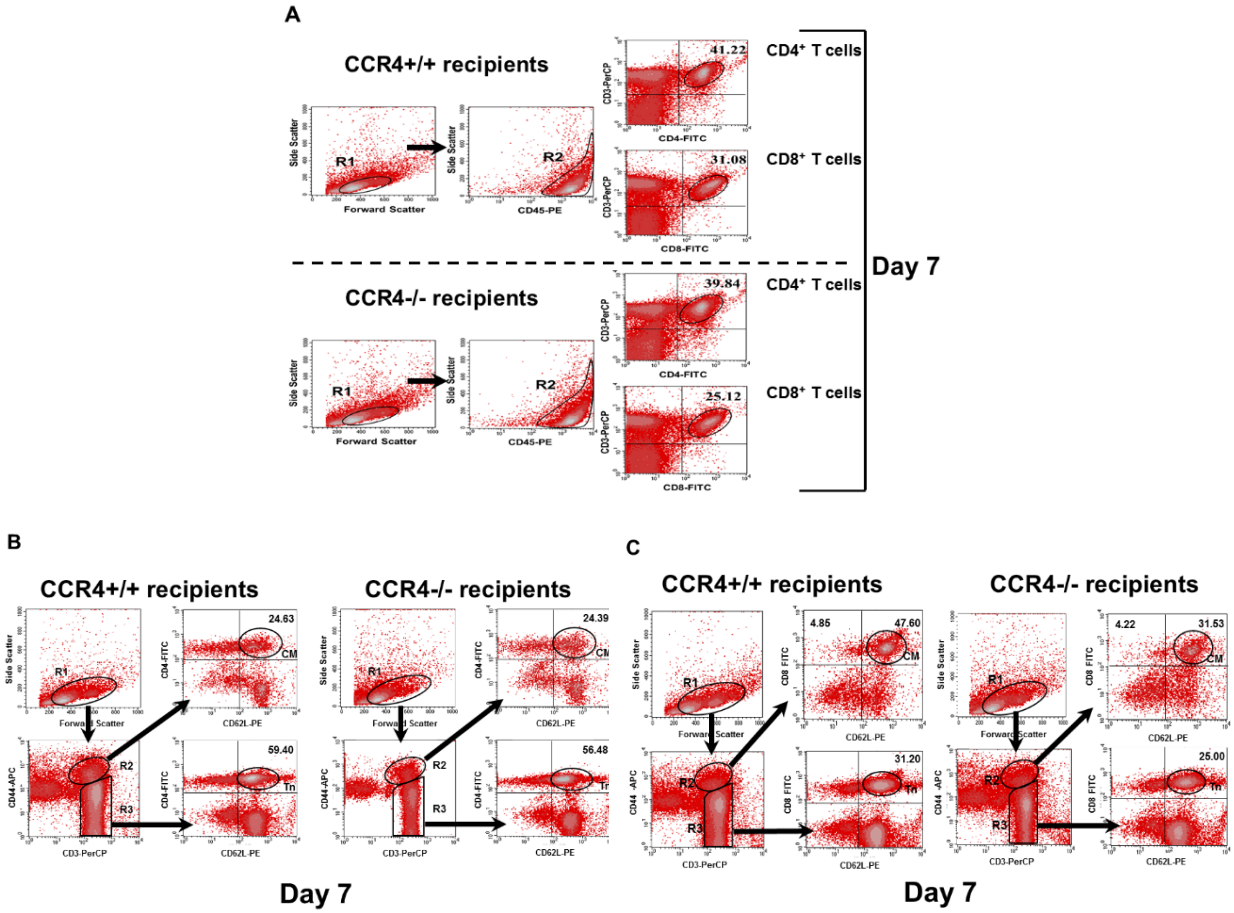
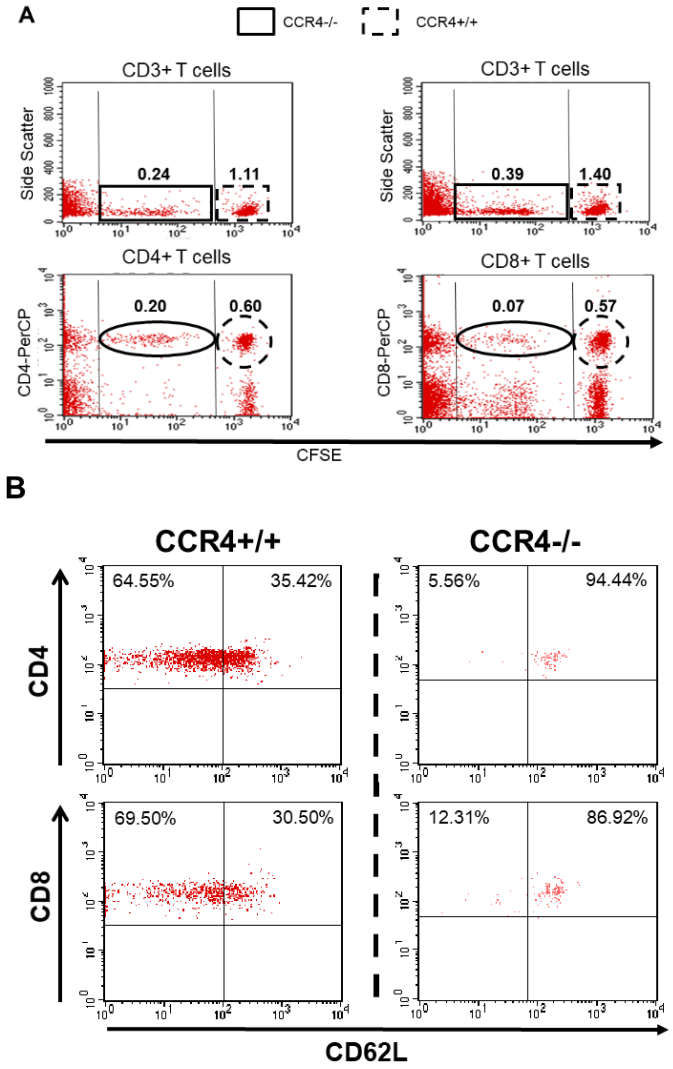
**SUPPLEMENTAL DATA**



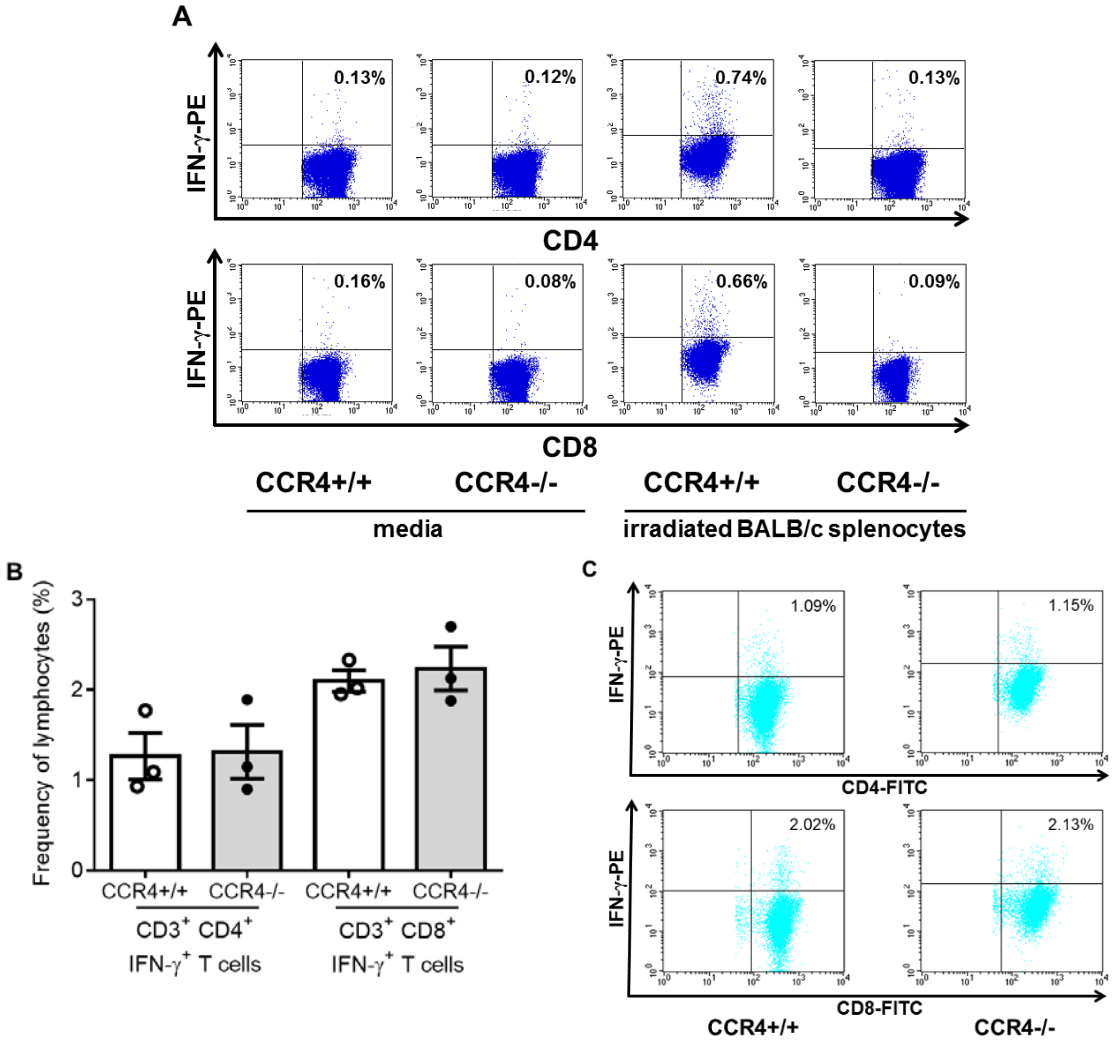

**Supplemental Figure 1. CCR4-/- recipients, but not CCR4-/- donors attenuate the development of murine airway allograft rejection.** BALB/c airways were subcutaneously transplanted into CCR4-/- versus CCR4+/+ recipients and in separate experiments CCR4-/- versus CCR4+/+ airways were transplanted into BALB/c recipients. (**A**) Representative H&E staining of airway allografts from CCR4-/- and CCR4+/+ recipients at day 28. Section of the airway allograft is magnified to show the presence of a virtually normal epithelial layer (green arrows) in the airway allografts from the CCR4-/- recipients. There is an absence of airway epithelial cells (purple arrows) and a presence of fibroblasts (pink arrows) causing fibro-obliteration of the airway allografts from the CCR4+/+ recipients. (**B**) Bar graph indicates rejection scores for CCR4-/- as compared to CCR4+/+ donor airways transplanted into BALB/c recipients at days 7, 14 and 21. (**C**) Representative H&E staining at day 21 demonstrates complete lumen fibro-obliteration from both CCR4-/- and CCR4+/+ donor airway allografts when transplanted into BALB/c recipients. Data is representative of 5-15 mice per group. Error bars indicate SEM. Significance was determined by Mann-Whitney, *$p<0.05$.



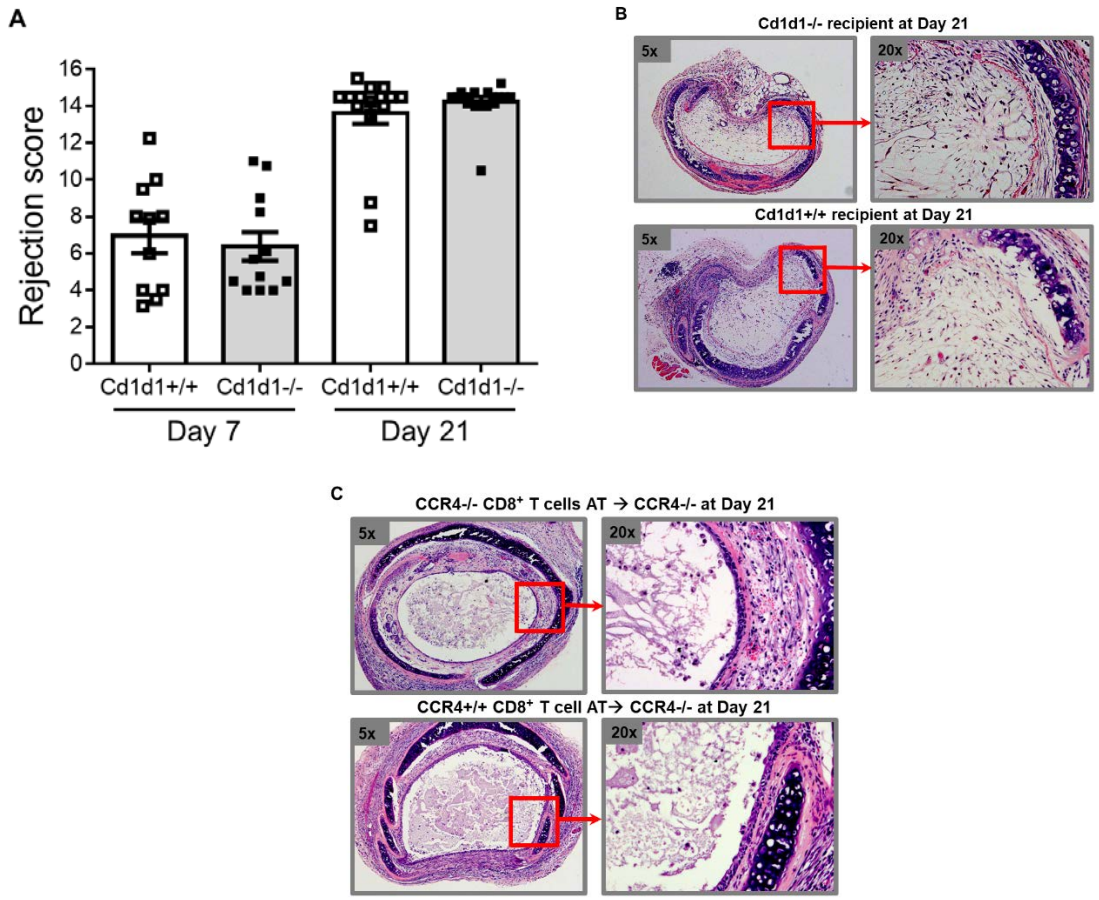

**Supplemental Figure 2. CCR4-/- allograft recipients have reduced T cells in draining lymph nodes at day 7.** CCR4-/- as compared to CCR4+/+ allograft recipient draining lymph nodes were harvested for flow cytometry. (**A-C**) Representative flow cytometry plots of whole draining lymph node single cell suspensions demonstrating the gating strategy used to evaluate the frequency of $CD4^+$ and $CD8^+$ T cells and their naïve (Tn) and central memory (CM) cell subpopulations.



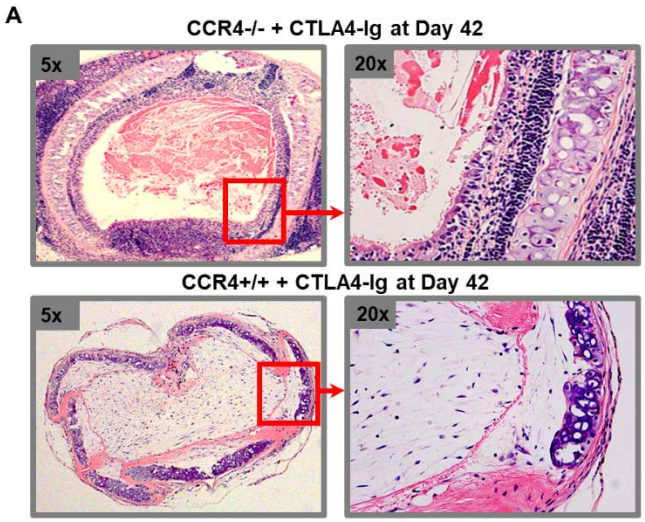

**Supplemental Figure 3. CCR4 expression on T cells is important for homing to draining lymph nodes as well as intranodal T cell activation.** CCR4+/+ and CCR4-/- T cells were labeled with (4.00 µM) and (0.25 µM) of CFSE; respectively, mixed 1:1 and delivered to day 7 CCR4+/+ allograft recipients. Eighteen hours later the draining nodes were prepared for flow cytometry. (**A**) Representative flow cytometry plots of whole lymph node single cell suspensions demonstrating the gating strategy used to evaluate the frequency of labeled $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ T cells after transfer from naïve CCR4-/- versus CCR4+/+ mice. (**B**) Representative flow cytometry plots of whole lymph node single cell suspensions demonstrating the gating strategy used to evaluate naïve T cell (Tn) shedding of the adhesion molecule, CD62L via gating on the CCR4-/- versus CCR4+/+ $CD4^+CFSE^+CD3^+CD44^{low/neg}$ and $CD8^+CFSE^+CD3^+CD44^{low/neg}$ subpopulations.




**Supplemental Figure 4. CCR4-/- allograft recipients have a reduction in the clonal expansion of CD4$^+$ and CD8$^+$ T cells.** Day 7 CCR4-/- versus CCR4+/+ airway allograft recipient's draining lymph nodes single cell suspensions were challenged ex vivo for 16 hours with donor irradiated BALB/c splenocytes and analyzed for T cell secretion of IFN-γ via flow cytometry. In separate experiments CCR4-/- versus CCR4+/+ T cells from naïve mice were challenged with superantigen Staphylococcal Enterotoxin B for 16 hours to determine T cell secretion of IFN-γ. (**A**) Representative example of the flow cytometry gating strategy evaluating recipient draining nodes alloresponsive CD4$^+$ and CD8$^+$ T cells secreting IFN-γ via gated on CD3$^+$CD4$^+$IFN-γ$^+$ and CD3$^+$CD8$^+$IFN-γ$^+$ cells. (**B**) Bar graph depicts CD4$^+$ and CD8$^+$ T cells from naïve CCR4-/- and CCR4+/+ mice that express IFN- γ after superantigen Staphylococcal Enterotoxin B challenge. (**C**) Representative example of flow cytometry gating strategy evaluating CD4$^+$ and CD8$^+$ T cells secreting IFN-γ from naïve mice after superantigen Staphylococcal Enterotoxin B challenge via gated on CD3$^+$CD4$^+$IFN-γ$^+$ and CD3$^+$CD8$^+$IFN-γ$^+$ cells. Data is representative of 3-12 mice per group. Error bars indicate SEM. Significance was determined by Mann-Whitney, *p<0.05.




**Supplemental Figure 5. Cd1d-/- recipients as well as the adoptive transfer of CCR4+/+ CD8$^+$ T cells to CCR4-/- recipients does not affect airway rejection.** BALB/c airways were transplanted into C57BL/6 Cd1d-/- versus Cd1d+/+ mice. In separate experiments ($5x10^6$) CD8$^+$ T cells from either CCR4-/- or CCR4+/+ naïve mice were transferred to CCR4-/- recipients of BALB/c airway grafts on day 0 and the allografts were analyzed for rejection scores at day 21. (**A**) Bar graph depicts the rejection scores of airway allografts from Cd1d-/- as compared to Cd1d+/+ recipients at days 7 and 21. (**B**) Representative hematoxylin and eosin (H&E) staining of transplanted BALB/c airway allografts from Cd1d-/- and Cd1d1+/+ recipients at day 21, both demonstrating intraluminal inflammation, loss of the epithelium and fibro-obliteration. (**C**) Representative H&E staining of allograft airways from CCR4-/- recipients after day 0 adoptive transfer of CD8$^+$ T cells from either CCR4+/+ or CCR4-/- naïve mice both demonstrating mild intraluminal inflammation, preserved epithelium and no fibro-obliteration at day 21. Data is representative of 10-16 mice per group. Error bars indicate SEM. Significance was determined by Mann-Whitney, *$p<0.05$.




**Supplemental Figure 6. CTLA4-Ig combined with CCR4-/- recipients leads to long-term airway allograft accommodation.** CTLA4-Ig given intraperitoneal (i.p.) at 0.2 mg on day 0 prior to airway transplantation and at days 2, 4, and 6 post-transplant to CCR4-/- versus CCR4+/+ recipients and grafts harvested to quantitate rejection at day 42. (**A**) Representative H&E staining of CCR4-/- and CCR4+/+ recipients given CTLA4-Ig at day 42. CTLA4-Ig given to CCR4-/- recipients demonstrate virtually normal airways with intact ciliated epithelium and no fibro-obliteration. CTLA4-Ig given to CCR4+/+ recipients leads to airways that are rejected and invaded by fibroblasts causing fibro-obliteration. Section of airway allograft is magnified to show the presence of the epithelial layer in CTLA4-Ig + CCR4-/- and absence of the epithelial layer with fibro-obliteration in the CTLA4-Ig + CCR4+/+ recipients.