# IL-27 induces CCL5 production by T cells that compromises anti-tumor activity

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## Abstract

Interleukin-27 (IL-27) is a pleiotropic cytokine that exhibits stimulatory/regulatory functions on multiple lineages of immune cells including T lymphocytes. In this work, we demonstrate that IL-27 directly induces CCL5 production by T lymphocytes, particularly CD8+ T cells in vitro and in vivo. IL-27-induced CCL5 production is IL-27R dependent. In CD4+ T cells, IL-27 induced CCL5 production is primarily dependent on Stat1 activation, as Stat1-deficiency abrogated CCL5 induction. While in CD8+ T cells, Stat1-deficiency did not abrogate CCL5 induction. Chromatin immunoprecipitation (ChIP) assay reveals that Stat3 is the dominant mediator of IL-27-induced activation of CCL5, since both the -200bp and -4700bp sites upstream of CCL5 transcription starting site exhibited significant binding of Stat3, while Stat1 displayed moderate binding to the -4700bp site but no binding to the other three putative sites. In IL-27 treated tumor bearing mice, IL-27 induced dramatic production of CCL5 in tumor infiltrating T cells. IL-27-induced CCL5 appears to inhibit IL-27-mediated anti-tumor effect, as significantly more tumor inhibition was observed in AAV-IL-27 treated CCL5-/- mice. Thus, IL-27 induces robust CCL5 production by T cells, which compromises anti-tumor activity.

### IL-27 induces CCL5 production by mouse and human T cells in vitro

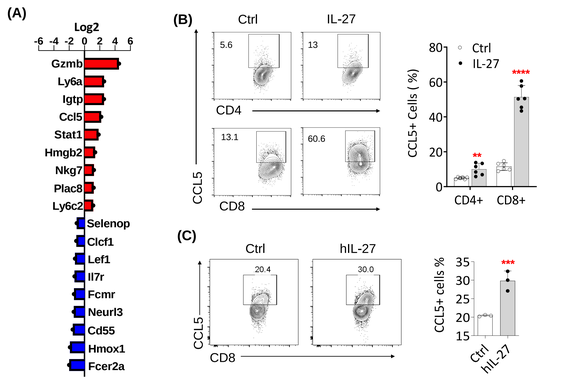


**Fig. 1 (A)** C57BL6 mice were treated with AAV-IL-27 or AAV-ctrl virus. Three weeks after AAV injection, mice were sacrificed, CD8+ T cells were purified from AAV-treated mice and submitted for RNAseq analysis. Major differentially expressed genes are shown. **(B)** Naïve CD4 or CD8 T cells were purified from spleen and lymph nodes from C57BL6 mice. Cells were activated with anti-CD3/CD28 beads with or without recombinant mouse IL-27 (50 ng/ml). Production of CCL5 was detected by flow cytometry on day 5 after T cell culture. **(C)** CD8+ T cells were purified from human PBMC. Cells were activated with anti-CD3/CD28 beads with or without recombinant hIL-27 (50 ng/ml). Production of CCL5 was detected by flow cytometry on day 5 after T cell culture. **P < 0.01, ***P < 0.001, ****P < 0.0001 by student's t test.

### IL-27 induced CCL5 production in T cells is IL-27R-dependent

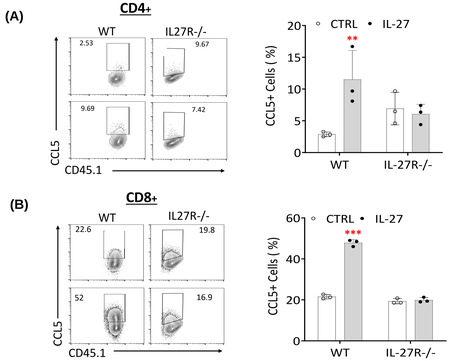


**Fig. 2 (A)** Naïve CD4+ and **(B)** CD8+ T cells were purified from spleen and lymph nodes from CD45.1+ C57BL6 mice or CD45.2+ IL-27Rα-/- mice. Cells were activated with anti-CD3/CD28 beads with or without recombinant mouse IL-27 (50 ng/ml). **P < 0.01, ***P < 0.001 by student's t test.

### IL-27 induces CCL5 production in T cells in the absence of Stat1

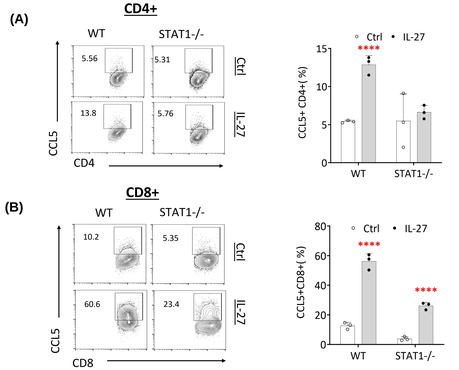


**Fig. 3** Naïve CD4 or CD8 T cells were purified from spleen and lymph nodes from Stat1-/- and control BALB/c mice. Cells were activated with anti-CD3/CD28 beads with or without recombinant mouse IL-27 (50 ng/ml). Production of CCL5 in **(A)** CD4+ and **(B)** CD8+ T cells were detected by flow cytometry on day 5 after T cell culture. ****P < 0.0001 by student's t test.

### ChIP assay of Stat1/Stat3 binding sites in CCL5 promotor

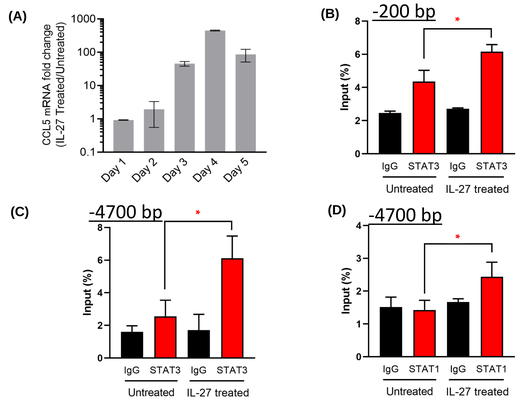


**Fig. 4** P1CTL TCR transgenic T cells were activated with 0.1 ug/ml of P1A35-43 with or without recombinant IL-27 (50 ng/ml). **(A)** The relative expression of CCL5 mRNA were determined by RT-qPCR. **(B)** ChIP assay was performed on P1CTL cells using qPCR primers designed to probe the two putative binding sties of Stat3. Both the -200bp and **(C)** -4700bp sites upstream of CCL5 TSS revealed significant binding of STAT3. **(D)** Moderate binding of Stat1 to the -4700bp site but not to the other putative sites were detected (data not shown). *P < 0.05 by student's t test.

### AAV–IL-27 treatment upregulates CCL5 in T cells in vivo

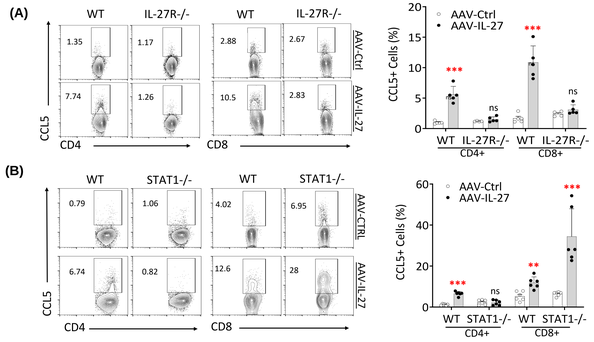


**Fig. 5 (A)** AAV-IL-27 or AAV-ctrl virus were injected into IL-27Rα-/- and control C57BL6, or **(B)** Stat1-/-BALB/c and control BALB/c mice i.m. at a dose of $2x10^{11}$ DRP. Three weeks later, mice were sacrificed and T cell production of CCL5 in spleens were analyzed by flow cytometry. **P < 0.01, ***P < 0.001 by student's t test.

### AAV–IL-27 therapy induces CCL5 production in tumor infiltrating T cells

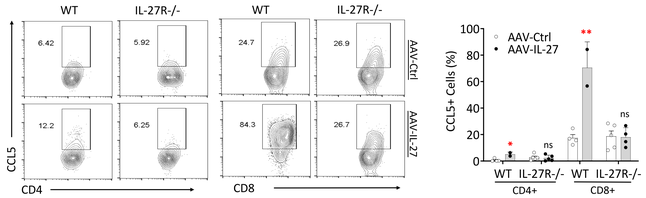


**Fig. 6** IL-27Rα-/- and control C57BL6 mice were first treated with AAV-IL-27 or AAV-ctrl virus ($2x10^{11}$ DRP/mouse i.m.). Mice were also challenged with B16.F10 tumor cells (1 x $10^5$ cells/mouse s.c.). Three weeks after AAV injection, T cell expression of CCL5 in tumors was evaluated by flow cytometry. *P < 0.05, **P < 0.01 by student's t test.

### IL-27-induced CCL5 production compromises anti-tumor immunity

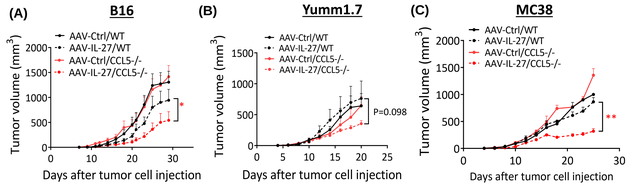


**Fig. 7** CCL5-/- mice and control C57BL6 mice were subcutaneously challenged with **(A)** B16.F10 melanoma cells (1 x $10^5$ cells/mouse; n=13/group), **(B)** Yumm1.7 melanoma cells (5 x $10^5$/mouse, n=5/group), or **(C)** MC38 colon tumor cells (5 x $10^5$/mouse, n=4/group). Mice were then treated with AAV-IL-27 or AAV-ctrl virus ($2x10^{11}$ DRP/mouse i.m.) four days later. The growth of tumors were monitored overtime. *P < 0.05, **P < 0.01 by One way ANOVA.

## Summary

- IL-27 directly induces CCL5 production by T lymphocytes, particularly CD8+ T cells in vitro and in vivo.
- IL-27-induced CCL5 production is IL-27R dependent and requires both Stat3 and Stat1 signaling.
- IL-27-induced CCL5 appears to inhibit IL-27-mediated anti-tumor effect. Thus, combination of IL-27 therapy with CCL5 blockade such as STAT1/STAT3 inhibitors could be a potential strategy in the treatment of tumors.