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Original article

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Distinct immune regulatory receptor profiles linked to altered monocyte subsets in sarcoidosis

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Key words: innate immunity, macrophage, monocyte, sarcoidosis

ABSTRACT

Background

In sarcoidosis, blood monocytes, circulating precursors of granuloma macrophages, display enhanced inflammatory cytokine production, reduced expression of the regulatory (inhibitory) receptor CD200R, and altered subsets defined by CD14 and CD16. Regulatory receptors serve to dampen monocyte and macrophage inflammatory responses. We investigated the relationship between monocyte subsets and regulatory receptor expression in sarcoidosis.

Methods

Multi-parameter flow cytometry was used to perform detailed analyses of cell surface regulatory molecules on freshly isolated blood immune cells from patients with chronic pulmonary sarcoidosis and age-matched healthy controls.

Results

Twenty-five patients with chronic pulmonary sarcoidosis (median duration of disease 22 months) who were not taking oral corticosteroids or other immunomodulators were recruited. Non-classical monocytes were expanded in sarcoidosis and exhibited significantly lower expression of regulatory receptors CD200R, SIRP-α, and CD47 than classical or intermediate monocytes. In sarcoidosis, all three monocyte subsets had significantly reduced CD200R and CD47 expression compared with healthy controls. A dichotomous distribution of CD200R was seen on classical and intermediate monocytes in the sarcoidosis population, with 14/25 (56%) sarcoidosis patients having a CD200R-low phenotype, and 11/25 (44%) CD200R-high. These distinct sarcoidosis monocyte phenotypes remained consistent over time.

Conclusion

Non-classical monocytes, which are expanded in sarcoidosis, express very low levels of regulatory receptors. Two distinct and persistent phenotypes of CD200R expression in classical and intermediate monocytes could be evaluated as sarcoidosis biomarkers.

INTRODUCTION

Sarcoidosis is characterized by granulomas composed of activated monocyte-derived macrophages (1). Studying circulating blood monocytes offers a window to granuloma macrophage biology and, unlike sarcoid tissue biopsies, allows repeated sampling. Research in sarcoidosis has identified alterations in circulating monocyte populations defined by expression of surface receptors CD14 (LPS co-receptor) and CD16 (low affinity receptor for IgG). Reduced classical ($CD14^{++}$, $CD16^{-}$) monocytes and increased CD16-expressing non-classical ($CD14^{+}$, $CD16^{++}$) or intermediate ($CD14^{++}$, $CD16^{+}$) monocytes have been reported (2-5). Enhanced production of inflammatory cytokines by sarcoidosis monocytes was associated with reduced expression of the regulatory receptor CD200R (2), which serves to dampen immune responses. CD200R deficiency in animals leads to enhanced production of TNF (6, 7), a critical driver of granulomatous inflammation. Therefore, relative lack of CD200R could contribute to the exaggerated inflammation seen in chronic sarcoidosis. The relationship between sarcoidosis monocyte subsets and expression of CD200R and other regulatory receptors is unknown.

METHODS

Subjects

Ethical approval was obtained (REC 16/YH/0118) and participants gave written informed consent. Patients with chronic pulmonary sarcoidosis were recruited from the sarcoidosis clinic at Hull University Teaching Hospitals NHS Trust. Patients were diagnosed by a specialist ILD physician according to international guidance (8). Exclusion criteria included current tobacco smoking or therapy with oral corticosteroids or other immunomodulatory drugs, which could independently affect monocyte phenotype. Healthy controls were age

and sex-matched with the patient cohort. Controls were volunteers without sarcoidosis or significant lung disease who were non-smokers and were not taking oral corticosteroids or immunosuppressant medication. Detailed medical histories were not obtained from healthy controls, nor were pulmonary function tests or blood investigations performed. A statistical power calculation was not performed for this exploratory study.

Immune cell phenotyping

Blood was collected in sodium heparin tubes (BD) and processing was started within 1 hour. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation (2). For immunophenotyping, live unfixed PBMCs were stained with saturating combinations of fluorescent antibodies (Supplementary Material Tables S1 and S2) and multi-parameter flow cytometry was used to analyse antibody binding, allowing discrimination of surface receptor expression on cellular subsets. Details of the immunophenotyping procedures can be found in the Supplementary Material.

Immunohistochemistry

The procedure for staining tissue sections for CD200L and CD200R was adapted from Cawkwell et al (9). Antibodies were a polyclonal goat CD200L antibody (R&D; AF2724), normal goat IgG control (R&D; AB-108-C), mouse monoclonal IgG1 anti-human CD200R (AbD Serotec/Biorad; OX108, MCA 2282T), and mouse monoclonal control IgG1 antibody (Biolegend; MOPC-21, 400101).

Statistical analyses

Statistical significance was determined using Mann-Whitney U or Kruskal-Wallis tests for non-parametric distributions and Student's T-test, one-way ANOVA or two-way ANOVA for parametric distributions. Multiple comparisons underwent Sidak or Tukey's or post-hoc analyses as appropriate. Pearson's chi-squared or Fisher's exact tests were used to examine associations between categorical variables. Analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, USA).

RESULTS

Patient demographics

Twenty-five treatment-naïve patients with chronic pulmonary sarcoidosis were recruited. Baseline characteristics of the patients are presented in **Table 1**. All participants were white, reflecting the local population.

Non-classical monocytes are increased in sarcoidosis and express low levels of CD200R

Sarcoidosis patients had significantly increased proportions of non-classical monocytes and significantly lower proportions of classical monocytes compared with controls (**Figure 1**). Non-classical monocytes expressed significantly lower levels (10-15%) of CD200R than intermediate or classical monocytes in both sarcoidosis patients and controls ($p<.0001$, **Supplementary Figure S1**).

Dichotomous populations of monocyte CD200R expression in sarcoidosis

Patients with sarcoidosis had significantly lower expression of CD200R on all monocyte subsets compared with controls (**Figure 2**). In the sarcoidosis cohort, CD200R expression on classical and intermediate monocytes was not normally distributed. Normal Q-Q plots

(**supplementary figure S2**) confirmed bimodal populations with inflection points delineating CD200R high from low at a GMFI of around 300.

To establish whether CD200R expression on monocytes was stable or varied over time, patients were tracked every two months for up to one year in a longitudinal study. The two distinct sarcoidosis patient populations with CD200R-high and CD200R-low classical and intermediate monocyte subsets maintained their phenotypes throughout follow-up (**Figure 3**). No individual switched between populations over time.

Eleven patients (44%) exhibited high CD200R expression on classical and intermediate monocytes (median GMFI 505, range 359-664), and 14 (56%) had low CD200R expression (median GMFI 157, range 70-276). Most control subjects had high CD200R expression on classical and intermediate monocytes, but we also observed a low classical monocyte CD200R phenotype in 3 of the 12 healthy controls.

CD200R high or low monocyte phenotypes in sarcoidosis patients were not associated with age, sex, extent of chest X-ray changes, lung function, serum ACE activity, C-reactive protein, or blood cell counts (**Table 2**). Comorbidities and medication use were evenly distributed across the two monocyte phenotypes (data not shown).

The effect of systemic corticosteroid therapy on monocyte subsets and CD200R expression was examined in three patients who progressed to need treatment with oral prednisolone during follow-up. Oral prednisolone therapy for two months (20 mg/day) tended to normalise monocyte subsets (**Figure 4**), with post-treatment increases in classical

monocytes and decreases in non-classical monocytes. Statistical significance could not be demonstrated with only three pairs of data. In contrast, prednisolone therapy had no demonstrable effect on monocyte CD200R expression (**Figure 4**).

Expression of regulatory receptors SIRP- α, CD47, and IL-10R on sarcoidosis monocytes

CD200R is one of several inhibitory receptors that dampen inflammatory responses in monocytes, so we studied whether other regulatory molecules were differentially expressed by monocyte subsets. We focused on molecules that, like CD200R, are known to regulate monocyte inflammatory responses, namely SIRP-α (signal regulatory protein α, which binds CD47 and surfactant proteins), CD47 (the ligand for SIRP-α and receptor for thrombospondin-1 that delivers an anti-phagocytic signal), and IL-10R (the receptor for interleukin-10, an anti-inflammatory cytokine). Expression of CD47 and SIRP-α was significantly lower on non-classical monocytes compared with classical or intermediate monocytes, whereas expression of IL-10R was generally low but was highest on intermediate monocytes (**Figure 5, Figure S1**).

Comparing patients with sarcoidosis and healthy controls demonstrated significantly reduced expression of CD47 on all monocyte subsets in sarcoidosis (**Figure 5**). IL-10R expression was reduced only on intermediate monocytes in patients with sarcoidosis compared with controls, but not on other subsets. SIRP-α expression was similar in controls and sarcoidosis patients.

Distribution of CD200R and CD200L in sarcoidosis granulomas

To gain a preliminary view of how macrophage regulatory receptors may interact with ligands within a sarcoidosis granuloma, we stained sarcoidosis biopsies for the receptor-ligand pairing CD200R and CD200L. Figure 6 shows the spatial relationship between CD200R-expressing granuloma macrophages and neighbouring CD200L-positive lymphocytes and fibroblasts.

DISCUSSION

Understanding the cellular and molecular drivers of persistent and progressive sarcoidosis will be an important step towards identifying new targets for treatment. Chronic, non-resolving sarcoidosis is characterised immunologically by heightened inflammatory responses of tissue granuloma macrophages and their precursors, circulating blood monocytes (2, 5, 10-14). Cell surface regulatory receptors such as CD200R serve to dampen inflammatory responses in monocytes and macrophages, including reducing production of TNF which is a key cytokine driving granuloma formation and persistence (15, 16). Altered regulatory receptor expression is a potential mechanism contributing to persistent and progressive granulomatous inflammation in sarcoidosis.

We provide the first description that monocyte subsets have distinct expression profiles of regulatory receptors. Non-classical monocytes exhibited much lower expression of CD200R, SIRP-α, and CD47 than classical or intermediate monocytes. Moreover, in sarcoidosis all monocyte subsets had reduced CD200R and CD47 expression compared with healthy controls, providing an additional explanation for the overall reduced regulatory receptor profile. However, there is not a global reduction of all regulatory molecules in sarcoidosis, since expression levels of SIRP-α or IL-10R were similar to controls.

In health, most (~90%) monocytes are classical $CD14^{++}/CD16^{-}$ cells (17). The remainder comprise CD16-positive non-classical monocytes which exhibit higher stimulated TNF production (18), and a low frequency intermediate ($CD14^{++}/CD16^{+}$) monocyte population. A developmental relationship between monocyte subsets has been described, with sequential increase in first intermediate then non-classical monocytes (19). Distinguishing non-classical from intermediate monocytes within the CD16-positive population can be challenging (4, 17, 20) so it is difficult to compare studies directly. In the present study, we identified a clear increase in non-classical monocytes in sarcoidosis, whereas previous studies reported that intermediate monocytes are increased (2, 5). Aside from differences between cohorts, the current findings can be explained by increased refinement of monocyte gating. By utilising a fluorophore with a high stain index for CD16, adopting an angled gate between intermediate and non-classical monocyte populations, and employing a robust gating protocol across all populations, the present study presents a more precise separation of monocyte subsets than we previously used. Although we did not refine discrimination of intermediate and non-classical monocytes with additional markers such as 6-sulfo LacNAc (slan) (4), we have shown that CD200R expression clearly distinguishes non-classical and intermediate monocytes, supporting the validity of our gating strategy and suggesting that CD200R has both functional and phenotypic importance and could be incorporated in future studies. Moreover, CD200R could be used in future studies as marker of non-classical monocytes.

Distribution of CD200R expression on classical and intermediate monocytes was dichotomous, such that patients with sarcoidosis could be divided into CD200R-high and

CD200R-low groups. The stability of this monocyte CD200R phenotype over time suggests two distinct and consistent phenotypes of sarcoidosis. In our previous study, there was a dichotomous distribution of CD200R on total monocytes in sarcoidosis, whereas all of the 17 healthy controls had a CD200R-high phenotype. In the present study, 3 of the 12 controls exhibited a CD200R-low phenotype on classical and intermediate monocytes. This difference between the studies may be explained by careful age-matching of the control with patient groups in the present study. We cannot rule out the possibility that the white population studied comprises two sub-populations, one with high and one with low CD200R expression and that individuals with low expression are over-represented within a sarcoid patient group.

Immunohistochemistry showed CD200R-expressing macrophages concentrated in the central core of the sarcoidosis granuloma, surrounded by CD200L-expressing lymphocytes and fibroblasts. We anticipate that within the dynamic milieu of a sarcoidosis granuloma, CD200R on macrophages will be ligated by CD200L on neighbouring cells as they migrate and interact with each other.

We provide the first description of significantly reduced regulatory receptor expression on non-classical monocytes. Expansion of these non-classical monocytes in sarcoidosis, together with reduced levels of specific regulatory molecules (CD200R and CD47) on all sarcoidosis monocyte subsets compared with controls, will favour heightened inflammatory responses. This sarcoidosis monocyte phenotype could be assessed as a prognostic or therapeutic biomarker in future studies.

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TABLES

Table 1. Demographic and clinical profiles of patients with sarcoidosis and healthy controls. Data are presented as median (range). * reference range 8-65 U/L; † reference range 0.8-3.4 $x10^9$ cells/L

	Sarcoidosis n=25	**Controls** n=12
Age (years)	**53** (38-73)	**51** (30-67)
Male/Female	**15/10**	**7/5**
Months since diagnosis	**22** (0-193)	
Scadding chest X-ray stage 0/1/2/3 (number of patients)	**3/5/12/5**	
Angiotensin converting enzyme (U/L)*	**94** (9-440)	
Blood lymphocyte count ($x10^9$/L) †	**0.86** (0.29-2.03)	
FEV1 percent predicted	**93** (54-126)	
FVC percent predicted	**95** (64-129)	
FEV1/FVC ratio	**0.81** (0.49-0.91)	

Table 2. Characteristics of sarcoidosis patients stratified by monocyte CD200R phenotype. Data are presented as median (interquartile range or *range). Statistical comparisons by nonparametric independent samples median test or Pearson's chi square.

	CD200R-low n=14	**CD200R-high n=11**	**p value**
Chest X-ray stage 0/1/2/4 (number of patients)	**1/4/5/4**	**2/1/7/1**	**.265**
Angiotensin converting enzyme (U/L)	**92** (52-136)	**104** (74-155)	**.695**
C-reactive protein (mg/L)	**2.0** (1.4-5.5)	**2.8** (0.6-9.6)	**.217**
Age (years)*	**53.5** (40-73)	**50.0** (38-67)	**1.00**
FEV1 percent predicted	**99** (69-110)	**92** (82-113)	**.428**
FVC percent predicted	**103** (76-115)	**90** (82-114)	**.428**
FEV1/FVC ratio	**0.79** (0.75-0.84)	**0.83** (0.76-0.86)	**.238**
Months since diagnosis	**20** (9-51)	**31** (10-73)	**.695**
Male/Female	**8/6**	**7/4**	**1.00**
White blood cell count ($x10^9$/L)	**5.4** (4.15-6.20)	**4.3** (3.50-5.60)	**.196**
Lymphocyte count ($x10^9$/L)	**0.93** (0.74-1.41)	**0.74** (0.62-1.02)	**.279**
Monocyte count	**0.55**	**0.42**	**.252**

($x10^9$/L)	(0.44-0.72)	(0.24-0.70)	
Neutrophil count ($x10^9$/L)	**3.42** (2.70-3.98)	**2.70** (2.33-3.97)	**.336**
Eosinophil count ($x10^9$/L)	**0.15** (0.13-0.23)	**0.20** (0.08-0.21)	**.797**
T lymphocyte count	**0.61** (0.36-0.91)	**0.48** (0.33-0.63)	**.403**
$CD4^+$ T lymphocyte count	**0.36** (0.22-0.70)	**0.30** (0.16-0.45)	**.596**
$CD8^+$ T lymphocyte count	**0.20** (0.11-0.40)	**0.17** (0.09-0.21)	**.290**
B lymphocyte count	**0.16** (0.07-0.21)	**0.14** (0.08-0.23)	**.499**
Natural Killer cell count	**0.17** (0.12-0.22)	**0.15** (0.09-0.26)	**.450**

FIGURE LEGENDS

Figure 1. Patients with sarcoidosis have increased non-classical monocytes and reduced classical monocytes compared with controls.

Flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) was used to define monocyte subsets based on expression of CD14 and CD16. Ungated PBMCs are shown here for illustrative purposes. a) Monocytes were defined as classical (CD14++ CD16-/low, labelled C), intermediate (CD14++ CD16+, labelled I) or non-classical (CD14+ CD16++, labelled NC). Proportions of b) classical, c) intermediate, and d) non-classical monocyte subsets within total blood monocytes for patients with sarcoidosis (red, n=25) and healthy controls (blue, n=12). Individual data points are presented along with median (horizontal line) and interquartile range. ** $p<0.01$ (Mann-Whitney U test).

Figure 2. Non-classical monocytes express low levels of CD200R, and CD200R expression is lower on all monocyte subsets in patients with sarcoidosis compared with healthy controls.

a) Representative flow cytometry histograms from a healthy control subject comparing CD200R expression on classical (blue), intermediate (teal) and non-classical (red) monocytes, compared with isotype control antibody (black). Representative heat-map dot plots from b) a healthy control subject demonstrating low CD200R on non-classical monocytes, and c) a patient with sarcoidosis showing reduced CD200R expression on sarcoidosis monocyte subsets compared with the healthy control. On these pseudocolor plots, orange indicates higher CD200R expression and green indicates lower CD200R expression. d-f) Expression of CD200R on monocyte subsets in sarcoidosis patients (red) and controls (blue). Note the small y-axis scale for non-classical monocytes. Individual data

points are presented along with median (horizontal line) and interquartile range (* p<0.05, ** p<0.01, Mann-Whitney U test).

Figure 3. CD200R expression on sarcoidosis classical and intermediate monocytes is dichotomous and consistent over time.

Patients with sarcoidosis in a longitudinal cohort (n = 19) underwent serial measurements of CD200R expression on a) classical, b) intermediate, and c) non-classical monocyte subsets. Each patient is presented as a different coloured line.

Figure 4. Oral corticosteroid therapy for sarcoidosis normalises monocyte subsets but has no effect on monocyte CD200R expression.

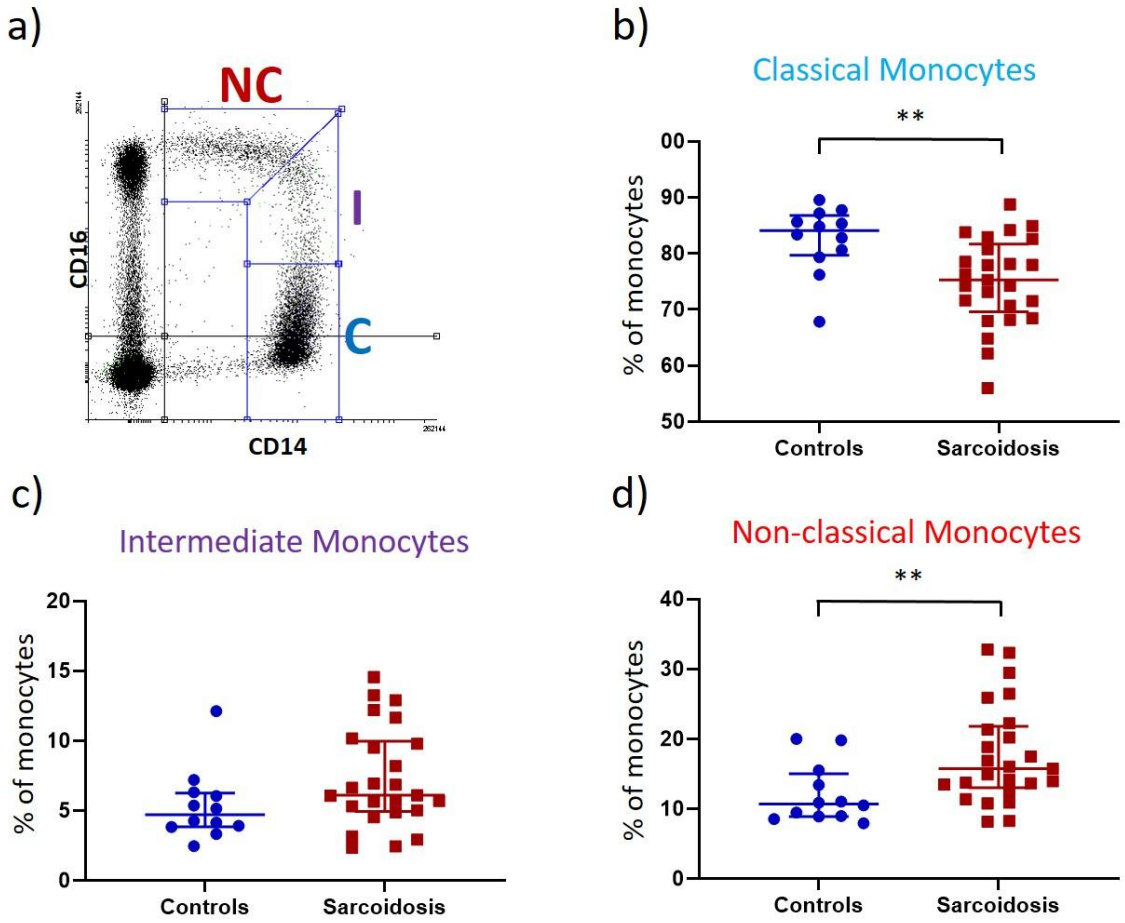
Pre- and two month post-steroid treatment data for three sarcoidosis patients who were treated with oral prednisolone 20mg/day. Panels A, C, and E show the percentages of classical, intermediate, and non-classical monocytes. Panels B, D, and F show CD200R expression on the respective monocyte subsets.

Figure 5. Monocyte subsets have distinct expression profiles of SIRP-α, CD47 and IL-10R in sarcoidosis and healthy controls

Expression of a) SIRP-α, b) CD47 and c) IL-10R on monocyte subsets was in patients with sarcoidosis (red, n=25) and healthy controls (blue, n=7). Individual data points are presented along with median (horizontal line) and interquartile range (** p<0.01, *** p=<0.001, **** p<.0001, Mann-Whitney U test).

Figure 6. Distribution of the regulatory receptor CD200R and its ligand CD200L in sarcoidosis granulomas

Transbronchial lung biopsy samples from two patients with sarcoidosis stained for a) CD200L and b) CD200R. Respective control antibody staining is shown in c) and d). Original magnification x100. f, fibroblasts; h, histiocytes (macrophages)


a)
NC
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CD16
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b)
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c)
Intermediate Monocytes
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Sarcoidosis
d)
Non-classical Monocytes
**
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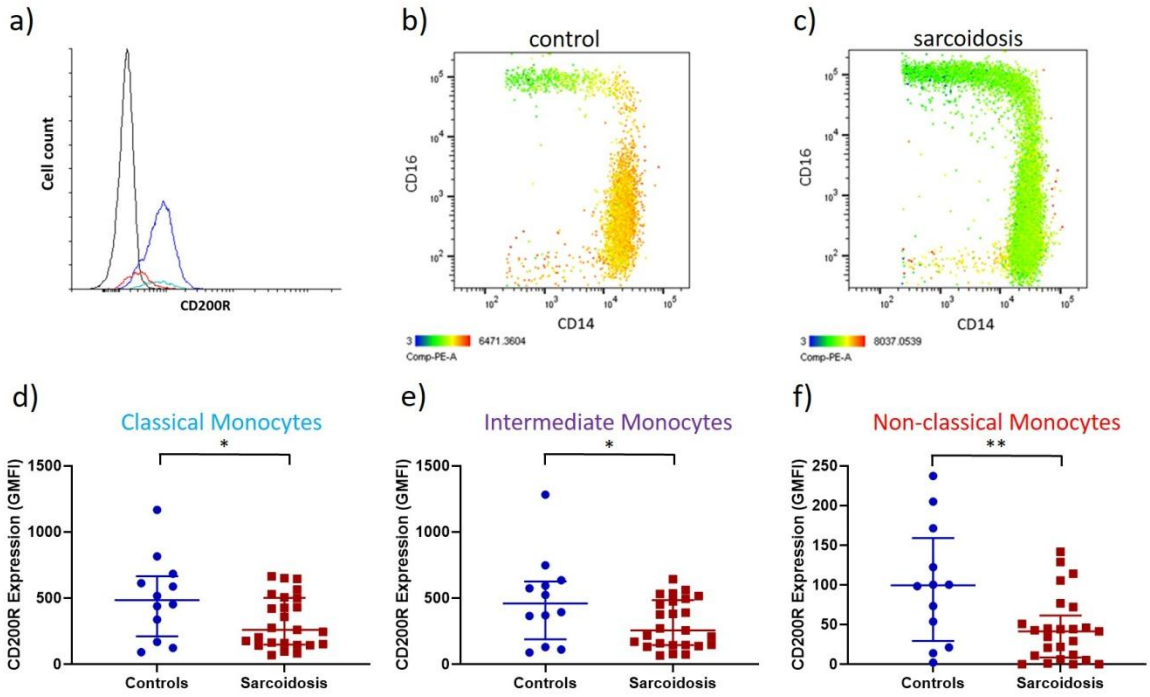
Sarcoidosis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Cell count
CD200R
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c)
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8037.0539
Comp-PE-A
d)
Classical Monocytes
*
CD200R Expression (GMFI)
1500
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Sarcoidosis
e)
Intermediate Monocytes
*
CD200R Expression (GMFI)
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500
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Sarcoidosis
f)
Non-classical Monocytes
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200
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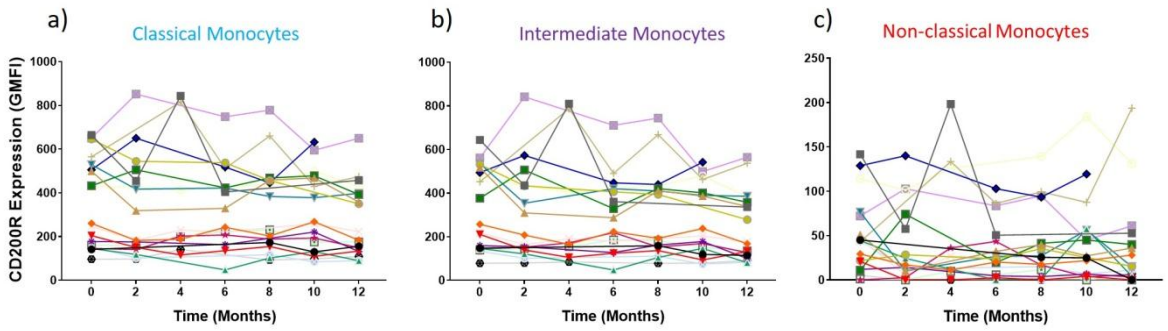
Controls
Sarcoidosis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Classical Monocytes
b)
Intermediate Monocytes
c)
Non-classical Monocytes
CD200R Expression (GMFI)
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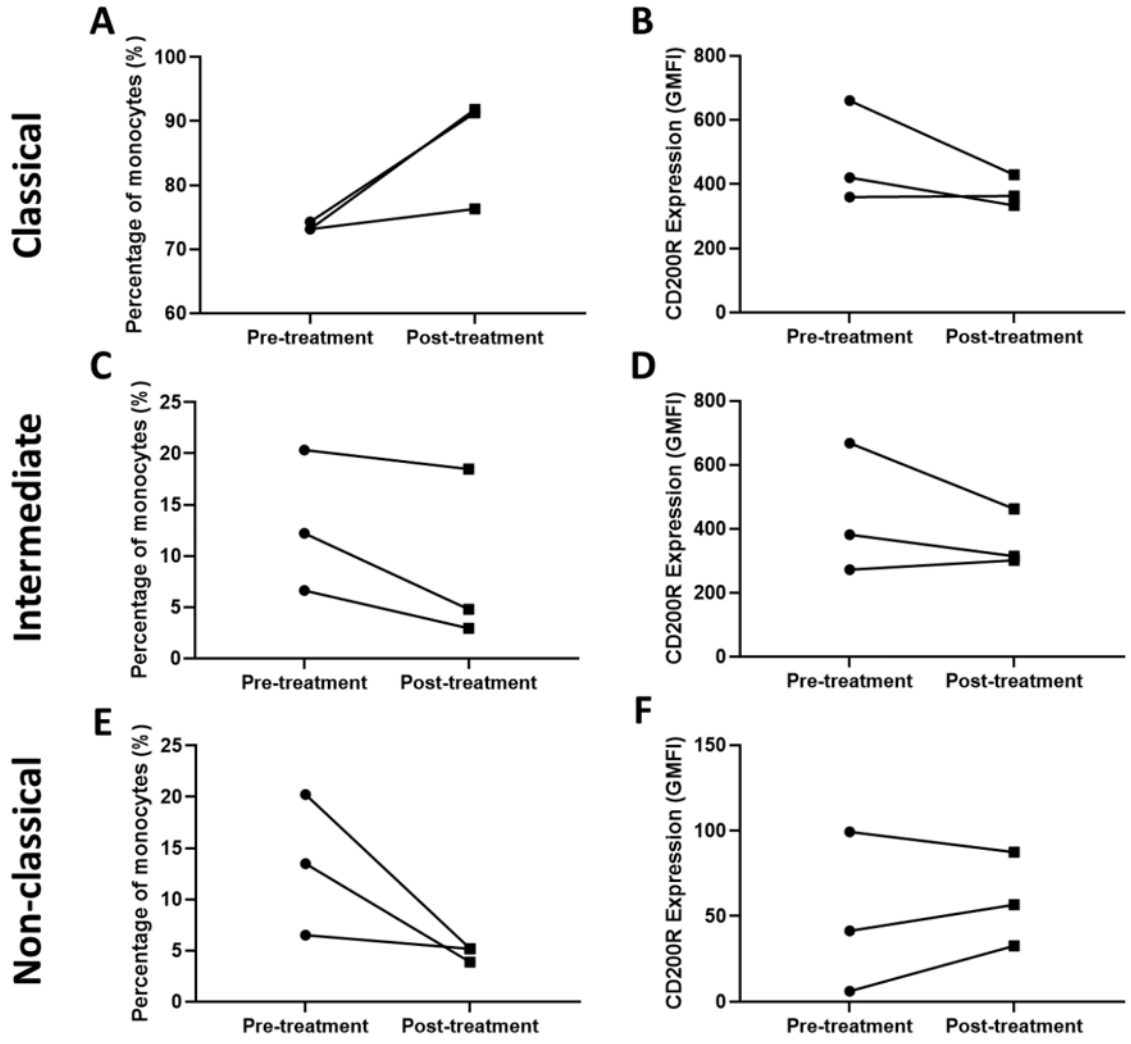
Classical
Intermediate
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A
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Percentage of monocytes (%)
CD200R Expression (GMFI)
Pre-treatment
Post-treatment
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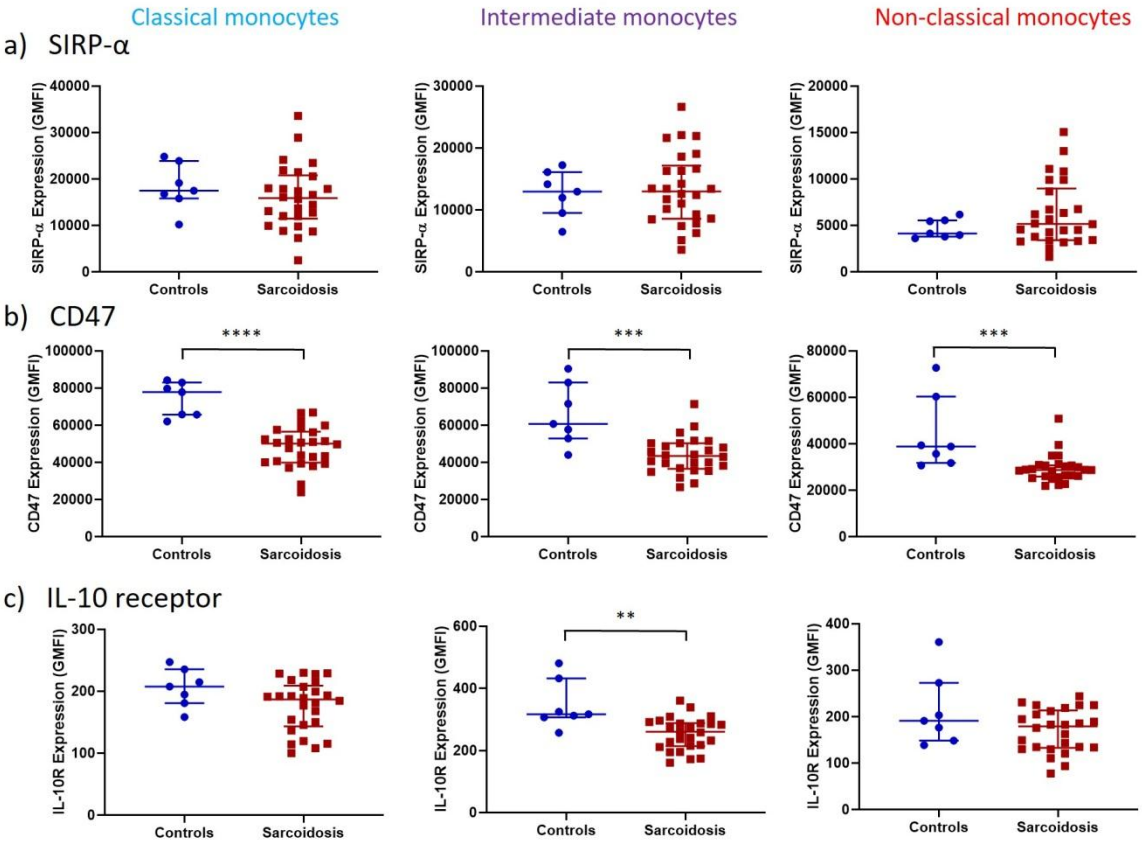
Classical monocytes
Intermediate monocytes
Non-classical monocytes
a) SIRP-α
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Sarcoidosis
SIRP-α Expression (GMFI)
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Sarcoidosis
SIRP-α Expression (GMFI)
20000
15000
10000
5000
0
Controls
Sarcoidosis
b) CD47
****
CD47 Expression (GMFI)
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40000
20000
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CD47 Expression (GMFI)
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20000
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Sarcoidosis
***
CD47 Expression (GMFI)
80000
60000
40000
20000
0
Controls
Sarcoidosis
c) IL-10 receptor
IL-10R Expression (GMFI)
300
200
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Controls
Sarcoidosis
**
IL-10R Expression (GMFI)
600
400
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Sarcoidosis
IL-10R Expression (GMFI)
400
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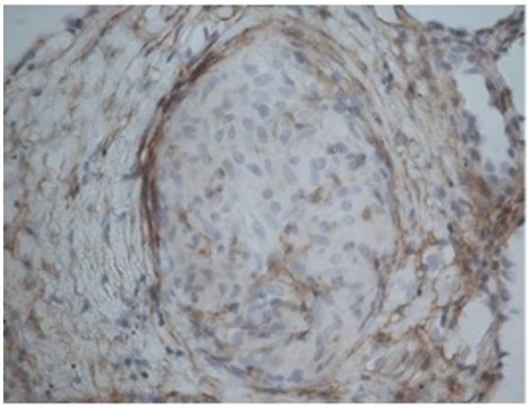
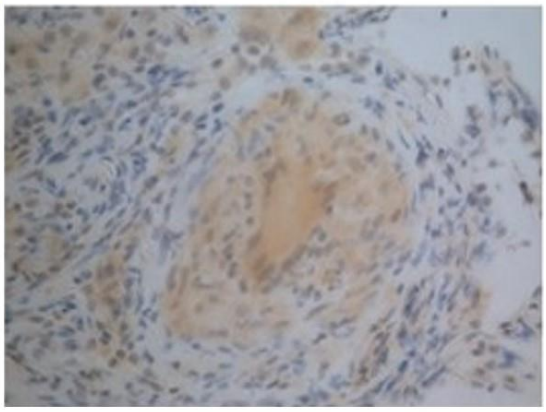
Controls
Sarcoidosis

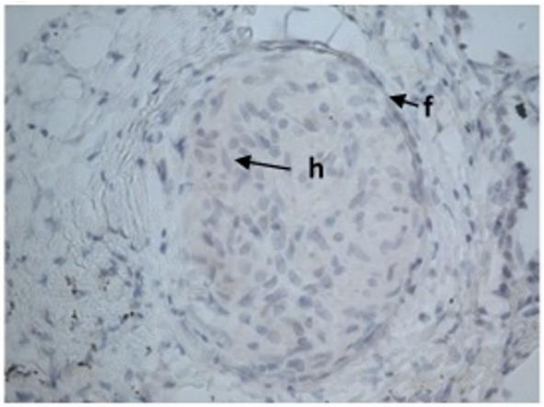
a) CD200L



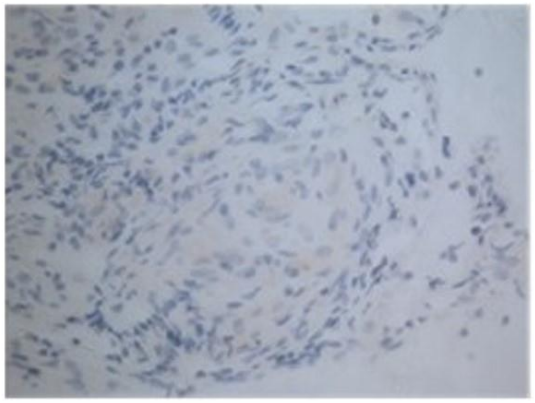
b) CD200R



c) Isotype control (CD200L)



d) Isotype control (CD200R)



Distinct immune regulatory receptor profiles linked to altered monocyte subsets in sarcoidosis

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Supplementary material

Immune cell phenotyping

For studying regulatory receptor expression on monocyte subsets, combinations of fluorophore-conjugated antibodies were carefully chosen that would have minimal or no overlap in their emission wavelengths. For all patients and healthy controls, the same combinations of antibodies were used (Supplementary Tables S1 and S2). PBMCs (2×10^5) were suspended in 50µl of FACS blocking solution (0.5% BSA in sterile PBS) for 1 hour at 4°C for blocking. Cells were centrifuged and resuspended in FACS blocking solution containing antibody combinations at optimum concentrations previously determined by titrations, typically 2.5 – 10 µg/ml, and incubated for 1 hour at 4°C in the dark. Cells were washed, resuspended and analysed using a FACS Aria II flow cytometer and FACS Diva software (BD, Oxford, UK). Compensation settings were optimised and kept constant throughout the study. The cytometer was calibrated before each sample analysis run using BD Cytometer Setup & Tracking Beads (ref 642412) to ensure consistency of results over time. Post-acquisition analyses were performed using Flowing Software 2.5.0 (Perttu Terho).

Flow cytometry gating and data analysis

Monocytes were defined by broad FSC and SSC gating followed by CD14+ gating. Lineage markers for T lymphocytes (CD3), B lymphocytes (CD19) and Natural Killer cells (CD3-CD56+) were absent from the monocyte gates. To define monocyte subsets, CD14 (APC) and CD16 (BV421) antibodies were used. Classical monocyte gates were defined using pooled data from controls and further monocyte subset populations were defined as described (1). Combining isotype control plots with density plots to determine the cut-off points allowed us to account for the strong CD16 fluorescence signal obtained from CD16 due to the high

stain index of BV421. All subsequent samples were calibrated using this fixed template to ensure consistent gating. An example of the gating is shown in Figure 1. Regulatory receptor expression was first calibrated using a mean isotype control GMFI in order to account for differences in acquisition between flow cytometer runs. Expression values were calculated as the calibrated GMFI minus the GMFI of the mean isotype control. Statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, USA).

1. Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. Front Immunol. 2013;4:23.

Table S1. Antibodies used for flow cytometry. PBMCs (2×10^5) were suspended in 50µl of FACS blocking solution (0.5% BSA in sterile PBS) for 1 hour at 4°C. Cells were pelleted and resuspended in FACS blocking solution containing antibody combinations at optimum concentrations (previously determined by titrations, typically 2.5–10 µg/ml) and incubated at 4°C in the dark for 1 hour. Cells were washed, re-suspended and analysed using a FACS Aria II flow cytometer and FACS Diva software (BD, Oxford, UK).

Markers	**Fluorescence**	**Reference (Biolegend)**	**Concentration used**
CD3	Brilliant Violet 421	300434	2.5 µg/ml
CD4	APC	300514	5 µg/ml
CD14	PE	325606	10 µg/ml
CD14	APC	325608	10 µg/ml
CD16	Brilliant Violet 421	302038	10 µg/ml
CD19	PerCP/CY5.5	302230	10 µg/ml
CD56	APC	318310	5 µg/ml
CD200R	PE	329306	10 µg/ml
IL-10R	PE	308804	10 µg/ml
SIRP-α	PE	323806	10 µg/ml
CD47	PE	323108	10 µg/ml
Isotype Controls Mouse IgG1	APC	400122	10 µg/ml
	PE	400114	10 µg/ml
	Brilliant Violet 421	400158	10 µg/ml
	PerCP/CY5.5	400150	10 µg/ml
Isotype Control Rat IgG2a	PE	400508	10 µg/ml

Table S2. Antibody combinations used for multi-parameter flow cytometry analysis of peripheral blood mononuclear cells.

Staining Target	BV421	PE	APC	PerCP/CY5.5
Immunophenotyping	CD3	CD14	CD56	CD19
	CD16	CD14	CD4	
CD200R	CD3	CD200R	CD14	CD19
	CD16	CD200R	CD14	

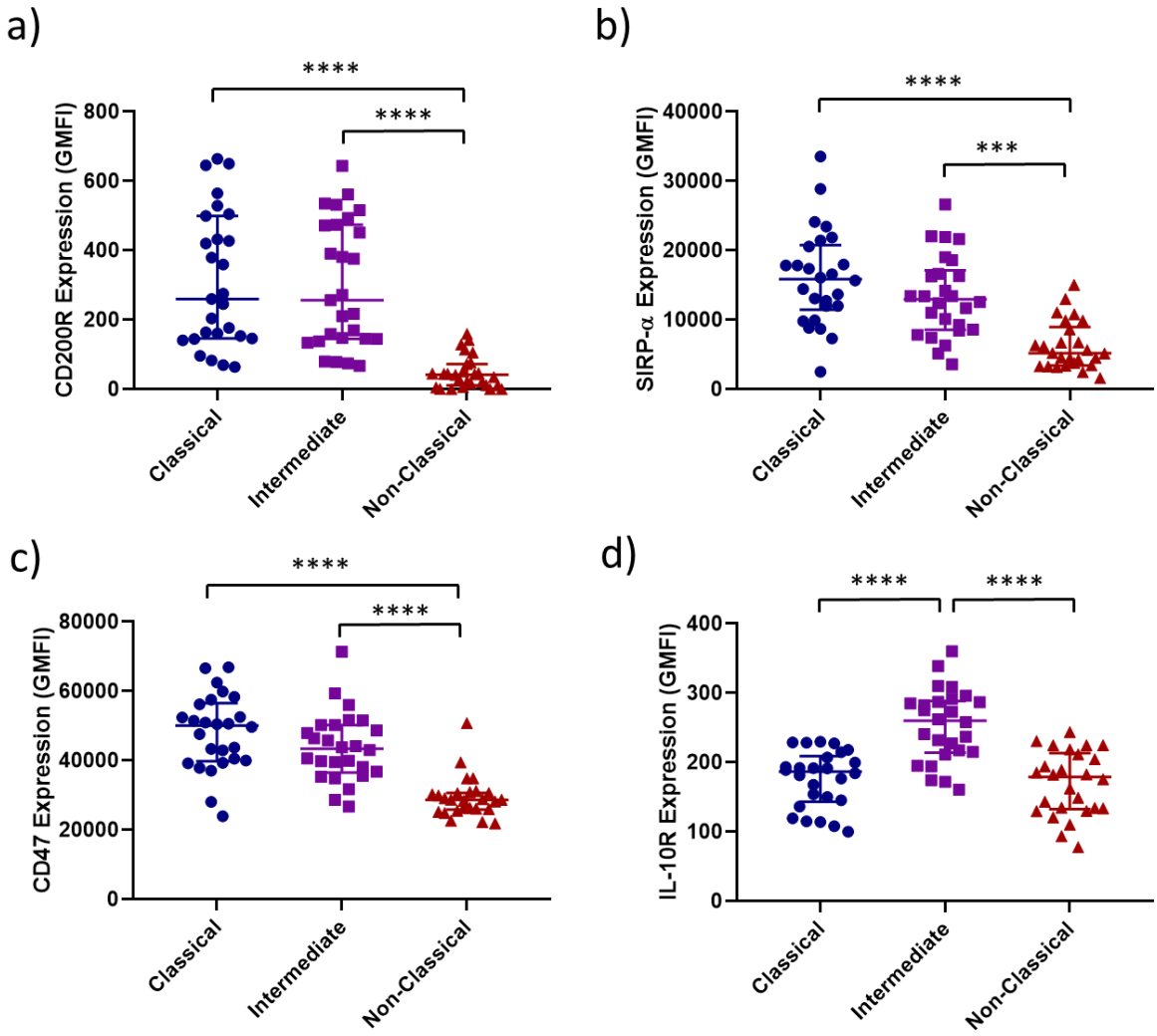

Figure S1. Comparison of regulatory receptor expression on monocyte subsets in patients with sarcoidosis.

Expression of a) CD200R, b) SIRP-α, c) CD47, and d) IL-10 receptor on classical (blue), intermediate (purple), and non-classical (red) monocytes. Receptor expression is presented as the geometric mean fluorescence intensity (GMFI). Individual data points are shown with medians (horizontal lines) with interquartile ranges. **** = $p < 0.0001$, statistics performed using the Kruskal-Wallis test with correction using the Dunn's multiple comparisons test.

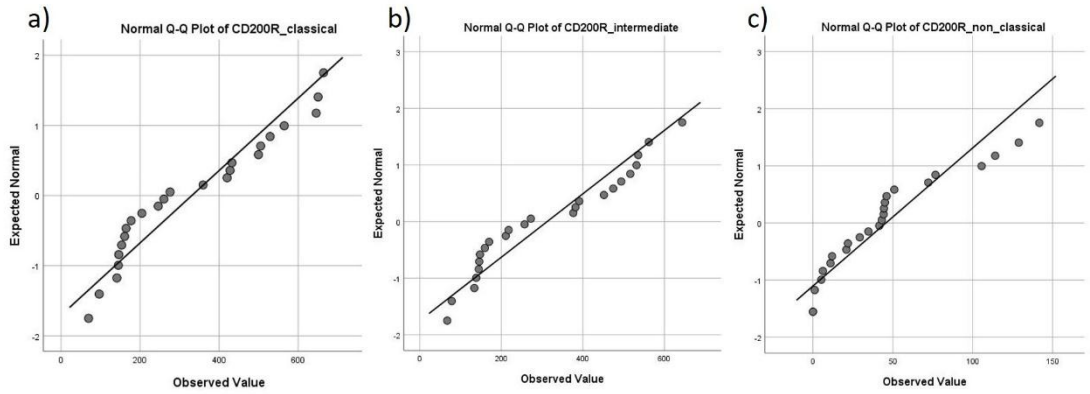

Figure S2. Normal quantile-quantile (Q-Q) plots for CD200R expression on a) classical, b) intermediate, and c) non-classical monocyte subsets from sarcoidosis patients at baseline. The Q-Q plots are consistent of bimodal distributions (with normally distributed data, the points would fall on the straight lines).