Poster
ID 315

TLR9 AGONIST ENHANCES B CELL DIFFERENTIATION AND ANTIBODY PRODUCTION IN HIV+ ADULTS

Mariane H. Schleimann[1]; Line K. Vibholm[1,2]; Maria-Louise R. Kobberø[1]; Manuel Schmidt[3]; Burghardt Wittig[4];Tine Damsgaard[5];
Peter Ahlburg[6]; Michel Hellfritzsch[7]; Rikke Olesen[1,2]; Lars Østergaard[1,2]; Paul W. Denton[1,2]; Martin Tolstrup[1,2]; Ole S. Søgaard[1,2]

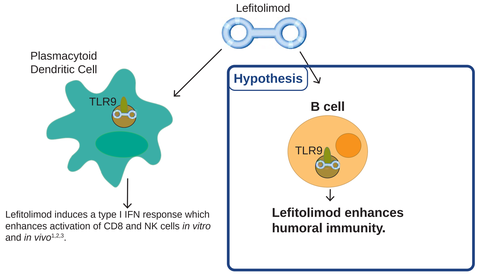
[1]Department of Infectious Diseases, Aarhus University Hospital, Skejby , Denmark; [2] Institute of Clinical Medicine, Aarhus University, Denmark; [3] Mologen AG, Berlin, Germany, [4]Foundation Institute Molecular Biology and Bioinformatics, Freie Universitaet Berlin, Germany; [5]Department of Plastic Surgery, Aarhus University, Denmark; [6]Department of Anesthesiology Aarhus University, Denmark; [7]Department of Radiology, Aarhus University, Denmark.

Introduction

Background

- In the context of HIV eradication we focus on the "kick and kill"strategy. While testing latency reversing agents (LRAs) it became apparent that additional enhancement of the functional immune system is required in order to obtain a sufficient killing of HIV-antigen presenting cells. Therefore, we have tested the TLR9 agonist Lefitolimod, that is described both as an LRA and an immune surveillance reactivator[1,2,3], in HIV-infected individuals on ART. The primary outcome was time to viral rebound after 24 weeks of treatment followed by ART treatment interruption. See POSTER ID 357 for details.

- It is well estabilished that chronic HIV-infected individuals on ART suffer from an impaired humoral immune system mainly due to persisting abnormalities in lymphoid tissue despite ART[4]. Since Lefitolimod binds TLR9 that is expressed in B cells, we wanted to investigate the impact of Lefitolimod on improvement of humoral immunity as well as lymph node architecture and fibrosis.



Aims

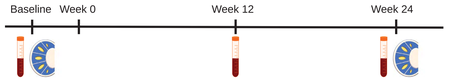
- Determine the impact of Lefitolimod on B cell differentiation in peripheral blood and lymph node.
- Assess the impact of Lefitolimod on lymph node arcitechture.

Methods

- Flow cytometry analyses was performed on freshly isolated PBMCs or lymph node mononuclear cells to assess B cell differentiation. Gating strategy: SSC singlets-FSC singlets-mononuclear cells-CD19+CD21+ B cells. Surface markers IgD, IgM, CD24, CD27 and CD38 was used to differentiate B cell subsets.
- Immunohistochemistry stains were performed to assess the impact of Lefitolimod on lymph node architecture. B cell follicles were visualized according to CD20 expression. Type I IFN responses was detected by visualization of the type I IFN stimulated gene-15 (ISG15). To determine the amount of collagen in the lymph nodes we used anti-Collagen I antibody.
- Quantification of total and subtype Immunoglobulin G in plasma was performed at the clinical biomedical lab at Aarhus University Hospital, Skejby, Aarhus, Denmark.
- We used 2-tailed Wilcoxon signed-rank test to analyze changes from baseline and Spearman rank test for correlations. The statistical comparisons and graphs were generated using GraphPad 7.

Study outline

- 12 HIV+ adults on ART received lefitolimod (60 mg s.c.) twice weekly for 24 weeks. Blood and lymph node sampling times are depicted on the graphic.



Results

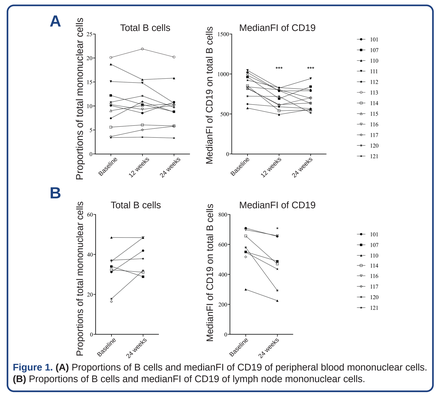


Figure 1. **(A)** Proportions of B cells and medianFI of CD19 of peripheral blood mononuclear cells. **(B)** Proportions of B cells and medianFI of CD19 of lymph node mononuclear cells.

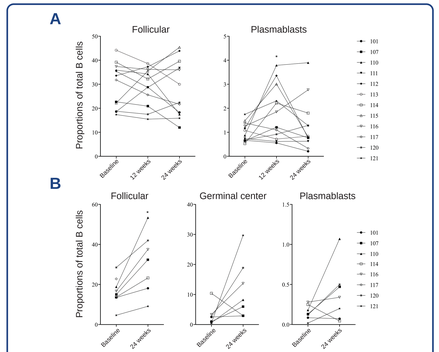


Figure 2 **(A)** Proportions of follicular B cells and plasmablasts of peripheral blood B cells. **(B)** Proportions of follicular B cells, germinal center B cells and plasmablasts of lymph node mononuclear cells.

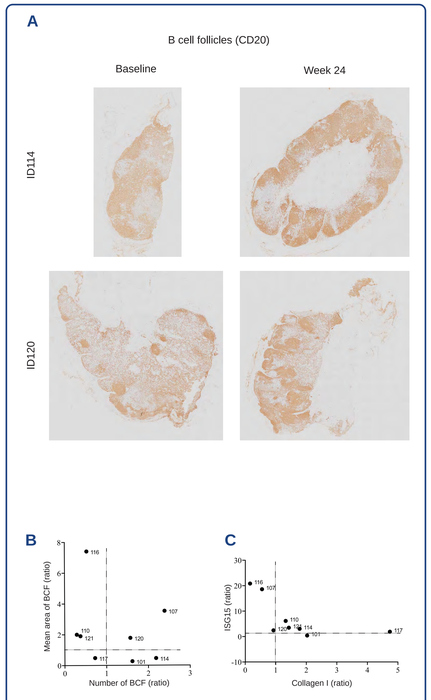


Figure 3 **(A)** Immunohistochemical stain of lymph nodes from ID 114 and ID120 at Baseline and 24 weeks of Lefitolimod treatment. Images show anti-CD20 antibody stain (Brown). Dence CD20 stain indicate a B cell follicle (BCF).
(B) Correlation of the ratio (24 weeks/baseline) of the mean area of BCFs against the ratio (24 weeks/baseline) of the number of BCFs.
(C) Correlation of the ratio (24 weeks/baseline)
of ISG15 expression against the ratio (24 weeks/baseline) of Collagen I expression. ISG15 and Collagen I was quantitated from immunohistochemical stains of the lymph node tissue.

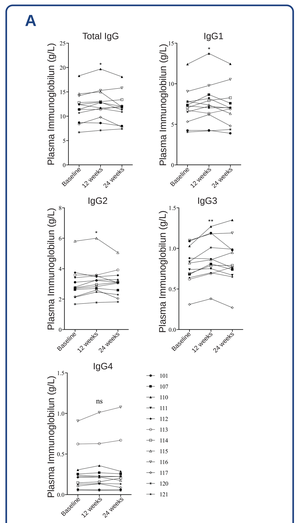


Figure 4 **(A)** Plasma Immunoglobulin G (IgG) measurements. Totals IgG and IgG subtype 1, 2, 3, and 4 plasma concentrations (g/L) was measured at baseline, week12 and week 24.

B cell subset	Surface markers
Naïve/Mature	CD19+CD21+IgD+IgM++ CD27-CD38-/+
Transitionale	CD19+CD21+IgD+IgM++ CD27-CD38++
Marginal Zone (like)	CD19+CD21+IgD$^{+/int}$IgM++ CD27+CD38-
Follicular	CD19+CD21+IgD+IgM$^{+/-}$ CD24+CD27-CD38+
Germinal Center	CD19+CD21+IgD-IgM-CD24++CD27+CD38+
Switched Memory	CD19+CD21+IgD-IgM-CD27+CD38-
Plasmablasts	CD19+CD21+IgD-IgM-CD27++CD38+++
Plasma cells	CD19-CD21-IgD-IgM-CD24-CD27++CD38+++

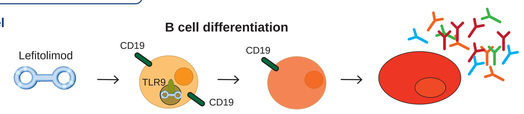
Table 1 Flow panel for B cell subsets

Results

- **Figure 1.** Lefitolimod treatment did not alter the proportions of total B cells in both peripheral blood (Fig. 1A left) or lymph nodes (Fig. 1B left). However, a significant decrease of CD19 expression was observed in both peripheral blood total B cells (Fig. 1A right) and lymph node total B cells (Fig.1B right). Since plasma cells do not express CD19 these data suggest that the B cells differentiate towards a plasma cell phenotype.
- **Figure 2.** Lefitolimod enhanced B cell differentiation in blood and lymph node. Lefitolimod significantly increased the proportions of plasmablast in the peripheral blood (Fig. 2A). In the lymph nodes we observed a significant increase in the proportions of follicular B cells and in 5 of 7 analyzed samples we also detected an increase in the proportions of germinal center associated B cells (Fig. 2B). These data strongly suggests that Lefitolimod is capable of directing B cells into a more differentiated state in HIV infected indivduals.
- **Figure 3.** Leftimod treatment altered lymph node architecture. Fig. 3A shows images of two paired lymph nodes from ID114 and ID120 at baseline and after 24 weeks of treatment. The lymph node biopsy was stained with CD20 antibody. CD20 rich areas indicate a B cell rich area, which is defined as a B cell follicle (BCF). We both counted the number of BCFs and measured the mean size of BCFs present in the lymph nodes. A correlation of the fold change in mean size versus the fold changes in number of BCFs is shown in Fig. 3B. CD20 staining reveals an apparent change in LN architecture, but does not inform whether primary follicles matured into secondary follicles.
In addition, we quantitated the number of ISG15 positive cell profiles (marker of treatment response) and the proportion of collagen deposition (measure of lymph node fibrosis). When correlating the fold changes of these two parameters the vast majority of the participants increase in ISG15, however only the two participants (ID107 and ID116) decrease in collagen levels (Fig. 3.C). Of note, ID107 and ID116 have the most robust increase in ISG15, which indicate that they had the strongest response to Lefitolimod.
- **Figure 4.** Lefitolimod increased plasma immunoglobulin G (IgG) levels. The fact that we were able to detect a signifcant increase in total IgG as well as subtypes IgG 1, 2 and 3 supports that Lefitolimod enhances the humoral immune response.

Conclusion

Model



- In summary, we detected a Lefitolimod-mediated differentiation of B cells in both the peripheral blood and lymph nodes potentially stabilizing B cell follicles. Consistent with this possibility, we observed that B cell populations shifted towards Ab-producing plasma cell phenotypes. That outcome would explain the increases in total IgG, including specific increases in IgG 1, 2 and 3 in plasma. However, whether these antibodies are HIV specific, remains to be determined. Overall these data support that Lefitolimod affects the humoral immune response *in vivo*.

Acknowledgements

- We thank all participants from this trial. Without your participation this study could not have been performed.
- We thank Mologen for providing the TLR9 agonist Lefitolimod.
- We thank Lene S. Jøhnke and Helene M. Andersen for technical assistance.

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marsch@rm.dk