Mechanisms of T-cell suppression by Mesenchymal Stromal Cells

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INTRODUCTION

In recent years, Mesenchymal Stem Cells (MSCs) have aroused the attention from scientific community for their capacity of suppress the T-cell proliferation. These immunological properties of MSC attracted the interest of basic and clinical investigators, in light of its potential therapeutical use in different immunological diseases.

AIMS

Uncover new immunomodulatory mechanisms on lymphocytes, mediated by MSC

METHODS

T-lymphocytes from 3 individuals were activated and cultured either in the absence or in the presence of MSC. Following a 5 day period, CD4 lymphocytes were purified and profiled by whole genome microarrays.

RESULTS

- Microarray analysis revealed many differentially expressed genes involved in immune response, among these, CD69 and adenosine receptor (ADORA2A) were at higher levels on co-cultured lymphocytes. These two independents results were separately explored.
- Proliferation of lymphocytes co-cultured with MSC was significantly inhibited (data not show)
- Lymphocytes cultivated with MSCs expresses higher levels of ADORA2A and lower levels of ADA (Fig. 1)

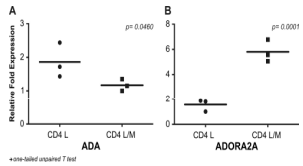

Figure 1. ADA and ADORA2A mRNA levels. CD3+ T cells were activated and cultured without or with MSCs. On the fifth day following activation, Real Time PCR was performed. (A) ADA mRNA levels; (B) ADORA2A mRNA levels.

- In co-cultures, the percentage of MSCs expressing CD39, and of T-cells expressing CD73, increased significantly (Fig. 2).

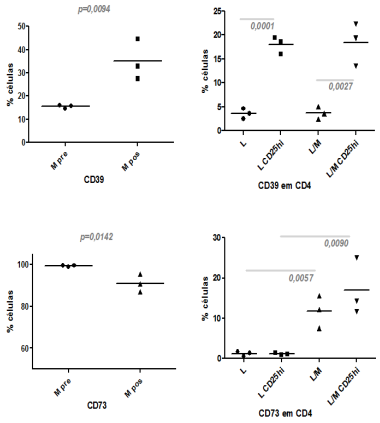

Fig. 2. CD39 and CD73 expression. CD39 and CD73 expression were analyzed in CD4+ T cells and CD4CD25hi cells obtained from cultures without MSCs (L and LCD25hi) or with MSCs (L/M and L/M CD25hi). CD39 and CD73 were also analyzed in MSC without (Mpre) or with (Mpos) activated lymphocytes.

- Incubation of MSCs with media conditioned by activated T lymphocytes induced the production of adenosine to levels similar to those observed in co-cultures, indicating that adenosine production was mainly derived from MSCs (Fig 3)

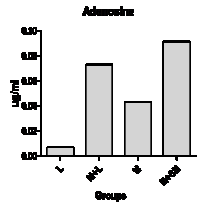

Fig. 3. Adenosine quantification. CD3+T cells were Activated and cultured without (L) or with (L/M) MSCs. T cells cultures supernatant presented higher levels of adenosine in the co-culture than in the culture without MSCs. In addition, MSCs culture produced 0.043µg/ml of adenosine, whereas in presence of supernatant of activated T cells, the adenosine production was 0.091µg/ml.

- blocking ADORA2A signaling raised lymphocyte proliferation significantly (Fig.4)

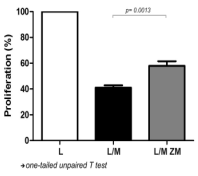

Fig. 4. Inhibition of ADORA2A Signaling. CD3+ T-cells were activated and cultured alone (L) or with MSCs (L/M). Alternatively, T-cells were pre-treated with ADORA2A antagonist ZM 241385 (L/M ZM). T-lymphocyte treatment with ZM 241385 induced an increase on T-cell proliferation by 29% compared to untreated T-lymphocytes .

- Genes with immunoregulatory functions, CD69 and non-canonical NF-kB subunits were all expressed at higher levels in lymphocytes co-cultured with MSCs (data not show)
- The frequency of CD69 cells among lymphocytes cultured alone progressively decreased after activation. In contrast, the frequency of CD69 cells increased significantly following activation in lymphocytes co-cultured with MSCs.

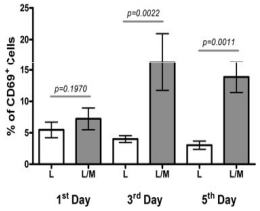

Fig. 5. Sustained increase of CD69. Flow cytometry evaluation of CD69 expression in lymphocytes cultured alone (L, white bars) or in the presence of MSCs (L/M, grey bars). Evaluations were performed on the first, third and fifth days following activation

- Inhibition of canonical NF-kB signalling by BAY immediately following activation blocked the induction of CD69; however, inhibition of canonical NF-kB signalling on the third day further induced the expression of CD69.

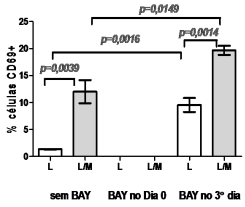

Fig. 6. Differential roles of canonical and non-canonical NF-B signalling in the early and late expression of CD69. CD3+T cells were activated and cultured in the presence (L/M) or absence (L) of MSCs. On the fifth day following activation, cells were collected, and the percentage of CD69 cells was evaluated by flow cytometry. The inhibitor of canonical NF-B signalling (BAY11-7082) was added to cultures immediately following activation or on the third day.

- Late expression of CD69 was inhibited by RELB siRNA.

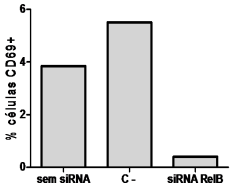

Fig.7. SiRNA agaisnt RELB. CD3+T cells were transfected with siRNA directed against the non-canonical NF-B subunit RELB (RELB siRNA) or with non-specific control siRNA (Ctr siRNA) prior to activation and culture. Because the expression of CD69 was absent in lymphocytes cultured alone, only the results for lymphocytes co-cultured with MSCs (L/M) are shown

CONCLUSION

1. **Our results suggest that some of the immunomodulatory properties of MSCs may, in part, be mediated through the modulation of components related to adenosine signaling.**
2. **Our results indicate that the canonical NF-kB pathway controls the early expression of CD69 after activation; however, in an immunoregulatory context, late and sustained CD69 expression is promoted by the non-canonical pathway and is inhibited by canonical NF-kB signalling.**

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