**Supplementary Material**

**Supplementary Table 1**. Antibodies used to sort regulatory cells populations.

|  |  |  |
| --- | --- | --- |
| **Antibody** | **Isotype** | **Purchased from** |
| **Tregs** |  |  |
| CD4 APC Cy7 | IgG1k | BD |
| CD25 APC | IgG1k | eBioscience |
| CD127 FITC | IgG1k | eBioscience |
| **CD8regs** |  |  |
| CD8 APC | IgG1k | eBioscience |
| CD56 FITC | IgG1k | eBioscience |
| CD161 Percp Cy5.5 | IgG1k | eBioscience |
| **Bregs** |  |  |
| CD19 APC Cy7 | IgG2ak | BD |
| CD38 APC | IgG1k | eBioscience |
| CD24 Percp Cy5.5 | IgG2ak | BD |
| **Tolerogenic dendritic cells** |  |  |
| CD11c Percp-efluor 710 | IgG1k | eBioscience |
| CD274 (B7H1) FITC | IgG1k | BD |
| **Intermediate monocytes** |  |  |
| CD14 Percp Cy5.5 | IgG1k | eBioscience |
| CD16 FITC | IgG1k | BD |

**Supplementary Table 2**. Primers and efficiency for each dopamine receptor type.

|  |  |  |
| --- | --- | --- |
| **Gene** | **Primer** | **Efficiency**  **(%)** |
| **DRD1** | Forward: 5'TGATGTCAAAGGCCACCCAG3'  Reverse: 5'GCCGTTATCAGGTTCCGACA3' | 100 |
| **DRD2** | Forward: 5'GCGTTATTGAGTCCGAAGAGGA3'  Reverse: 5'AATACGCGCTACAGCTCCA3' | 93 |
| **DRD3** | Forward: 5'CTATTCACGTAGCCCAGCCA3'  Reverse: 5'CTGCCCTTCTTCTTGACCCAT3' | 102 |
| **DRD4** | Forward: 5'TGCGCTACAACCGGCAG3'  Reverse: 5'ACGGACGAGTAGACCACGTA3' | 106 |
| **DRD5** | Forward: 5'CCTCATCTCCTTCATTCCGGT3'  Reverse: 5'GTTCTCTGCATTCACGTCGG3' | 108 |
| **Reference gene** | |  |
| **HPRT1** | Forward: 5'GAGATGTGATGAAGGAGATGGGA3'  Reverse 5'AATCCAGCAGGTCAGCAAAGA3' | 105 |

**Supplementary Table 3**. Statistical power analysis of regulatory populations when compared controls and patients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Phenotype** | **CTR** | **PD** | ***Common SD*** | ***β*** |
| **Gated**  **(%)** |  |  |  |  |
| Tregs | 6.73 ± 3.81 | 7.85 ± 1.68 | 2.81 | 0.15 |
| CD8regs | 21.78 ± 12.16 | 18.61 ± 8.76 | 10.26 | 0.11 |
| Bregs | 7.19 ± 4.90 | 4.75 ± 3.01 | 4.05 | 0.29 |
| TDCs | 15.47 ± 6.92 | 14.45 ± 7.22 | 6.92 | 0.05 |
| Intermediate monocytes | 3.22 ± 1.29 | 4.22 ± 2.61 | 2.13 | 0.19 |
|  |  |  |  |  |
| **†Absolute numbers**  **(× 10−4)** |  |  |  |  |
| Tregs | 9 ± 16 | 7 ± 7 | 2 | 0.05 |
| CD8regs | 24 ± 45 | 4 ± 4 | 31 | 0.32 |
| Bregs | 3 ± 6 | 1 ± 1 | 4 | 0.27 |
| TDCs | 59 ± 30 | 64 ± 60 | 47 | 0.05 |
| Intermediate monocytes | 23 ± 28 | 21 ± 17 | 22 | 0.04 |

SD = Standard deviation

β = Statistical power

TDCs = Tolerogenic dendritic cells

**Supplementary Table 4**. Statistical power analysis of dopamine receptor expression when compared male and female patients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Population** | **Male**  **(Ratio)** | **Female**  **(Ratio)** | ***Common SD*** | ***β*** |
| **Tregs** |  |  |  |  |
| DRD1 | 1.08 ± 0.39 | 0.56 ± 0.06 | 0.37 | 0.68 |
| DRD2 | 1.15 ± 0.10 | 0.64 ± 0.07 | 0.28 | 0.89 |
| DRD3 | 1.06 ± 0.26 | 0.59 ± 0.11 | 0.30 | 0.76 |
| DRD4 | 1.20 ± 0.30 | 0.64 ± 0.16 | 0.37 | 0.75 |
| DRD5 | 1.32 ± 0.37 | 0.60 ± 0.08 | 0.45 | 0.79 |
|  |  |  |  |  |
| **CD8regs** |  |  |  |  |
| DRD1 | 1.33 ± 0.39 | 0.79 ± 0.10 | 0.41 | 0.63 |
| DRD2 | 1.33 ± 0.30 | 0.82 ± 0.14 | 0.36 | 0.69 |
| DRD3 | 1.25 ± 0.20 | 0.71 ± 0.06 | 0.35 | 0.77 |
| DRD4 | 1.53 ± 0.42 | 0.77 ± 0.45 | 0.56 | 0.65 |
| DRD5 | 1.89 ± 0.98 | 0.75 ± 0.08 | 0.86 | 0.63 |
|  |  |  |  |  |
| **Bregs** |  |  |  |  |
| DRD1 | 1.48 ± 0.96 | 0.71 ± 0.37 | 0.77 | 0.4 |
| DRD2 | 1.36 ± 0.30 | 0.69 ± 0.43 | 0.50 | 0.63 |
| DRD3 | 0.99 ± 0.13 | 0.65 ± 0.36 | 0.32 | 0.44 |
| DRD4 | 1.08 ± 0.51 | 0.58 ± 0.18 | 0.43 | 0.52 |
| DRD5 | 1.16 ± 0.34 | 0.54 ± 0.31 | 0.45 | 0.67 |
|  |  |  |  |  |
| **TDCs** |  |  |  |  |
| DRD1 | 1.16 ± 0.28 | 0.60 ± 0.10 | 0.35 | 0.79 |
| DRD2 | 1.23 ± 0.16 | 0.64 ± 0.11 | 0.33 | 0.86 |
| DRD3 | 1.16 ± 0.16 | 0.66 ± 0.13 | 0.29 | 0.83 |
| DRD4 | 1.13 ± 0.26 | 0.57 ± 0.11 | 0.34 | 0.80 |
| DRD5 | 1.29 ± 0.18 | 0.66 ± 0.25 | 0.38 | 0.80 |
|  |  |  |  |  |
| **Intermediate monocytes** |  |  |  |  |
| DRD1 | 1.71 ± 0.31 | 0.89 ± 0.42 | 0.56 | 0.72 |
| DRD2 | 1.51 ± 0.30 | 0.78 ± 0.18 | 0.45 | 0.81 |
| DRD3 | 1.63 ± 0.14 | 0.86 ± 0.18 | 0.43 | 0.87 |
| DRD4 | 1.72 ± 0.35 | 0.69 ± 0.25 | 0.61 | 0.83 |
| DRD5 | 1.75 ± 0.35 | 0.66 ± 0.44 | 0.69 | 0.79 |

SD = Standard deviation

β = Statistical power

TDC = Tolerogenic dendritic cells

**Supplementary Figure 1.** Sorting strategy for regulatory populations. The cells were gated according to the expression of CD4+, CD8+, and CD19+ and their forward-scatter properties. For Tregs, CD4+ cells were gated for the double expression of CD25 and CD127, and the CD4+CD25+CD127− cells were sorted. For CD8regs, CD8+ cells were gated for the double expression of CD56 and CD161, and the cells CD8+CD56+CD161− were sorted. CD19+ cells were gated for the double expression of CD38 and CD24, and Breg cells were defined as CD19+CD24+CD38+ cells. For tolerogenic dendritic cells (TDC) and intermediate monocytes, the cells were first gated according to forward-side scatter properties. CD11c+B7H1+ cells were considered as TDC, and CD14HICD16+ cells were considered as intermediate monocytes.

**Supplementary Figure 2.** Reference gene selection strategy. Four reference genes were tested in all patients and controls. Mean Ct for HPRT1, B2M, GUSB, and RPLP0 is shown for **A)** each regulatory population, and **B)** the global Ct for controls and patients. The reference gene with the least coefficient of variation (CV) among patients and controls was selected. Considering only the CV of the reference genes in each population, all genes seemed to be eligible as a reference. But when global CV was evaluated, HPRT1 showed to be the least variable between patients and controls.

SD = Standard deviation.

CV = Coefficient of variation.

**Supplementary Figure 3.** Variation in the relative expression of dopamine receptors in patients and controls. The results are shown as individual data in patients and controls for the expression of DRD1-DRD5 on Tregs, Bregs, CD8regs, TDCs, and intermediate monocytes. The 2−ΔCt method was used to report individual values. HPRT1 was used as a reference gene.

**Supplementary Figure 4.** Relative expression of dopaminergic receptors on Tregs, CD8regs, Bregs, TDCs, and intermediate monocytes. Data are reported as the individual relative expression of dopamine gene receptors in each PD patient with respect to the controls. Patients were classified according to **A)** age, **B)** Hoehn and Yahr score, **C)** treatment, and **D)** disease duration. HPRT1 was used as a reference gene. *n* = 11 for Tregs, Bregs, TDCs, and intermediate monocytes; *n* = 10 for CD8regs.

**Supplementary Figure 5.** Dopamine receptors are present on the membrane surface of regulatory populations. A) Histogram showing background fluorescence and DR+ PE fluorescence with respect to the isotype. Left to right: DRD1 (isotype IgG1k), DRD2 (isotype IgG2bk), DRD3 (isotype IgG1k), DRD4 (isotype IgG1k), and DRD5 (isotype IgG1k). B) Mean ± SD of two patients and two controls is shown for each dopamine receptor and immune regulatory population.