Cannabidiol Enhances Intestinal CB2 Receptor Expression and Activation Increasing Regulatory T Cells and Reduces Murine Acute Graft-Versus-Host Disease without Interfering with The Graft-Versus-Leukemia Response

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Supplemental Figures

Supplemental Figure 1. Antibodies used in immunofluorescence. Information regarding antibodies used in immunofluorescence assay, their dilution and source.

Supplemental Figure 2. Flow cytometry analysis of spleen. (A) Mix 1 regards the analysis of cell surface markers, such as CD3, CD4, CD8 and the activation marker CD28. (B) Mix 2 includes CD4 and CD25 as cell surface markers, and the intracellular FoxP3 for the characterization of Tregs.

Supplemental Figure 3. Flow cytometry analysis of small intestine. (A) Mix 1 comprehends CD3, CD4, CD8 and CD28, all cellular surface markers. (B) Mix 2 includes CD4, CD25 and FoxP3 in order to characterize T regulatory lymphocytes.

Supplemental Figure 4. Flow cytometry analysis of liver. (A) Mix 1 included CD3, CD4 and CD8 for the characterization of local lymphocytes, and CD28 as an activation marker. (B) Mix 2 regards T regs and was composed of CD4, CD25 and FoxP3 markers.

Supplemental Figure 5. Immunofluorescence of CD4/CB2 in small intestine. CD4 marker is stained with FITC (green arrow) and CB2 receptor with AlexaFluor594 (red arrow). In yellow it is possible to perceive colocalization of these markers (yellow arrow). For the staining of cell nuclei DAPI was used (blue marker). The last column displays a zoomed detailed part of the Merge image.

Supplemental Figure 6. Immunofluorescence of FoxP3/CB2 in small intestine. FoxP3 marker is stained with PE (red arrow) and CB2 receptor with AlexaFluor488 (green arrow). In yellow it is possible to perceive colocalization of these markers (yellow arrow). For the staining of cell nuclei DAPI was used (blue marker). The last column displays a zoomed detailed part of the Merge image.
**Supplemental Figure 7.** Immunofluorescence of CD8/CB2 in small intestine. CD8 marker is stained with PerCP (red arrow) and CB2 receptor with AlexaFluor488 (green arrow). In yellow it is possible to perceive colocalization of these markers (yellow arrow). For the staining of cell nuclei DAPI was used (blue marker). The last column displays a zoomed detailed part of the Merge image.

**Supplemental Figure 8.** Intravital analysis after GVHD was induced in Balb/c mice using C57BL/6J GFP+ mice as donors. At day 7 after transplant, mice were anesthetized intraperitoneally with 15 mg/Kg of xylazine and 80mg/Kg of Ketamine diluted in PBS autoclaved, and the mesentery exposed in a perfusion system containing PBS (pH 7.4) at 37° C. Only, 30 minutes before intravital assay, mice that received C57BL/6J GFP+ splenocytes were treated with 200µL of CBD vehicle (5% of Tween 80) or CBD 30 mg/kg. An intravital confocal microscope (Nikon, ECLIPSE 50i, 20x objective lens) was used to examine the mesenteric microcirculation. The images were recorded for playback analysis using Fiji-ImageJ with plugin NIS Elements-Nikon Imaging software (NIS ELEMENTS-NIKON). Intestinal venules (±40 µm) were selected and the number of rolling and adherent leukocytes determined offline during video playback analysis. Rolling leukocytes were defined as those cells moving at velocity less than that of erythrocytes within a given vessel. The flux of rolling cells was measured as the number of rolling cells passing by a given point in the venule per minute. A leukocyte was adherent if it remained stationary for at least 30s, and total leukocyte adhesion was quantified as the number of cells in the intravascular space within area of 100 µm. Number of rolling cells per minute and number of adherent cells per 100um is presented as the mean ± SEM (n= 6-8). * for P<0.05 when compared to the vehicle and CBD treated group.
Supplemental Figure 1. Antibodies used in immunofluorescence.

**Figure S1**

<table>
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<tr>
<th>Antibody</th>
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<th>Source</th>
<th>Catalog Number</th>
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**Secondary**

- AlexaFluor 488 donkey anti-goat Invitrogen - ThermoFischer A11055 1:500
- AlexaFluor 594 donkey anti-goat Invitrogen - ThermoFischer A11058 1:500

Supplemental Figure 2. Flow cytometry analysis of spleen.

**Figure S2**
Supplemental Figure 3. Flow cytometry analysis of small intestine.

Figure S3

Supplemental Figure 4. Flow cytometry analysis of liver

Figure S4
Supplemental Figure 5. Immunofluorescence of CD4/CB2 in small intestine.
Supplemental Figure 6. Immunofluorescence of FoxP3/CB2 in small intestine.
Supplemental Figure 7. Immunofluorescence of CD8/CB2 in small intestine.
Supplemental Figure 8. Intravital analysis after GVHD was induced in Balb/c mice using C57BL/6J GFP+ mice as donors.

Figure S8

![Graphs showing number of rolling cells per minute and number of adherent cells per 100 m for Vehicle and CBD treatments.](image)

**Intestinal microvessels of GVHD mouse**

**Intestinal microvessels of CBD treated mouse**

*Legend:*
- Green = Intestinal Venules
- Blue = Transmigrated leukocytes
- Yellow = Rolling or adherent leukocytes
- Red = Scale bar 100 μm

*P-values for comparisons:*
- Number of rolling cells per minute: P=0.4375
- Number of adherent cells per 100 m: P=0.1572