Alteration of CD83 Expression and sCD83 Secretion during the Course of Pregnancy

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Abstract

The 45 kDa transmembrane molecule CD83, originally known as activation marker on Dendritic Cells, owns immunosuppressive features: B-Cells overexpressing CD83 show a high Interleukin 10 release, while CD83 positive T-Cells are able to suppress the proliferation of effector CD83 negative T-Cells. The membrane-bound form of CD83 generates a soluble protein (sCD83) with therapeutic potential in autoimmune diseases. We aimed to investigate if those features play a role in murine pregnancy.

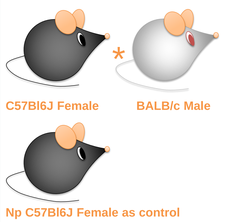
C57Bl/6 female mice were paired to BALB/c males and sacrificed on days 7, 14 or 18 of pregnancy. Non pregnant C57Bl/6 females served as control. CD83 expression on lymphocytes from spleen, thymus and lymph nodes was measured using flow-cytometry. Additionally, the CD83 expression was analyzed after in-vitro stimulation. The supernatants were collected and the amount of sCD83 was examined by ELISA. Diagrams were generated with GraphPad Prism®, the statistics were performed with ANOVA or non-parametric Kruscal-Wallis test (*p<0,05; **p<0,01; ***p<0,001).

We were able to detect an upregulation of CD83 molecule on splenic B and T-Cells in the middle of pregnancy. However, after stimulation, the strongest CD83 upregulation was detectable on B-Cells at the end of pregnancy. Remarkably, lymphocytes from day 18 of pregnancy showed a significantly higher release of sCD83 after stimulation compared to non-pregnant mice. Except an upregulation on B-cells from paraaortal lymph nodes, no significant changes were observed in B- or T-Cells from thymus and lymph nodes.

Our data demonstrates an increase of CD83 expression during pregnancy, supporting our thesis of its involvement in immunological adaptions to pregnancy. We hypothesize that the higher sCD83 release after lymphocyte stimulation can protect the fetus from an inadequate immune response to different pathogens.

Mouse Models and Methods

Allogenic mating of mice



7/14/18 dpp (day post plug) or np (non pregnant): Removal of different lymphoid organs and blood

- **Spleen**
- **Thymus**
- **Lymph Nodes**
- **Blood**

- Isolation of lymhocytes and FACS staining for CD83 expression
- Cell-culture of lymhocytes for FACS staining and collection of supernatants
- ELISA for sCD83

Fig.1: Mouse model and methods.

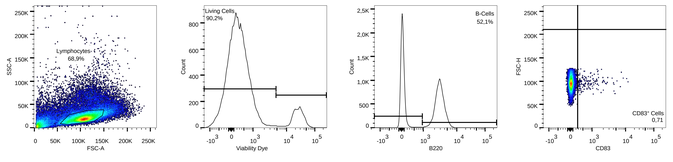
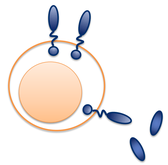


Fig.2: Example of FACS-Gating strategy: Gating of CD83⁺ B-Cells.

B-Cells



- **Release sCD83 (by cutting the extracellular domain)**
- **CD83 overexpressing B-Cell:**
  - **Higher IL10 Release**
  - **Reduced Antibody Secretion**

Fig.3: Functions of CD83 on B-Cells.

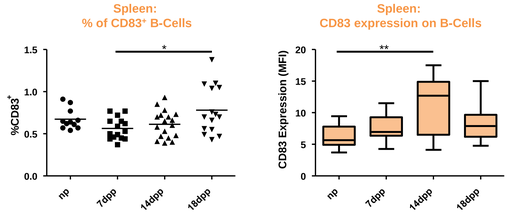


Fig.4-5: Percentage of B220⁺ CD83⁺ B-Cells and CD83 expression on splenic B-Cells.Similar results were found in Marginal-Zone and Follicular-Zone B-Cells (Data not shown).

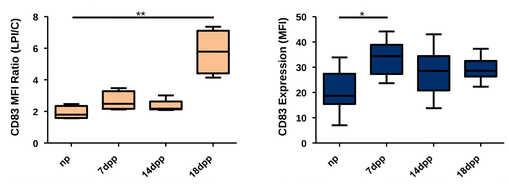


Fig.6: Ratio (Stimulated/Unstimulated) of the CD83 expression on CD19⁺ splenic B-Cells after stimulation. C=Control; LPI=LPS, PMA and Ionomycin.

Fig.7: CD83 expression on B220⁺ B-Cells from paraaortal lymph nodes.

T-Cells

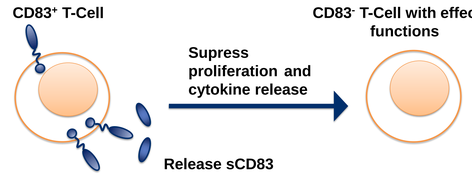


Fig.8: Functions of CD83 on T-Cells.

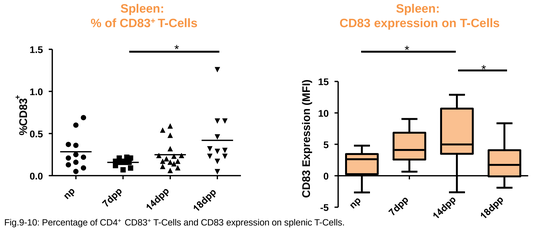


Fig.9-10: Percentage of CD4⁺ CD83⁺ T-Cells and CD83 expression on splenic T-Cells.

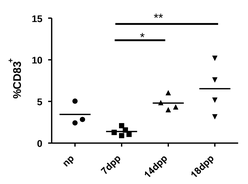
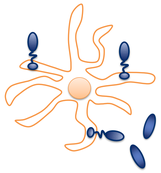


Fig.11: Percentage of CD83+ splenic CD4⁺ CD25⁺ T-Cells.

Dendritic Cells



- **Activation Marker**
- **Release sCD83**
- **Increased expression – improved stimulation of CD8⁺ Cells?**

Fig.12: Functions of CD83 on Dendritic Cells.

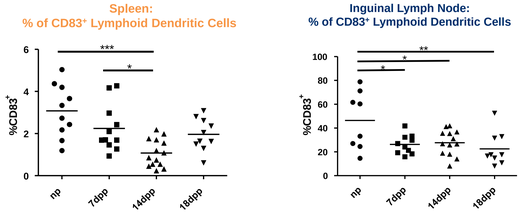


Fig.13: Percentage of 33D1⁺ CD83⁺ Dendritic Cells from Spleen.

Fig.14: Percentage of 33D1⁺ CD83⁺ Dendritic Cells from inguinal lymph nodes.

sCD83

- **Inhibits lymphocyte proliferation in vitro**
- **Supresses Dendritic Cell mediated T-Cell activation**
- **Increases number of regulatory T-Cells**
- **Therapeutical potential in autoimmune diseases**
- **Inhibits graft versus host reactions**

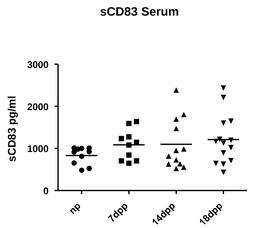


Fig.15: sCD83 concentration in murine serum.

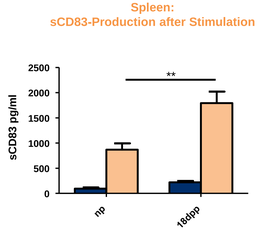


Fig.16: sCD83 production from splenic lymphocytes cultivated for 48 hours. Analysis was performed with Two-way ANOVA and Bonferroni posttest.

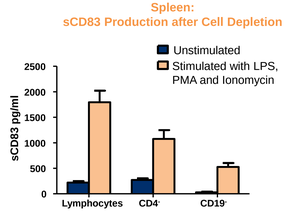


Fig.17: sCD83 production after depletion of CD4 or CD19 fraction of splenic lymphocytes.

Conclusion & Perspectives

CD83 has different functions on different cell types.
Our data demonstrates an increase in CD83 expression on B- and T-Cells during pregnancy, supporting our thesis of its involvement in immunological adaptions to pregnancy.
As expected, there is a decrease of CD83⁺ (activated) dendritic cells in spleen and lymph nodes.
We hypothesize that the higher sCD83 release after lymphocyte stimulation can help protecting the fetus from an inadequate immune response to different pathogens.

To identify the cells which react to sCD83 we will use a labeled CD83-Fluorosome. This will not only enlarge information about immune reactions in pregnancy, but also expand the knowledge about the molecule itself.

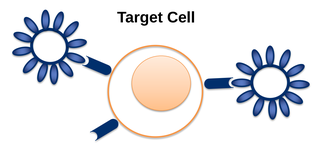


Abb.18: Perspectives: Identification of CD83 binding cells with CD83-Fluorosomes. The Fluorosomes express a recombinant CD83-extracellular domain.

Acknowledgements

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Animal experiments were carried out according to institutional guidelines and approved by the LALLF-MV Institution (7221.3-1-068/13).