

POSTER PRESENTATION

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Expression of Semaphorin4C by Th2-stimulated B cells

Jean-Philippe Drolet^{1,2*}, Julie Guay¹, Yasaman Nouhi¹, Bruce Mazer^{1,2}

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Background

Semaphorins are a family of proteins implicated in neurogenesis, angiogenesis and axonal migration. Previous work from our laboratory has shown that Th2-stimulated B cells express a unique semaphorin, SEMA4C. Given the roles of other semaphorins, we hypothesized that SEMA4C was expressed on Th2-stimulated B cells and might play a role in B cell trafficking and tissue homing therefore being expressed stronger in terminally differentiated B cells, such as memory and plasma cells.

Experiment and results

B cells were purified from tonsils removed from children undergoing routine tonsillectomy. They were cultured for 24 hours or 7 days at a concentration of 5×10^4 cells/mL in complete medium in the presence of α CD40 with either IFN γ , IL-4, IL-21 or both IL-4 and IL-21. RNA was extracted, cDNA was made, and SEMA4C mRNA was amplified by qPCR and compared to the housekeeping gene GAPDH. After 24h culture, SEMA4C mRNA expression was detected only in IL-4-stimulated cells but after 7 days, the expression was much stronger in both conditions containing IL-21, and decreased in IL-4-only stimulated cells.

B cells were also similarly cultured for 72 hours at a concentration of 0.5×10^6 cells/mL in chamber slides. Slides were stained for SEMA4C with a fluorescent Alexa488 antibody and then visualized using fluorescent microscopy. As expected, stimulation with IL-21 yielded

much higher SEMA4C expression than IL-4 alone whereas IFN γ did not induce significant staining. Figures 1 and 2.

Conclusion

SEMA4C is induced in B cells upon Th2 stimulation but increases further upon follicle-like differentiation triggered by IL-21. This data confirms that SEMA4C is specific to terminally-differentiated B cells and supports our hypothesis that it might play a role in B-cell trafficking and homing in peripheral tissue follicles. SEMA4C could therefore be a key player in local allergic inflammation.

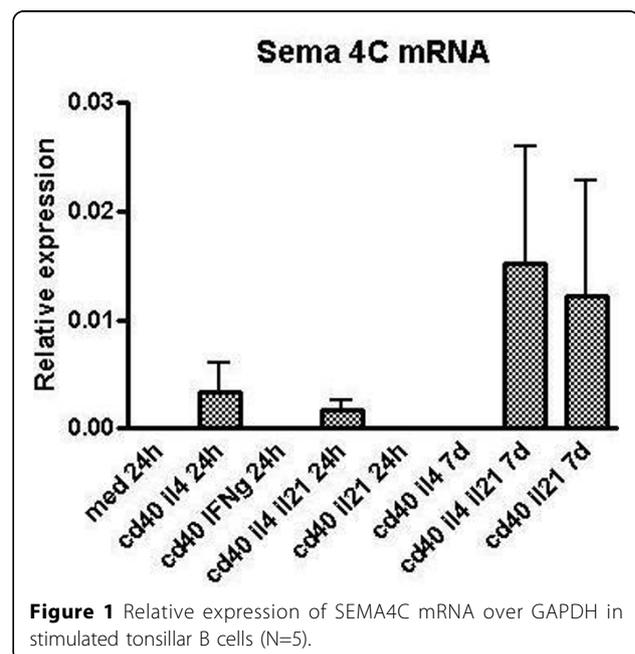


Figure 1 Relative expression of SEMA4C mRNA over GAPDH in stimulated tonsillar B cells (N=5).

* Correspondence: jean-philippe.drolet@mail.mcgill.ca

¹Meakins-Christie Laboratories, McGill University, Montreal, H2X 2P2, Canada
Full list of author information is available at the end of the article

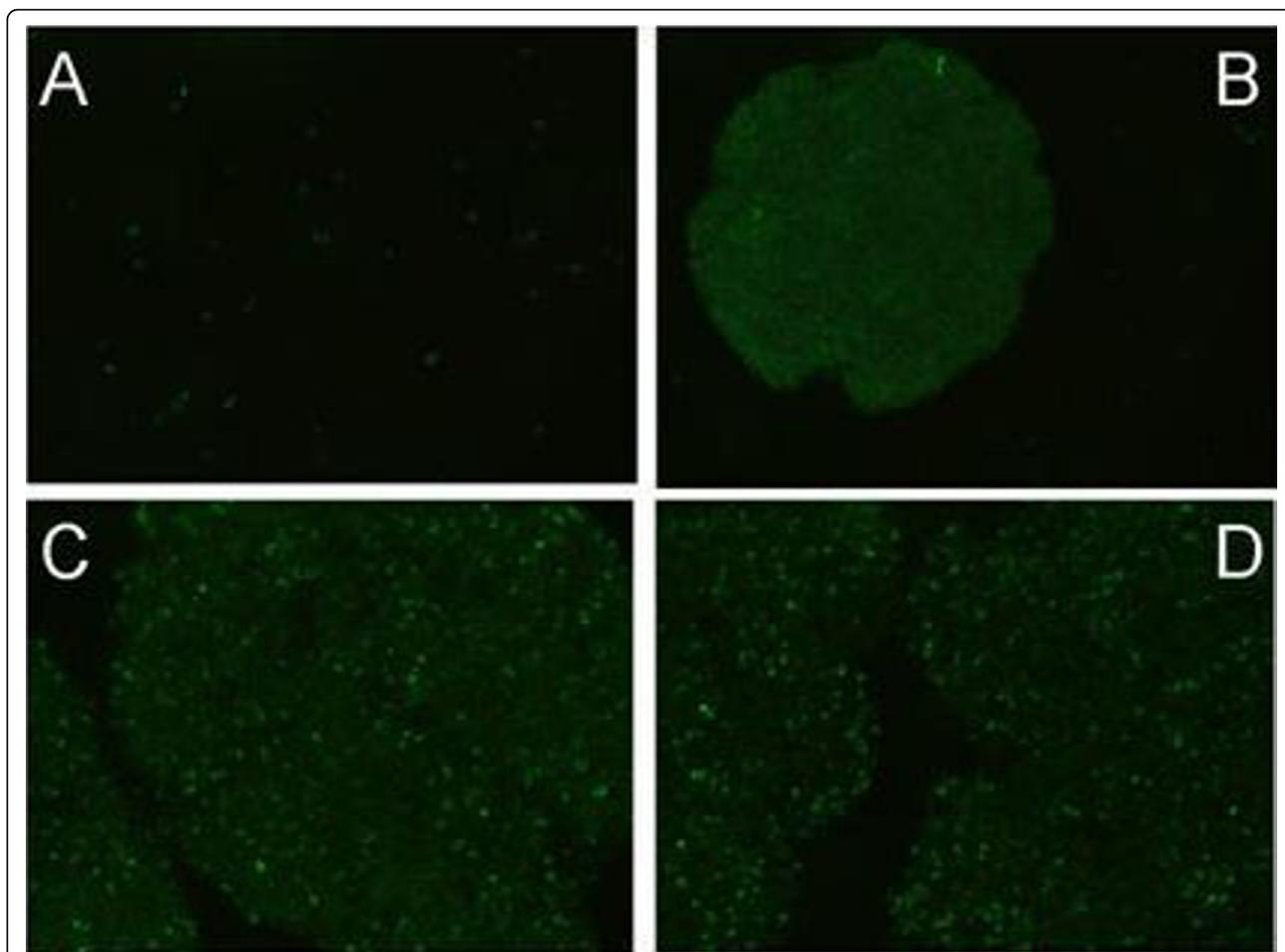


Figure 2 Immunofluorescent staining at 10X magnification for SEMA4C in B cell cultures is associated with "follicle-like" clones.
Stimulations: A) α CD40 + IFN γ , B) α CD40 + IL-4, C) α CD40 + IL-4 + IL-21, D) α CD40 + IL-21.

Author details

¹Meakins-Christie Laboratories, McGill University, Montreal, H2X 2P2, Canada.

²Division of Pediatric allergy and clinical immunology, McGill University Health Center, Montreal, Quebec, Canada, H3H 1P3.

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