Inhibition of the Jagged-1/Notch pathway increases the hematopoiesis-supportive activity of mesenchymal stem cells

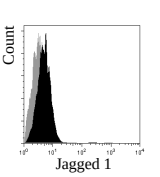
Alexandra Briquet, Céline Grégoire, Yves Beguin, André Gothot
GIGA-Research, Hematology Unit, University of Liège, Belgium

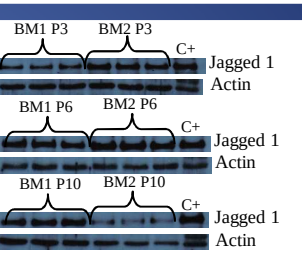
Introduction

Mesenchymal stem cells (MSC) are able to support hematopoiesis ex vivo by providing components of the extracellular matrix and essential growth signals allowing proliferation and differentiation of hematopoietic stem cells. The aim of this work consisted in determining the contribution of the Notch/Jagged-1 pathway in the ex vivo hematopoiesis-supportive activity of MSC.

Expression of Jagged 1 by MSC

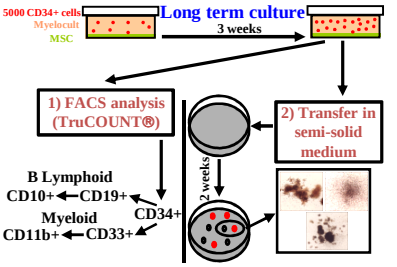
It is well known that Notch is expressed in CD34+ hematopoietic precursors. By qRT-PCR, we demonstrated the expression of Jagged 1 by cultured human MSC. By Western blot and intracellular flow cytometric analysis, we confirmed the expression of Jagged 1 by MSC at several passages (P) and from two bone marrow (BM). For positive control (C+), Caski cells were used.

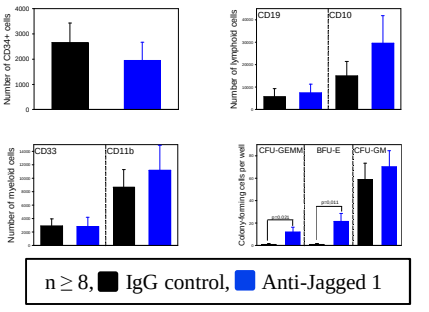




Outgrowth of lymphoid and myeloid cells was not affected by Jagged-1 inhibition

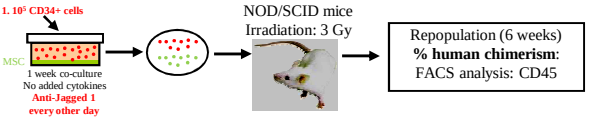
Dexter-type long term cultures were carried out with MSC and cord blood CD34+ cells in the presence of neutralising anti-human Jagged-1 antibody (anti-Jagged-1). After 3 weeks, absolute numbers of CD34+, CD10+, CD19+, CD11b+ and CD33+ cells grown in culture were determined by flow cytometric analysis using TruCount tubes. In second time, persistence of primitive progenitors was assessed by transferring cells in a semi-solid medium to allow development of hematopoietic colonies. Compared to culture with irrelevant IgG, outgrowth of lymphoid and myeloid cells was not affected by Jagged-1 inhibition. The number of CFU-GEMM and BFU-E was increased by Jagged-1 neutralisation (Student's t-test).

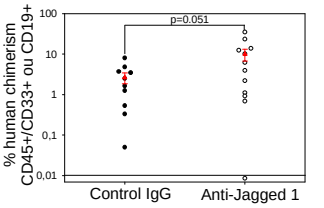




NOD/SCID mice repopulating activity was increased by Jagged-1 neutralisation

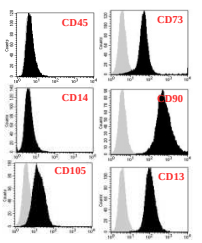
Repopulation assays in irradiated NOD/SCID mice were set with the expansion product of CD34+ cells co-cultured for one week in contact with MSC and in the presence of anti-Jagged-1 or non specific IgG. Compared to infusion of CD34+ cells cultured in the presence of control IgG, NOD/SCID mice repopulating activity was increased by Jagged-1 neutralisation (p=0.051).

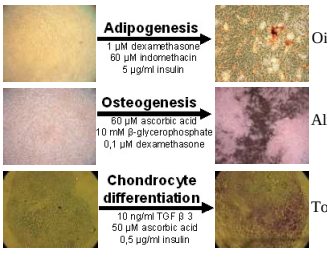


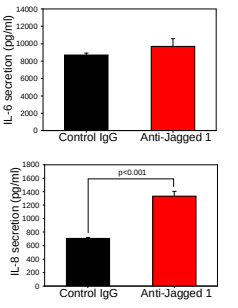


IL-8 secretion by MSC was increased by Jagged-1 neutralisation

In further experiments, to determine whether this enhancement of repopulating activity was due to direct or indirect effects, the influence of anti-Jagged-1 on MSC was studied. The phenotype, adipogenic, chondrogenic and osteogenic differentiation capacity of MSC were not affected by Jagged-1 inhibition. However, we noted a 2-fold increase of IL-8 secretion ($p<0.001$) in the presence of anti-Jagged-1. In contrast, IL-6 secretion did not significantly change.







Conclusion

In conclusion, these data suggest that, in our conditions, the Jagged-1/Notch pathway inhibits the supportive activity of MSC toward NOD/SCID-repopulating cells. This is not paralleled by changes in the phenotype or differentiation potential of MSC but may be related to inhibition of IL-8 secretion.