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DISSERTATION

CD38 als neues therapeutisches Target bei Systemischem Lupus Erythematodes
–
CD38 as a novel therapeutic treatment target for Systemic Lupus Erythematosus

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1. Abstract (Zusammenfassung)

1.1. Zusammenfassung

Der Systemische Lupus erythematodes (SLE) ist eine systemische Autoimmunerkrankung mit bisher nur zum Teil geklärter Ätiopathogenese. Ein wichtiger Aspekt der Krankheitsentstehung ist die Entwicklung von antinukleären Autoantikörpern, die von kurz- und langlebigen Plasmazellen sezerniert werden. Langlebige Plasmazellen tragen zur Chronizität der Erkrankung bei und stellen eine therapeutische Herausforderung dar, da sie durch konventionelle immunsuppressive oder B-Zell-gerichtete Therapien nur unzureichend unterdrückt werden können.

CD38 ist ein Oberflächenprotein, das auf unterschiedlichen Gruppen von Immunzellen entweder konstitutiv oder nach Stimulation exprimiert wird und insbesondere von Plasmazellen stark exprimiert wird. In dieser Arbeit wurde zunächst die Expression von CD38 auf verschiedenen Immunzellen im Blut von 35 SLE Patienten und 20 gesunden Kontrollen (HC) analysiert. Dabei konnte eine signifikant erhöhte CD38 Expression auf verschiedenen Immunzellsubsets von SLE-Patienten nachgewiesen werden, insbesondere bei plasmazytoiden dendritischen Zellen (pDC), Gedächtnis- B- und T-Zellen und auch zirkulierenden Plasmazellen. Zusätzlich wurde der therapeutische Effekt des gegen CD38 gerichteten monoklonalen Antikörpers Daratumumab bei zwei SLE-Patientinnen mit refraktärem, lebensbedrohlichen SLE untersucht. Dabei erhielten die Patientinnen 4 wöchentliche Infusionen mit Daratumumab und wurden für ein Jahr klinisch und immunologisch nachbeobachtet. Die Daratumumab Behandlung resultierte bei beiden Patientinnen in einer klinisch relevanten Verbesserung der Krankheitsaktivität bei insgesamt guter Verträglichkeit. Insbesondere konnte ein günstiger Effekt auf Lupus-Nephritis und autoimmun-hämolytische Anämie beobachtet werden. Die therapeutischen Effekte waren assoziiert mit einem Abfall von Autoantikörpern und Impftitern, was auf eine relevante Depletion von langlebigen Plasmazellen hindeutet. Zusätzlich war eine Reduktion der Typ-I Interferon-Aktivität und eine modulierte Genexpression von Gedächtnis T-Zellen nachweisbar. Die Depletion von autoreaktiven Plasmazellen ist nicht nur beim SLE ein wichtiges Therapiekonzept – so berichten wir zusätzlich über die Behandlung eines Patienten mit einer therapierefraktären Anti-CASPR2-Antikörper vermittelten Enzephalitis mit Daratumumab. Auch bei diesem Patienten kam es unter der Therapie zu klinischer Besserung der neurologischen Symptomatik und Reduktion der Autoantikörper. Allerdings entwickelte dieser Patient in der Rehabilitation eine Sepsis und verstarb wenig später an der Infektion.

Insgesamt weisen diese Daten auf ein hohes Potenzial einer gegen CD38 gerichteten Therapie bei SLE, was möglicherweise auch auf andere Autoantikörper-vermittelte

Erkrankungen übertragbar ist. Allerdings muss die Effektivität und Sicherheit von Daratumumab in diesen Erkrankungen in klinischen, kontrollierten Studien untersucht werden.

1.2. Abstract

Systemic Lupus erythematosus (SLE) is a rheumatologic autoimmune disease, the pathogenesis and aetiology of which are only partially understood. One important aspect of the mechanisms of disease is the generation and maintenance of short- and long-lived, autoreactive plasma cells, which produce autoantibodies. Long-lived plasma cells with their continuous secretion of autoantibodies contribute to the chronicity of the disease and are not sufficiently targeted by conventional immunosuppressants or B cell targeted therapies.

CD38 is a surface protein that is expressed either constitutionally or upon stimulation on a host of different immune cell subsets and that is particularly highly expressed on plasma cells. In this work, we examined the expression of CD38 on different immune cell subsets in the peripheral blood of 35 SLE patients and 20 healthy controls. We were able to show that the expression of CD38 is increased on various subsets of peripheral blood leukocytes of SLE patients, such as plasmacytoid dendritic cells, memory B and T cells, as well as plasma blasts was increased.

Additionally, we investigated the therapeutic effects of the anti-CD38 monoclonal antibody Daratumumab in two SLE patients with refractory, life-threatening disease with the anti-CD38 monoclonal antibody Daratumumab. The patients received four weekly infusions of Daratumumab and were followed up clinically and immunologically for one year. The treatment of two SLE patients with Daratumumab was, apart from the expected hypogammaglobulinemia, safe and induced a clinical and immunological improvement of the disease that was sustained after one year of follow-up. Especially, amelioration of lupus nephritis and autoimmune haemolytic anaemia were observed. The therapeutic effects were associated with a decline in the titres of both autoantibodies and vaccine-induced protective antibodies declined, indicating a depletion of (long-lived) antibody-secreting cells. Additionally type 1 interferon activity and single-cell transcription analysis of memory T cells showed a downregulation of T cell transcripts associated with chronic T cell activation.

The depletion of autoreactive plasma cells could also be an important framework work the treatment of other autoimmune diseases – here, we additionally report the treatment of a patient with refractory anti-CASPR2 antibody mediated encephalitis with daratumumab. Similar to the SLE patients, the treatment resulted in clinical improvement of the neurological manifestations as well as a reduction in autoantibody titres. However, this patient developed septicemia after discharge into a neurological rehabilitation centre and subsequently died.

Our results indicate that a CD38-target therapy is a modality that has large potential for the treatment of SLE and likely also many other autoantibody-mediated diseases. The safety and

efficacy of Daratumumab for these diseases, however, will need to be investigated in clinical-controlled trials

2. Introduction

2.1. Classification and epidemiology of SLE

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that is most prevalent in younger women.[1] It is characterized by a multitude of clinical manifestations. From the eponymous butterfly rash and typical UV light sensitive dermatitis to fulminant organ failure, SLE can present with inflammation of almost any tissue and organ. Apart from inflammation of the skin, the most commonly affected organs include the joints, serous membranes, kidney and blood.[2] Serologically, autoantibodies against nuclear antigens (ANAs) are found in almost all SLE patients and while autoantibodies against double-stranded DNA (dsDNA) are found only in approximately 70% of patients[3], they aid the diagnosis due to their high specificity. The disease prevalence ranges from 20 to 150 per 100,000[4], with wide geographical variability, mostly likely due to ethnic differences, methods of case identification and access to specialty care.[5] While some progress has been made in the treatment of SLE, this population of mostly younger women still has a 2.4 fold increase in mortality compared to the general population[6] and better treatment modalities are urgently needed.

A joint European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) initiative recently updated the classification criteria of SLE, aiming to identify SLE as early as possible, while excluding lupus mimicker.[7] The criteria include positivity for antinuclear antibodies as entry criterion and require a total score of 10 or more to classify SLE and provided a sensitivity of 96.1% and specificity of 93.4%.[7]

2.1. Systemic Lupus Erythematosus –– Current understanding of aetiology and pathogenesis

The aetiology of SLE is complex and incompletely understood. As for most autoimmune diseases, both genetic and environmental influences contribute to SLE. Monozygotic twin studies showed approximately 25-50% concordance[8,9] and genome-wide association studies (GWAS) identified many risk loci, although the attributable risk per locus is generally rather low. [10] Loci typically associated with SLE risk include genes involved in the clearance of dead cells and extracellular DNA such as the NADPH oxidase, genes of the complement system, major histocompatibility complex (especially *HLA-DRB1*) and genes that are in nucleic acid sensing and type 1 interferon signalling. [11]

As approximately 90% of SLE patients are female and most are diagnosed at “childbearing age”,[1] hormones seem to play an important role in the pathogenesis of SLE. Estrogens as well as other steroid hormones have been shown to exert direct immunomodulatory effects; for example estradiol was shown to increase type I interferon production.[12] Additionally,

environmental factors such as ultraviolet light, cigarette smoking and viral infections modify the risk of SLE. [2]

Genetic and environmental factors lead to a reduced capacity for the clearance of nuclear antigens in SLE patients;[13] apoptotic bodies circulate for a longer time, undergo secondary necrosis and nuclear antigens are thus liberated.[14] The production of neutrophil extracellular traps (NETs) is increased in SLE patients and NET clearance is reduced due to reduced DNAse activity in the serum. [15,16] In the process of NET generation, nuclear antigens are modified which increases their potential to break self-tolerance. [17]

The presence of free nucleic acids in circulation also mimics the presence of viruses and thus induces anti-viral immunity: nucleic acids engages toll-like receptors (TLR) 7 and 9, which activates immune cells like monocytes, B cells and plasmacytoid dendritic cells (pDCs).[18] Plasmacytoid dendritic cells are the main producers of type I interferons, the hallmark cytokines in the SLE pathogenesis, which likely directly cause constitutional symptoms such as fatigue and malaise and correlate with disease activity.[19]

Complexes of protein and nucleic acid are also taken up by antigen-presenting cells, which stimulate antigen-specific T cells. Within the lymph node, stimulated T follicular helper cells then provide help to B cells with an affinity against the autoantigens. In a process called affinity maturation, B cells introduce random mutations into their B cell receptors and are selected for higher binding strength to the antigen. Consequently, these antigen-experienced B cells can differentiate into memory B cells and long-lived plasma cells, leading to long-lived memory against the autoantigens. Autoreactive B cells are common, also in healthy people, [20] but usually do not receive T cell help and are thus not able to undergo affinity maturation or differentiate into long-lived memory cells.

The germinal centre reaction has inbuilt control mechanisms against the generation of autoreactive B cells and it is not entirely clear how these mechanisms are evaded in SLE.[21] One observation that might explain this is the presence of so-called “tertiary lymphoid structures” (TLS), aggregates of lymphocytes that are found not within lymphoid organs like the spleen and lymph nodes, but within the inflamed organs themselves.[22] TLS have been observed in many autoimmune diseases, for example in the meninges in multiple sclerosis [23] or the synovium in rheumatoid arthritis [24] and they have also been found in different organs in SLE. [25,26] Affinity maturation has been shown to occur also in TLS, however they lack the organized structure of lymph nodes that is necessary for the mechanisms protecting against the generation of autoreactive B cells. TLS could thus be a location where tolerance break occurs in SLE. [22]

As a consequence of T cell help, antigen-specific memory B cells and plasma cells are generated and can then seed dedicated survival niches in the bone marrow as well as the inflamed organs themselves. They continuously produce antibodies against nuclear

autoantigens and thereby contribute to the chronicity of the disease. As SLE patients have deficient clearance of apoptotic cells, NETs and other nuclear detritus, autoantibodies have ample binding opportunities. Conglomerates of nuclear antigens and autoantibodies form circulating immune complexes that deposit in organs like the skin and the kidney and induce an innate immune response.[2] Antibodies against nuclear antigens are often detected years before the onset of clinical disease [27] and it is not entirely clear, which mechanisms lead to the pathogenicity of autoantibodies. Glycosylations of the Fab and Fc fragments of the antibodies can modulate their inflammatory potential and have been shown to contribute to the switch from asymptomatic autoantibodies to clinical disease in rheumatoid arthritis. [28] Furthermore, additional epitopes are targeted by autoantibodies over time ("epitope spread") – potentially spreading to more pathogenic epitopes with time. Mouse experiments clearly indicate a direct pathogenic role for plasma cells and their antibodies as the transfer of plasma cells from a lupus mouse model to an unaffected mouse induced lupus-like manifestations. [29]

2.2. Targeting immunological memory in Systemic Lupus erythematosus

As illustrated by the importance of autoantibodies in the pathogenesis, antibody-producing cells such as plasma cells are an attractive target for the treatment of SLE, however the depletion of these long-lived cells is a therapeutic challenge.[29–31] As they do not proliferate, they are not targeted by chemotherapeutic agents such as cyclophosphamide and as they mostly do not express CD20, they evade the B cell depleting effect of rituximab and other anti-CD20 monoclonal antibodies.[32] The anti-BAFF (B Cell activating factor) antibody belimumab inhibits an important differentiation signal for B cells to become plasma cells, however it does not efficiently deplete long-lived plasma cells. [33]

Previous work investigating immunoablation followed by autologous stem cell transplantation (ASCT) as a treatment for SLE demonstrated that the ablation of the immunological memory with high dose chemotherapy and anti-thymocyte globulin (ATG) lead to a depletion of bone marrow plasma cells and induced treatment-free clinical and immunological remission in a subset of patients.[34] However, this "immune reset" may be associated with considerable morbidity and mortality which limits its use to the most severe SLE cases.

The proteasome inhibitor bortezomib has also been investigated in SLE and other autoantibody-mediated diseases. [35,36] Originally used in multiple myeloma, a malignant disease of plasma cells, it was able to deplete autoreactive plasma cells and decrease SLE disease activity. However, the plasma cells relatively quickly regenerated from autoreactive plasmablasts and Bortezomib, too, was associated with significant toxicities such as peripheral neuropathy and gastrointestinal symptoms, limiting its use.

While the ideal method for the depletion of humoral immune memory has yet to be identified, a new treatment modality for an intervention that can deplete plasma cells without significant

side-effects could advance the treatment of many diseases. [30] The promise of an ablation of the established humoral immune memory is to achieve a long-lasting remission of disease activity without continued immunosuppressive therapy. The experiences with ASCT in SLE and other autoimmune diseases like multiple sclerosis and ANCA-associated vasculitis have shown that this is possible and novel mechanisms to target the pathogenic immunological memory can hopefully achieve this goal with a more favourable side effect profile.[37] Potential targets for plasma cell include survival signals such as APRIL (A proliferation-inducing ligand) and integrin interactions (via VLA-4 and LFA-1), the chemokine receptor CXCR4 that is important for the homing and retention of plasma cells to their niches, or surface proteins that are highly expressed on plasma cells such as CD138, SLAMF7 (CD319) or CD38.[30]

2.3. CD38 – A multifunctional surface glycoprotein

CD38 is a surface glycoprotein that is expressed on a variety of cells of the immune system but also non-immune cells. Initially it was described as a marker of activation on lymphocytes.[38] It functions both as a receptor and a multifunctional enzyme. As a receptor, it binds to CD31 (Platelet endothelial cell adhesion molecule, PECAM-1), which is expressed on endothelial cells and platelets. The CD38-CD31 interaction mediates the adhesion of CD38 expressing leukocytes on endothelial cells, while at the same time, ligation of CD38 induces the production of pro-inflammatory cytokines, like IL-6 or IFNγ. [39,40] As an enzyme, it catalyses the degradation of NAD+ (Nicotinamide adenine dinucleotide) and synthesis and hydrolysis of cyclic ADP ribose in the extracellular environment. [39] CD38 mediated NAD+ degradation also seems to play a role in metabolic aging. CD38 knockout (KO) mice are unable to mount an adaptive immune response, primarily because CD38 deficient dendritic cells are unable to follow cytokine gradients into the inflamed tissue and into the lymph node to start the immune response. [41,42]

Some previous works have investigated the expression of CD38 on leukocytes in SLE. Pavón et al found an increase in CD38 on T cells in SLE patients as well as autoantibodies against CD38 that showed a negative correlation with disease activity. [43] Subsequent work found that CD38 positive $CD8^+$ T cells have decreased cytotoxic function and their frequency correlated with infections in SLE patients. [44] Additionally, $CD16^+$ non-classical monocytes had increased CD38 expression in a study investigating the CD38 expression of monocyte subsets in SLE. [45]

CD38 is highly expressed on plasma cells and specifically also on malignant cells in multiple myeloma. [46] Thus, CD38-targeting therapeutics have been developed and specifically the anti-CD38 monoclonal antibody daratumumab has been successfully investigated for its efficacy in the treatment of multiple myeloma. [47,48] The rationale for this study has thus been that, especially due to the availability of a licensed drug, CD38 is an attractive target to deplete

autoreactive plasma cells in patients with SLE and other autoantibody mediated diseases. As a first step, we investigated the differential expression of CD38 on leukocyte subsets in SLE in order to better understand potential pleiotropic effects of daratumumab in SLE. [49] Consequently we evaluated the therapeutic effect of daratumumab in two SLE patients and one patient with anti-CASPR2 antibody-mediated encephalitis, including characterization of clinical, serologic and immunologic changes upon treatment.[50,51]

3. Methods

3.1. Cohort Description

We analysed the expression of CD38 on common lymphocyte subsets on peripheral blood mononuclear cells (PBMCs) from 35 SLE patients and 20 healthy controls. Additionally, paired blood and urine samples of 9 patients with lupus nephritis were analysed. The median age was 40.0 for the SLE patients (range 20-64), 27.5 for the healthy controls (range 22-48) and 37.0 for the lupus nephritis patients (range 18-19). 92.3% of the SLE patients and 100% of the lupus nephritis patients were female, as well as 60.0% of the healthy controls. The SLE patients had a median disease history of 14 years (range 1-50 years) and a median SLEDAI-2K disease activity score of 4 (range 0-14). All patients received some immunosuppressive therapy, with glucocorticoid treatment (32/35) and hydroxychloroquine (25/35) being the most commonly used agents. The median prednisolone dose was 5.0mg daily (range 0-25). All patients provided written informed consent and the study was approved by the ethics committee of the Charité Universitätsmedizin Berlin (EA1/104/17).

3.2. Isolation of Peripheral blood mononuclear cell (PBMC)

The peripheral blood mononuclear cells (PBMCs) are a subset of the leukocytes in the blood, they contain mostly T and B lymphocytes, NK cells, monocytes, and dendritic cells. In order to analyse these subpopulations, they have to be separated from the most prevalent corpuscular elements in the blood: neutrophilic granulocytes and erythrocytes. This is achieved by density-gradient separation, which uses the fact that neutrophils and erythrocytes both have slightly higher density than the PBMCs. To this end, 20ml of heparinized blood was mixed with 15ml phosphate buffered saline (PBS) with 0.3% bovine serum albumin (BSA) and carefully layered onto 15ml Ficoll®-Paque Premium (Sigma Aldrich) in a 50ml Falcon tube (Sigma Aldrich). It is centrifuged at 400g for 20 minutes at room temperature and without break. Consequently, the PBMCs are seen as a thin white layer in the middle of the tube, which can then be collected with a pipette. The PBMCs are washed twice by adding PBS + BSA to a volume of 50ml, centrifuging for 10 minutes at 300g and 4°C and discarding the supernatant.

3.3. Flow Cytometry

For flow cytometry analysis, the cells of interest are labelled with antibodies that are coupled to a range of fluorochromes with different excitation and emission characteristics. The cells are then acquired on a flow cytometer which uses a fluidics system to form the sample into a stream of individual cells that is moved through multiple laser beams.[52] Scattered light from the laser impulses is used to estimate cell size and granularity, while fluorescence in response to laser excitation is used to evaluate the presence of certain antigens on the cells. [52] To

prepare PBMCs for cytometric analysis, approximately 10^6 cells and the antibodies of interest are to a total volume of 100µl in phosphate buffered saline (PBS). For surface antigens, the mix is incubated for 15 minutes in the dark at 4°C. Staining of chemokine receptors, especially CCR7 (CD197), is more efficient at 37°C. Afterwards, cells are washed in 1ml PBS and resuspended to a volume of approximately 300µl for acquisition.
For the evaluation of intracellular antigens such as cytokines or transcription factors, the cells have to be fixated and permeabilized prior to staining. To this end, cells are placed in the eBioscience FoxP3 staining buffer (ThermoFisher) according to the manufacturer's protocol. The incubation period for staining is increased to 30 minutes for the staining for intracellular antigens.

3.4. Single-Cell transcriptome and T cell receptor sequencing

Recent technological advances allow for the sequencing of transcriptomes of thousands of individual cells.[53] For this, stained cells as indicated above and FACS sorted at least 10'000 $CD3^+$ living memory T cells (not $CD45RA^+CCR7^+$) from patient 1. At baseline, we additionally sorted the cells according to CD38 surface expression. Follow-up sequencing was performed on days 0, 41 and 74. The sorted T cells were counted and adjusted to a concentration of 1'000 cells/µl in PBS with bovine serum albumin. The cells were then applied to the 10X Genomics platform with the Chromium Single Cell 5' Library & Gel Bead Kit (10X Genomics) according to manufacturer's instructions. After 16 cycles of PCR of amplified cDNA, the sample was used for library preparation for gene expression and T cell receptor sequencing (using the Chromium Single Cell V(D)J Enrichment Kit for Human T cells (10X Genomics). The samples were sequenced on a NextSeq500 machine (Illumina). This work and the subsequent bioinformatic analysis of the data was done in cooperation with Gitta Heinz, Frederik Heinrich, Pawel Durek and Mir-Farzin Mashreghi, Deutsches Rheuma-Forschungszentrum Berlin.

3.5. Bioinformatic analysis of Single-Cell transcriptome data

After alignment of reads and deconvolution of the data using the cell-range software (10X Genomics), subsequent analysis was performed using the Seurat package Version 3 in R. [54,55] Cells with a high percentage of mitochondrial reads were excluded and the cells from all time points plotted as a UMAP (Uniform Manifold Approximation and Projection). We manually separated the sequenced memory T cells into three clusters (conventional $CD4^+$ T cells, regulatory $CD4^+$ T cells and $CD8^+$ T cells) according to expression of key marker genes (CD4, CD8, IKZF2 (which encodes for Helios) and FOXP3) and proximity in the UMAP. Consequent analyses were performed on each subset separately, mainly the analysis of differentially expressed genes between the different time points. Additionally, differential gene expression

analysis was performed focussing specifically on $CD8^+$ memory T cells with a T cell receptor that was within the 10 most frequent TCR clones shared between all timepoints.

3.6. Treatment with daratumumab and follow-up

Daratumumab is a monoclonal IgG1 kappa antibody against CD38 that is licensed for use in multiple myeloma.[47] Two patients with long-standing, refractory and life-threatening SLE gave informed written consent to an off-label treatment with daratumumab. They received intravenous daratumumab infusions of 16mg/kg body weight once weekly for four weeks in an inpatient setting. To prevent infusion reactions, patients were pre-treated with 20mg dexamethasone and dimetindene intravenously. For the first three months, patients received antiviral and -fungal prophylaxis with acyclovir (200mg, daily) and trimethoprim/sulfamethoxazole (960 mg, three times a week). Baseline immunosuppressive therapy was continued and on day 120 both patients were started on belimumab (an anti-BAFF antibody, weekly 200mg subcutaneous injections).

We followed both patients at least monthly for a duration of 12 and 11 months, respectively. Follow-up investigations included clinical evaluation for disease activity and potential side effects, laboratory analysis of established markers of disease activity such as levels of complement factors, autoantibody titres and SIGLEC1 on monocytes (a surrogate marker of type I interferon activity).[19,56] The immunological follow-up included the analysis of leukocyte subsets and their CD38 expression with flow and mass cytometry as well as the analysis of sorted memory T cells via single-cell transcriptome and T cell receptor analysis.

4. Essential Results

4.1. CD38 expression is elevated on peripheral blood leukocyte subsets in SLE

We investigated the expression of CD38 on the surface of peripheral blood mononuclear cells (PBMCs) in patients with SLE compared to a cohort of healthy controls. CD38 was expressed on all major PBMC subsets. In the canonical definition of B-cell subsets, CD38 is used to identify "Transitional B-cells" ($CD24^{high}CD38^{high}$) as well as plasmablasts ($CD19^{low}CD24^{low}CD27^{high}CD38^{high}$). Excluding these subgroups, total "mature B-cell" MFI (median fluorescence intensity) was still increased in SLE patients compared to healthy controls. Defining plasmablasts independent of CD38 expression as $CD19^{low}CD27^{high}$ showed that also plasmablast CD38 expression was significantly increased in SLE patients. Additionally, CD38 MFI was higher on SLE plasmacytoid dendritic cells ($CD123^{+}$HLA-$DR^{+}Lineage^{-}$) compared to healthy controls. Monocytes ($CD14^{+}$), myeloid dendritic cells ($CD11c^{+}$HLA-$DR^{+}Lineage^{-}$) and natural killer (NK) cells ($CD56^{+}CD3^{-}$) all showed a trend towards higher expression of CD38; however, these differences did not reach statistical significance.

On naive ($CD45RA^{+}CCR7^{+}$) T cells, CD38 expression is uniformly low to intermediate and in healthy controls, only a small fraction of memory T cells (not expressing both CD45RA and CCR7) expresses CD38. In SLE patients however, the proportion of $CD38^{+}$ memory T cells was higher both in $CD4^{+}$ and $CD8^{+}$ T cells. To investigate whether these cells are also found in the inflamed kidney, we measured the CD38 expression on memory T cells in paired blood and urine samples of lupus nephritis. In addition, these cells showed high expression of the chemokine receptor CXCR3 that is required for T cells to enter inflamed tissue. [57]

In summary, CD38 expression is not only high on plasma cells, but also increased on plasmacytoid dendritic cells and memory T cells in SLE. As these three cell types are all integral components of our current understanding of SLE pathogenesis, they provide an additional rationale for a CD38-targeted treatment in SLE beyond the depletion of auto-antibody-secreting plasma cells.

4.2. Treatment of two patients with the CD38 antibody daratumumab

Two patients with long-standing, treatment refractory, and life- and organ threatening SLE received 4 infusions of 16mg/kg body weight daratumumab after informed consent was obtained. After 120 days, additionally the anti-BAFF antibody belimumab was started.

Patient 1 was a 50-year-old woman with a 17-year history of refractory SLE. She had previously been treated with cyclophosphamide, mycophenolate mofetil, ciclosporin A, azathioprine, bortezomib, prednisolone and hydroxychloroquine. At baseline, she showed clinical and serological activity with WHO class III+V lupus nephritis, dermatitis, pericarditis

and arthritis as well as elevated anti double-stranded DNA antibody levels and decreased complement factor levels. Her baseline immunosuppressive therapy of 20mg of prednisolone and 2000mg of mycophenolate mofetil daily was continued.

Patient 2 was a 32-year-old woman with a 16-year history of SLE. She had previously been treated with cyclophosphamide, mycophenolate mofetil, ciclosporin A, bortezomib, intravenous immunoglobulins, belimumab, rituximab, azathioprine, prednisolone and hydroxychloroquine. At baseline, she had immune-mediated haemolytic anaemia and thrombocytopenia as well as arthritis, oral ulcerations, arthritis, alopecia and vasculitis skin lesions. Historically she had also had liver and CNS manifestations. Her baseline treatment was 10mg of prednisolone daily.

Both patients had prompt and sustained clinical improvements at the time of the fourth Daratumumab infusion. The SLEDAI-2K (Systemic Lupus erythematosus disease activity index 2000) decreased from 22 and 21 to 15 at the time of the fourth infusion and decreased further to 6 and 4 respectively at the time of the last follow up (360 and 330 days after the first infusion). The proteinuria in patient 1 decreased from 6362 mg/g creatinine to 1197 mg/g creatinine, her cardiac output function improved, the pericardial effusion, arthritis and skin rash resolved, even though her daily prednisolone dose was gradually decreased to 4mg/day. In patient 2, the haemoglobin and thrombocyte levels increased and the direct antigen test was negative on multiple occasions. Her vasculitic skin lesions, arthritis and skin manifestations resolved.

The daratumumab infusions were generally well-tolerated. In patient 1, serum immunoglobulin levels decreased below 400 mg/dl (normal range 700-1600 mg/dl) early after daratumumab infusions and she received two infusions of 30g intravenous immunoglobulins. Patient 2 had an uncomplicated upper respiratory tract infection without need for specific treatment around day 90. No other adverse events were noted.

In summary, daratumumab treatment with four infusions of 16mg/kg body weight led to sustained clinical responses in two SLE patients with highly active and refractory disease and the treatment was well-tolerated.

4.3. Immunological effects of Daratumumab treatment

To investigate the immunological effects of daratumumab, we combined flow cytometry and mass cytometry approaches for immunophenotyping of peripheral blood leukocytes. The levels of all major leukocyte subsets in the blood remained stable over the follow-up period, except for a gradual and sustained decrease in $CD19^+$ B cell counts. Additionally, the fraction of $CD19^{low}CD27^{high}$ plasmablasts decreased early after the treatment but overall underwent strong fluctuations, while the fraction of IgD^+CD27^+ naive B cells gradually decreased over the follow-up period in both patients. $CD3^-CD56^+$ NK cell and $CD123^+HLA\text{-}DR^+$ $lineage^-$ pDC

counts dropped early after treatment but recovered to baseline levels between 90 and 150 days after the initiation of Daratumumab treatment. SIGLEC1 expression on monocytes, a surrogate parameter for type 1 interferon activity and disease activity in SLE, decreased directly after completion of the Daratumumab infusions and remained low over the follow-up period.

To assess the capacity of daratumumab to deplete $CD38^+$ memory T cells, we evaluated absolute T cell counts, the fraction of memory T cells and $CD38^+$ memory T cells. Daratumumab treatment abrogates the binding of conventional anti-CD38 antibodies for flow cytometric analysis, thus, we used a polyclonal anti-CD38 antibody that is marketed specifically for the detection of CD38 after anti-CD38 targeted therapy. While CD38 expression on T cells was almost completely abrogated directly after daratumumab treatment, total T cell counts as well as memory T cell counts remained unchanged. These results could either indicate that $CD38^+$ memory T cells were depleted and replaced by other T cells or that the $CD38^+$ cells persisted but lost the surface expression of CD38. In order to differentiate between these competing hypotheses, we turned to single-cell RNA and T cell receptor sequencing.

4.4 Effects of Daratumumab on $CD38^+$ Memory T cells

As outlined above, conventional, protein-based immune-monitoring did not reveal whether $CD38^+$ memory T cells were depleted by daratumumab treatment or if they lost CD38 surface expression. Single-cell T cell receptor sequencing can potentially answer this question, as the T cell receptor (TCR) can serve as an endogenous barcode to follow clones of T cells over time. We sequenced single-cell transcriptomes and TCRs of patient 1 at days 0, 41, and 74 and clustered the cells into $CD4^+$ conventional T cells, $CD4^+$ regulatory T cells and $CD8^+$ T cells. As expected from the flow cytometric results, at baseline most CD38 expressing cells were found within the $CD8^+$ T cell cluster. Within the $CD8^+$ T cells, the sequenced TCRs showed remarkable oligoclonality. Especially within the $CD38^+CD8^+$ T cells, 54% of T cells expressed one of the ten most common TCRs. The frequency of these TCRs within the $CD8^+$ did not change during the follow-up period, indicating that $CD38^+$ memory T cells were not depleted by daratumumab.

Next, we investigated the transcriptional profile of these TCR-barcoded $CD8^+$ T cells. After daratumumab treatment, $CD8^+$ T cells had lower mean expression of genes associated with interferon exposure (like *IRF7, IFI44L, ISG15*), which is in line with our observations of the interferon-surrogate parameter SIGLEC1 on monocytes. Additionally, the expression of genes associated with T cell activation (like *DUSP2, CD40LG, CD69*) and also CD38 was decreased after daratumumab treatment in both memory $CD4^+$ and $CD8^+$ T cells. Interestingly, the expression of *GZMA, GZMB, NKG7* and other genes associated with cytotoxic function in T cells increased after daratumumab treatment. This is in line with a recent report, describing a

decreased cytotoxic potential in $CD38^+$ $CD8^+$ T cells, which was associated with an increased risk of infection in these patients.[44] Our data could indicate that the CD38-targeted treatment with daratumumab led to a normalization of the defective cytotoxic capacity of these cells.

4.5 Daratumumab in autoantibody-mediated encephalitis

In addition to the use of daratumumab in SLE, we recently treated a patient with an anti-CASPR2 autoimmune encephalitis,[51] a disease in which autoantibodies against the synaptic protein Contactin-associated protein-like 2 (CASPR2) induces brain inflammation and neuronal dysfunction.[58] Similar to SLE, long-lived plasma cells are believed to be important producers of pathogenic antibodies in this disease and thus represent a potential target for daratumumab.[59]

A 60-year-old male patient with a two-year history of anti-CASPR2 encephalitis and a squamous-cell carcinoma of the tongue clinically deteriorated after a steroid taper and concomitant soft-tissue infection. He developed a central breathing disorder and aggressive behavioural changes, requiring sedation and mechanical ventilation. Anti-CASPR2 antibody levels were detected >1:10'000 both in the cerebrospinal fluid (CSF) and the serum. Treatment with methylprednisolone, rituximab, bortezomib and immunoadsorption was initiated but he remained refractory to these treatments over five months. Evidence of tumour recurrence was found neither clinically nor in PET or MRI imaging. He then received a total of 13 cycles of 16mg/kg body weight daratumumab, first in weekly intervals and after the 8^{th} infusion in biweekly intervals. After 8 cycles of daratumumab, the patient showed signs of clinical improvement, allowing the discontinuation of sedation and weaning from mechanical ventilation. His autoantibodies in the serum were reduced by multiple titre levels, while they only changed from >1:10'000 to 1:10'000 in the CSF.

We observed immunologic changes similar to the treated SLE patients, with a depletion of NK cells, a reduction in total and vaccine-specific immunoglobulin levels and a decrease in HLA-DR^+ and $Ki67^+$ memory T cells, as markers of a reduced activation of $CD4^+$ and $CD8^+$ T cells. Four months after the initiation of daratumumab treatment, the patient was transferred to a neurological rehabilitation centre, where he died of sepsis.

5. Discussion and outlook

5.1. Significance of results in SLE

In this work, we investigated the expression of CD38 on leukocytes of SLE patients and report the treatment of two SLE patients with the anti-CD38 antibody daratumumab. Both patients had significant disease activity despite best available treatment and after failure of other investigational treatment, a situation that is associated with high risks of morbidity and mortality and where only few treatment options are available. [60] In these two patients, daratumumab was both safe and efficacious, inducing long-lasting clinical remissions.

As CD38 is expressed on many different subsets of leukocytes and, as demonstrated here, also increased on a large number of disease-relevant leukocyte populations, the beneficial effect of daratumumab is likely due to pleiotropic effects. Apart from the plasma cell depletion that was induced by the anti-CD38 treatment, we found evidence of a depleting effect of daratumumab on pDCs, NK cells and for a modulatory effect on $CD38^{+}$ memory T cells. Further work is required to characterize the effects of daratumumab and other anti-CD38 antibodies on the different cells of the immune system in SLE patients.

Notably, the increased expression of CD38 on peripheral blood leukocytes was not homogenous among all SLE patients investigated. This notion, together with the relatively high intraindividual correlation of the expression of CD38, could indicate that there is a $CD38^{high}$ subgroup of SLE patients. This subset could also feature specific patterns of clinical manifestations as well as an increased response upon anti-CD38 targeted treatment. Nevertheless, the patients in our study were chosen for daratumumab for ethical reasons, as they had insufficient response to established SLE therapies. For future studies, the ideal target population for anti-CD38 treatment remains to be defined. Based on the assumption that long-lived plasma cells play a pathogenic role in the diseases, ideal candidates for daratumumab are those with persistent serologic activity despite conventional therapies. Since pathogenic immune memory accumulate over years, daratumumab may provide higher efficacy in patients with long-lasting disease with high antibody titres. Nevertheless, to prevent accumulating organ damage, a “hit hard and early” approach with an anti-CD38 antibody could potentially lead to long-lasting remissions, comparable to the use of the anti-CD20 antibody rituximab in early arthritis.[61]

The dose of four daratumumab infusions of 16mg/kg body weight was chosen based on the dosing scheme for the treatment of multiple myeloma, but it is not clear whether the dose needs to be adjusted for use in SLE. Based on the observed reduction of autoantibody titres by 30-50%, it can be speculated that continued daratumumab treatment may lead to an even stronger reduction in autoimmunity and potentially superior clinical efficacy. However, a higher dosage would have likely been associated with more severe side effects, especially more

severe hypogammaglobulinemia. As the burden of autoreactive plasma cells can be estimated by the titre levels of autoantibodies, individualised dosing according to antibody titres could be tried in the future. Additionally, the anti-BAFF (B cell activating factor) antibody belimumab was started as weekly subcutaneous injections with the intention of inhibiting the generation of new autoreactive antibody-secreting cells. The relative contribution of this drug in the sustained partial remission of the patient remains unclear and will require further investigation. The efficacy of daratumumab for SLE as well as the ideal dose and target population will have to be established in a randomized and controlled clinical trial.

5.2 Significance for other autoimmune diseases

While our results are encouraging as novel therapeutic approach in SLE, our results from the treatment of a patient with an anti CASPR2 antibody-mediated autoimmune encephalitis highlights that the concept of long-lived plasma cells as drivers of chronic autoimmunity is valid for many different autoimmune diseases and that CD38-targeted therapies could fill a need for additional effective treatments in these disorders. The report, however, also highlights the potential risks that are associated with daratumumab treatment. The CASPR2-patient was heavily pre-treated with immunosuppressive drugs and received 13 infusions of daratumumab (compared to the 4 infusions, the SLE patients received). This combination resulted in clinical improvement but also heavy immunosuppression. Nevertheless, the prognosis of autoantibody encephalitis that is treatment-refractory for multiple months is bleak, so that daratumumab may still be considered as a rescue therapy in these patients.

Further work is needed to evaluate the safety and efficacy as well as the right dosing of Daratumumab or other anti-CD38 agents in auto-antibody mediated disease, of which there are plenty. While a depletion of plasma cells using an anti-CD38 antibody was successful in this study, it also affects the protective functions of humoral immunity by depleting plasma cells induced by vaccinations or previous infections. An ideal treatment would deplete only those plasma cells that produce pathogenic antibodies. This is complicated by the fact that IgG^+ plasma cells do not express significant levels of immunoglobulins on the surface.

A conjugate of an anti-CD138 antibody (targeting plasma cells) and the antigen was able to induce labelling of antigen-specific plasma cells with their secreted immunoglobulin and induced depletion of these cells.[62] This is however only feasible in those diseases where autoantibodies against a single, well defined antigen drive the pathogenesis, such as antibodies against acetylcholinesterase in myasthenia gravis or against phospholipase A2 receptor in primary membranous glomerulonephritis. In other diseases like SLE or idiopathic CD4 lymphopenia[63] , where there are autoantibodies against diverse targets, a specific approach seems less feasible.

In summary, while our results presented in this thesis are a promising start, many more steps are required to offer new safe and effective plasma-cell targeted treatments to patients with SLE and other autoantibody-mediated diseases. We hope that this work and future contributions can help to fill this need and induce long-standing, treatment-free remission in different autoimmune diseases.

Bibliography

1. Rees, F., Doherty, M., Grainge, M. J., Lanyon, P. & Zhang, W. The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies. *Rheumatol. Oxf. Engl.* **56**, 1945–1961 (2017).
2. Tsokos, G. C. Systemic Lupus Erythematosus. *N. Engl. J. Med.* 12 (2011).
3. Haugbro, K., Nossent, J. C., Winkler, T., Figenschau, Y. & Rekvig, O. P. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann. Rheum. Dis.* **63**, 386–394 (2004).
4. Lewis, M. J. & Jawad, A. S. The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus. *Rheumatology* kew399 (2016) doi:10.1093/rheumatology/kew399.
5. Carter, E. E., Barr, S. G. & Clarke, A. E. The global burden of SLE: prevalence, health disparities and socioeconomic impact. *Nat. Rev. Rheumatol.* **12**, 605–620 (2016).
6. Bernatsky, S., Boivin, J.F., Joseph, L., Manzi, S., Ginzler, E., Gladman, D.D., Urowitz, M., Fortin, P.R., Petri, M., Barr, S., Gordon, C., Bae, S.C., Isenberg, D., Zoma, A., Aranow, C., Dooley, M.A., Nived, O., Sturfelt, G., Steinsson, K., Alarcón, G., Senécal, J.L., Zummer, M., Hanly, J., Ensworth, S., Pope, J., Edworthy, S., Rahman, A., Sibley, J., El-Gabalawy, H., McCarthy, T., St Pierre, Y., Clarke, A. & Ramsey-Goldman, R. Mortality in systemic lupus erythematosus. *Arthritis Rheum.* **54**, 2550–2557 (2006).
7. Aringer, M., Costenbader, K., Daikh, D., Brinks, R., Mosca, M., Ramsey-Goldman, R., Smolen, J.S., Wofsy, D., Boumpas, D.T., Kamen, D.L., Jayne, D., Cervera, R., Costedoat-Chalumeau, N., Diamond, B., Gladman, D.D., Hahn, B., Hiepe, F., Jacobsen, S., Khanna, D., Lerstrøm, K., Massarotti, E., McCune, J., Ruiz-Irastorza, G., Sanchez-Guerrero, J., Schneider, M., Urowitz, M., Bertsias, G., Hoyer, B.F., Leuchten, N., Tani, C., Tedeschi, S.K., Touma, Z., Schmajuk, G., Anic, B., Assan, F., Chan, T.M., Clarke, A.E., Crow, M.K., Czirják, L., Doria, A., Graninger, W., Halda-Kiss, B., Hasni, S., Izmirly, P.M., Jung, M., Kumánovics, G., Mariette, X., Padjen, I., Pego-Reigosa, J.M.,

Romero-Diaz, J., Rúa-Figueroa Fernández, Í., Seror, R., Stummvoll, G.H., Tanaka, Y., Tektonidou, M.G., Vasconcelos, C., Vital, E.M., Wallace, D.J., Yavuz, S., Meroni, P.L., Fritzler, M.J., Naden, R., Dörner, T. & Johnson, S.R. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann. Rheum. Dis.* **78**, 1151–1159 (2019).

8. Grennan, D.M., Parfitt, A., Manolios, N., Huang, Q., Hyland, V., Dunckley, H., Doran, T., Gatenby, P. & Badcock, C. Family and twin studies in systemic lupus erythematosus. *Dis. Markers* **13**, 93–98 (1997).
9. Block, S. R., Winfield, J. B., Lockshin, M. D., D'Angelo, W. A. & Christian, C. L. Studies of twins with systemic lupus erythematosus: A review of the literature and presentation of 12 additional sets. *Am. J. Med.* **59**, 533–552 (1975).
10. Cui, Y., Sheng, Y. & Zhang, X. Genetic susceptibility to SLE: recent progress from GWAS. *J. Autoimmun.* **41**, 25–33 (2013).
11. Rullo, O. J. & Tsao, B. P. Recent insights into the genetic basis of systemic lupus erythematosus. *Ann. Rheum. Dis.* **72 Suppl 2**, ii56-61 (2013).
12. Kovats, S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell. Immunol.* **294**, 63–69 (2015).
13. Baumann, I., Kolowos, W., Voll, R.E., Manger, B., Gaipl, U., Neuhuber, W.L., Kirchner, T., Kalden, J.R. & Herrmann, M. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum.* **46**, 191–201 (2002).
14. Muñoz, L.E., Janko, C., Grossmayer, G.E., Frey, B., Voll, R.E., Kern, P., Kalden, J.R., Schett, G., Fietkau, R., Herrmann, M., Gaipl, U.S.Remnants of secondarily necrotic cells fuel inflammation in systemic lupus erythematosus. *Arthritis Rheum.* **60**, 1733–1742 (2009).

15. Martinez-Valle, F., Balada, E., Ordi-Ros, J., Bujan-Rivas, S., Sellas-Fernandez, A. & Vilardell-Tarres, M.DNase 1 activity in patients with systemic lupus erythematosus: relationship with epidemiological, clinical, immunological and therapeutical features. *Lupus* **18**, 418–423 (2009).

16. Yasutomo, K., Horiuchi, T., Kagami, S., Tsukamoto, H., Hashimura, C., Urushihara, M. & Kuroda, Y. Mutation of DNASE1 in people with systemic lupus erythematosus. *Nat. Genet.* **28**, 313–314 (2001).

17. Bouts, Y.M., Wolthuis, D.F., Dirkx, M.F., Pieterse, E., ,Simons E.M., van Boekel, A.M., Dieker, J.W., Apoptosis and NET formation in the pathogenesis of SLE. *Autoimmunity* **45**, 597–601 (2012).

18. Rahman, A. H. & Eisenberg, R. A. The role of toll-like receptors in systemic lupus erythematosus. *Springer Semin. Immunopathol.* **28**, 131–143 (2006).

19. Rose, T., Grützkau, A., Hirseland, H., Huscher, D., Dähnrich, C., Dzionek, A., Ozimkowski, T., Schlumberger, W., Enghard, P., Radbruch, A., Riemekasten, G., Burmester, G.R., Hiepe, F. & Biesen, R. IFNα and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. *Ann. Rheum. Dis.* **72**, 1639–1645 (2013).

20. Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E. & Nussenzweig, M.C. Predominant Autoantibody Production by Early Human B Cell Precursors. *Science* **301**, 1374–1377 (2003).

21. Victora, G. D. & Nussenzweig, M. C. Germinal Centers. *Annu. Rev. Immunol.* **30**, 429–457 (2012).

22. Hutloff, A. T Follicular Helper-Like Cells in Inflamed Non-Lymphoid Tissues. *Front. Immunol.* **9**, (2018).

23. Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E. & Aloisi, F. Detection of Ectopic B-cell Follicles with Germinal Centers in the Meninges of Patients with Secondary Progressive Multiple Sclerosis. *Brain Pathol.* **14**, 164–174 (2004).

24. Timmer, T.C., Baltus, B., Vondenhoff, M., Huizinga, T.W., Tak, P.P., Verweij, C.L., Mebius, R.E. & van der Pouw Kraan, T.C. Inflammation and ectopic lymphoid structures in rheumatoid arthritis

synovial tissues dissected by genomics technology: identification of the interleukin-7 signaling pathway in tissues with lymphoid neogenesis. *Arthritis Rheum.* **56**, 2492–2502 (2007).

25. Stock, A.D., Der, E., Gelb, S., Huang, M., Weidenheim, K., Ben-Zvi, A. & Putterman, C. Tertiary lymphoid structures in the choroid plexus in neuropsychiatric lupus. *JCI Insight* **4**, (2019).
26. Dorraji, S.E., Kanapathippillai, P., Hovd, A.K., Stenersrød, M.R., Horvei, K.D., Ursvik, A., Figenschau, S.L., Thiyagarajan, D., Fenton, C.G., Pedersen, H.L. & Fenton, K.A. Kidney Tertiary Lymphoid Structures in Lupus Nephritis Develop into Large Interconnected Networks and Resemble Lymph Nodes in Gene Signature. *Am. J. Pathol.* **190**, 2203–2225 (2020).
27. Eriksson, C., Kokkonen, H., Johansson, M., Hallmans, G., Wadell, G. & Rantapää-Dahlqvist, S. Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. *Arthritis Res. Ther.* **13**, R30 (2011).
28. Rombouts, Y., Ewing, E., van, d.e., Selman, M.H., Trouw, L.A., Deelder, A.M., Huizinga, T.W., Wuhrer, M., van Schaardenburg, D., Toes, R.E. & Scherer, H.U. Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann. Rheum. Dis.* **74**, 234–241 (2015).
29. Cheng, Q., Mumtaz, I.M., Khodadadi, L., Radbruch, A., Hoyer, B.F. & Hiepe, F. Autoantibodies from long-lived 'memory' plasma cells of NZB/W mice drive immune complex nephritis. *Ann. Rheum. Dis.* **72**, 2011–2017 (2013).
30. Hiepe, F. & Radbruch, A. Plasma cells as an innovative target in autoimmune disease with renal manifestations. *Nat. Rev. Nephrol.* **12**, 232–240 (2016).
31. Maschmeyer, P., Chang, H.D., Cheng, Q., Mashreghi, M.F., Hiepe, F., Alexander, T. & Radbruch, A. Immunological memory in rheumatic inflammation — a roadblock to tolerance induction. *Nat. Rev. Rheumatol.* **17**, 291–305 (2021).
32. Hiepe, F., Dörner, T., Hauser, A.E., Hoyer, B.F., Mei, H. & Radbruch, A. Long-lived autoreactive plasma cells drive persistent autoimmune inflammation. *Nat. Rev. Rheumatol.* **7**, 170–178 (2011).

33. Stohl, W., Hiepe, F., Latinis, K.M., Thomas, M., Scheinberg, M.A., Clarke, A., Aranow, C., Wellborne, F.R., Abud-Mendoza, C., Hough, D.R., Pineda, L., Migone, T.S., Zhong, Z.J., Freimuth, W.W., Chatham, W.W & BLISS-52 Study Group; BLISS-76 Study Group..Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis Rheum.* **64**, 2328–2337 (2012).

34. Alexander, T., Thiel, A., Rosen, O., Massenkeil, G., Sattler, A., Kohler, S., Mei, H., Radtke, H., Gromnica-Ihle, E., Burmester, G.R., Arnold, R., Radbruch, A. & Hiepe, F. Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through de novo generation of a juvenile and tolerant immune system. *Blood* **113**, 214–223 (2009).

35. Alexander, T., Sarfert, R., Klotsche, J., Kühl, A.A., Rubbert-Roth, A., Lorenz, H.M., Rech, J., Hoyer, B.F., Cheng, Q., Waka, A., Taddeo, A., Wiesener, M., Schett, G., Burmester, G.R., Radbruch, A., Hiepe, F. & Voll, R.E. The proteasome inhibitior bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. *Ann. Rheum. Dis.* **74**, 1474–1478 (2015).

36. Scheibe, F., Prüss, H., Mengel, A.M., Kohler, S., Nümann, A., Köhnlein, M., Ruprecht, K., Alexander, T., Hiepe, F. & Meisel, A. Bortezomib for treatment of therapy-refractory anti-NMDA receptor encephalitis. *Neurology* **88**, 366–370 (2017).

37. Alexander, T., Greco, R. & Snowden, J. A. Hematopoietic Stem Cell Transplantation for Autoimmune Disease. *Annu. Rev. Med.* **72**, 215–228 (2021).

38. Deterre, P., Berthelier, V., Bauvois, B., Dalloul, A., Schuber, F. & Lund, F. CD38 in T- and B-Cell Functions. *Hum. CD38 Relat. Mol.* **75**, 146–168 (2000).

39. Hogan, K. A., Chini, C. C. S. & Chini, E. N. The Multi-faceted Ecto-enzyme CD38: Roles in Immunomodulation, Cancer, Aging, and Metabolic Diseases. *Front. Immunol.* **10**, (2019).

40. Deaglio, S., Morra, M., Mallone, R., Ausiello, C.M., Prager, E., Garbarino, G., Dianzani, U., Stockinger, H. & Malavasi, F. Human CD38 (ADP-Ribosyl Cyclase) Is a Counter-Receptor of CD31, an Ig Superfamily Member. *J. Immunol.* **160**, 395–402 (1998).

41. Partida-Sánchez, S., Cockayne, D.A., Monard, S., Jacobson, E.L., Oppenheimer, N., Garvy, B., Kusser, K., Goodrich, S., Howard, M., Harmsen, A., Randall, T.D. & Lund, F.E. Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nat. Med.* **7**, 1209–1216 (2001).

42. Partida-Sánchez, S., Goodrich, S., Kusser, K., Oppenheimer, N., Randall, T.D. & Lund, F.E. Regulation of Dendritic Cell Trafficking by the ADP-Ribosyl Cyclase CD38. *Immunity* **20**, 279–291 (2004).

43. Pavón, E.J., Zumaquero, E., Rosal-Vela, A., Khoo, K.M., Cerezo-Wallis, D., García-Rodríguez, S., Carrascal, M., Abian, J., Graeff, R., Callejas-Rubio, J.L., Ortego-Centeno, N., Malavasi, F., Zubiaur, M. & Sancho, J. Increased CD38 expression in T cells and circulating anti-CD38 IgG autoantibodies differentially correlate with distinct cytokine profiles and disease activity in systemic lupus erythematosus patients. *Cytokine* **62**, 232–243 (2013).

44. Katsuyama, E., Suarez-Fueyo, A., Bradley, S.J., Mizui, M., Marin, A.V., Mulki, L., Krishfield, S., Malavasi, F., Yoon, J., Sui, S.J.H., Kyttaris, V.C. & Tsokos, G.C. The CD38/NAD/SIRTUIN1/EZH2 Axis Mitigates Cytotoxic CD8 T Cell Function and Identifies Patients with SLE Prone to Infections. *Cell Rep.* **30**, 112-123.e4 (2020).

45. Amici, S.A., Young, N.A., Narvaez-Miranda, J., Jablonski, K.A., Arcos, J., Rosas, L., Papenfuss, T.L., Torrelles, J.B., Jarjour, W.N. & Guerau-de-Arellano, M. CD38 Is Robustly Induced in Human Macrophages and Monocytes in Inflammatory Conditions. *Front. Immunol.* **9**, (2018).

46. Krejcik, J., Casneuf, T., Nijhof, I.S., Verbist, B., Bald, J., Plesner, T., Syed, K., Liu, K., van de Donk, N.W., Weiss, B.M., Ahmadi, T., Lokhorst, H.M., Mutis, T. & Sasser, A.K. Daratumumab depletes

CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. *Blood* **128**, 384–394 (2016).

47. Lokhorst, H.M., Plesner, T., Laubach, J.P., Nahi, H., Gimsing, P., Hansson, M., Minnema, M.C., Lassen, U., Krejcik, J., Palumbo, A., van de Donk, N.W., Ahmadi, T., Khan, I., Uhlar, C.M., Wang, J., Sasser, A.K., Losic, N., Lisby, S., Basse, L., Brun, N. & Richardson, P.G.Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. *N. Engl. J. Med.* **373**, 1207–1219 (2015).

48. Facon, T., Kumar, S., Plesner, T., Orlowski, R.Z., Moreau, P., Bahlis, N., Basu, S., Nahi, H., Hulin, C., Quach, H., Goldschmidt, H., O'Dwyer, M., Perrot, A., Venner, C.P., Weisel, K., Mace, J.R., Raje, N., Attal, M., Tiab, M., Macro, M., Frenzel, L., Leleu, X., Ahmadi, T., Chiu, C., Wang, J., Van Rampelbergh, R., Uhlar, C.M., Kobos, R., Qi, M., Usmani, S.Z & MAIA Trial Investigators. Daratumumab plus Lenalidomide and Dexamethasone for Untreated Myeloma. *N. Engl. J. Med.* **380**, 2104–2115 (2019).

49. Burns, M., Ostendorf, L., Biesen, R., Grützkau, A., Hiepe, F., Mei, H.E. & Alexander, T. Dysregulated CD38 Expression on Peripheral Blood Immune Cell Subsets in SLE. *Int. J. Mol. Sci.* **22**, 2424 (2021).

50. Ostendorf, L., Burns, M., Durek, P., Heinz, G.A., Heinrich, F., Garantziotis, P., Enghard, P., Richter, U., Biesen, R., Schneider, U., Knebel, F., Burmester, G., Radbruch, A., Mei, H.E., Mashreghi, M.F., Hiepe, F. & Alexander, T. Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus. *N. Engl. J. Med.* **383**, 1149–1155 (2020).

51. Scheibe, F., Ostendorf, L., Reincke, S.M., Prüss, H., von Brünneck. A.C., Köhnlein, M., Alexander, T., Meisel, C. & Meisel, A.Daratumumab treatment for therapy-refractory anti-CASPR2 encephalitis. *J. Neurol.* (2019) doi:10.1007/s00415-019-09585-6.

52. Cossarizza, A., Chang, H.D., Radbruch, A., Acs, A., Adam, D., Adam-Klages, S., Agace, W.W., Aghaeepour, N., Akdis, M., Allez, M., Almeida, L.N., Alvisi, G., Anderson, G., Andrä, I., Annunziato, F., Anselmo, A., Bacher, P., Baldari, C.T., Bari, S., Barnaba, V., Barros-Martins, J., Battistini, L., Bauer, W., Baumgart, S., Baumgarth, N., Baumjohann, D., Baying, B., Bebawy, M., Becher, B.,

Beisker, W., Benes, V., Beyaert, R., Blanco, A., Boardman, D.A., Bogdan, C., Borger, J.G., Borsellino, G., Boulais, P.E., Bradford, J.A., Brenner, D., Brinkman, R.R., Brooks, A.E.S., Busch, D.H., Büscher, M., Bushnell, T.P., Calzetti, F., Cameron, G., Cammarata, I., Cao, X., Cardell, S.L., Casola, S., Cassatella, M.A., Cavani, A., Celada, A., Chatenoud, L., Chattopadhyay, P.K., Chow, S., Christakou, E., Čičin-Šain, L., Clerici, M., Colombo, F.S., Cook, L., Cooke, A., Cooper, A.M., Corbett, A.J., Cosma, A., Cosmi, L., Coulie, P.G., Cumano, A., Cvetkovic, L., Dang, V.D., Dang-Heine, C., Davey, M.S., Davies, D., De, B.i.a.s.i., Del, Z.o.t.t.o., Dela, C.r.u.z., Delacher, M., Della, B.e.l.l.a., Dellabona, P., Deniz, G., Dessing, M., Di, S.a.n.t.o., Diefenbach, A., Dieli, F., Dolf, A., Dörner, T., Dress, R.J., Dudziak, D., Dustin, M., Dutertre, C.A., Ebner, F., Eckle, S.B.G., Edinger, M., Eede, P., Ehrhardt, G.R.A., Eich, M., Engel, P., Engelhardt, B., Erdei, A., Esser, C., Everts, B., Evrard, M., Falk, C.S., Fehniger, T.A., Felipo-Benavent, M., Ferry, H., Feuerer, M., Filby, A., Filkor, K., Fillatreau, S., Follo, M., Förster, I., Foster, J., Foulds, G.A., Frehse, B., Frenette, P.S., Frischbutter, S., Fritzsche, W., Galbraith, D.W., Gangaev, A., Garbi, N., Gaudilliere, B., Gazzinelli, R.T., Geginat, J., Gerner, W., Gherardin, N.A., Ghoreschi, K., Gibellini, L., Ginhoux, F., Goda, K., Godfrey, D.I., Goettlinger, C., González-Navajas, J.M., Goodyear, C.S., Gori, A., Grogan, J.L., Grummitt, D., Grützkau, A., Haftmann, C., Hahn, J., Hammad, H., Hämmerling, G., Hansmann, L., Hansson, G., Harpur, C.M., Hartmann, S., Hauser, A., Hauser, A.E., Haviland, D.L., Hedley, D., Hernández, D.C., Herrera, G., Herrmann, M., Hess, C., Höfer, T., Hoffmann, P., Hogquist, K., Holland, T., Höllt, T., Holmdahl, R., Hombrink, P., Houston, J.P., Hoyer, B.F., Huang, B., Huang, F.P., Huber, J.E., Huehn, J., Hundemer, M., Hunter, C.A., Hwang, W.Y.K., Iannone, A., Ingelfinger, F., Ivison, S.M., Jäck, H.M., Jani, P.K., Jávega, B., Jonjic, S., Kaiser, T., Kalina, T., Kamradt, T., Kaufmann, S.H.E., Keller, B., Ketelaars, S.L.C., Khalilnezhad, A., Khan, S., Kisielow, J., Klenerman, P., Knopf, J., Koay, H.F., Kobow, K., Kolls, J.K., Kong, W.T., Kopf, M., Korn, T., Kriegsmann, K., Kristyanto, H., Kroneis, T., Krueger, A., Kühne, J., Kukat, C., Kunkel, D., Kunze-Schumacher, H., Kurosaki, T., Kurts, C., Kvistborg, P., Kwok, I., Landry, J., Lantz, O., Lanuti, P., LaRosa, F., Lehuen, A., LeibundGut-Landmann, S., Leipold, M.D., Leung, L.Y.T., Levings, M.K., Lino, A.C., Liotta, F., Litwin, V., Liu, Y., Ljunggren, H.G., Lohoff, M.,

Lombardi, G., Lopez, L., López-Botet, M., Lovett-Racke, A.E., Lubberts, E., Luche, H., Ludewig, B., Lugli, E., Lunemann, S., Maecker, H.T., Maggi, L., Maguire, O., Mair, F., Mair, K.H., Mantovani, A., Manz, R.A., Marshall, A.J., Martínez-Romero, A., Martrus, G., Marventano, I., Maslinski, W., Matarese, G., Mattioli, A.V., Maueröder, C., Mazzoni, A., McCluskey, J., McGrath, M., McGuire, H.M., McInnes, I.B., Mei, H.E., Melchers, F., Melzer, S., Mielenz, D., Miller, S.D., Mills, K.H.G., Minderman, H., Mjösberg, J., Moore, J., Moran, B., Moretta, L., Mosmann, T.R., Müller, S., Multhoff, G., Muñoz, L.E., Münz, C., Nakayama, T., Nasi, M., Neumann, K., Ng, L.G., Niedobitek, A., Nourshargh, S., Núñez, G., O'Connor, J.E., Ochel, A., Oja, A., Ordonez, D., Orfao, A., Orlowski-Oliver, E., Ouyang, W., Oxenius, A., Palankar, R., Panse, I., Pattanapanyasat, K., Paulsen, M., Pavlinic, D., Penter, L., Peterson, P., Peth, C., Petriz, J., Piancone, F., Pickl, W.F., Piconese, S., Pinti, M., Pockley, A.G., Podolska, M.J., Poon, Z., Pracht, K., Prinz, I., Pucillo, C.E.M., Quataert, S.A., Quatrini, L., Quinn, K.M., Radbruch, H., Radstake, T.R.D.J., Rahmig, S., Rahn, H.P., Rajwa, B., Ravichandran, G., Raz, Y., Rebhahn, J.A., Recktenwald, D., Reimer, D., Reis, e., Remmerswaal, E.B.M., Richter, L., Rico, L.G., Riddell, A., Rieger, A.M., Robinson, J.P., Romagnani, C., Rubartelli, A., Ruland, J., Saalmüller, A., Saeys, Y., Saito, T., Sakaguchi, S., Sala-de-Oyanguren, F., Samstag, Y., Sanderson, S., Sandrock, I., Santoni, A., Sanz, R.B., Saresella, M., Sautes-Fridman, C., Sawitzki, B., Schadt, L., Scheffold, A., Scherer, H.U., Schiemann, M., Schildberg, F.A., Schimisky, E., Schlitzer, A., Schlosser, J., Schmid, S., Schmitt, S., Schober, K., Schraivogel, D., Schuh, W., Schüler, T., Schulte, R., Schulz, A.R., Schulz, S.R., Scottá, C., Scott-Algara, D., Sester, D.P., Shankey, T.V., Silva-Santos, B., Simon, A.K., Sitnik, K.M., Sozzani, S., Speiser, D.E., Spidlen, J., Stahlberg, A., Stall, A.M., Stanley, N., Stark, R., Stehle, C., Steinmetz, T., Stockinger, H., Takahama, Y., Takeda, K., Tan, L., Tárnok, A., Tiegs, G., Toldi, G., Tornack, J., Traggiai, E., Trebak, M., Tree, T.I.M., Trotter, J., Trowsdale, J., Tsoumakidou, M., Ulrich, H., Urbanczyk, S., van de Veen W, van den Broek M, van der Pol E, Van Gassen S, Van Isterdael G, van Lier RAW, Veldhoen M, Vento-Asturias S, Vieira P, Voehringer D, Volk HD, von Borstel A, von Volkmann, K., Waisman, A., Walker, R.V., Wallace, P.K., Wang, S.A., Wang, X.M., Ward, M.D., Ward-Hartstonge, K.A., Warnatz, K., Warnes, G., Warth, S., Waskow, C.,

Watson, J.V., Watzl, C., Wegener, L., Weisenburger, T., Wiedemann, A., Wienands, J., Wilharm, A., Wilkinson, R.J., Willimsky, G., Wing, J.B., Winkelmann, R., Winkler, T.H., Wirz, O.F., Wong, A., Wurst, P., Yang, J.H.M., Yang, J., Yazdanbakhsh, M., Yu, L., Yue, A., Zhang, H., Zhao, Y., Ziegler, S.M., Zielinski, C., Zimmermann, J. & Zychlinsky, A. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). *Eur. J. Immunol.* **49**, 1457–1973 (2019).

53. Efremova, M., Vento-Tormo, R., Park, J.-E., Teichmann, S. A. & James, K. R. Immunology in the Era of Single-Cell Technologies. *Annu. Rev. Immunol.* **38**, 727–757 (2020).

54. R Core Team. *R: A Language and Environment for Statistical Computing*. (R Foundation for Statistical Computing, 2013).

55. Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W.M., Hao, Y., Stoeckius, M., Smibert, P. & Satija, R.. Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902.e21 (2019).

56. Biesen, R., Demir, C., Barkhudarova, F., Grün, J.R., Steinbrich-Zöllner, M., Backhaus, M., Häupl, T., Rudwaleit, M., Riemekasten, G., Radbruch, A., Hiepe, F., Burmester, G.R. & Grützkau, A. Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. *Arthritis Rheum.* **58**, 1136–1145 (2008).

57. Groom, J. R. & Luster, A. D. CXCR3 in T cell function. *Exp. Cell Res.* **317**, 620–631 (2011).

58. Lancaster, E., Huijbers, M.G., Bar, V., Boronat, A., Wong, A., Martinez-Hernandez, E., Wilson, C., Jacobs, D., Lai, M., Walker, R.W., Graus, F., Bataller, L., Illa, I., Markx, S., Strauss, K.A., Peles, E., Scherer, S.S. & Dalmau, J. Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann. Neurol.* **69**, 303–311 (2011).

59. Prüss, H. Autoantibodies in neurological disease. *Nat. Rev. Immunol.* (2021) doi:10.1038/s41577-021-00543-w.

60. Campar, A., Farinha, F. & Vasconcelos, C. Refractory disease in Systemic Lupus Erythematosus. *Autoimmun. Rev.* **10**, 685–692 (2011).

61. Gerlag, D.M., Safy, M., Maijer, K.I., Tang, M.W., Tas, S.W., Starmans-Kool, M.J.F., van Tubergen, A, Janssen, M., de Hair, M., Hansson, M., de Vries, N., Zwinderman, A.H. & Tak, P.P. Effects of B-cell directed therapy on the preclinical stage of rheumatoid arthritis: the PRAIRI study. *Ann. Rheum. Dis.* **78**, 179–185 (2019).

62. Cheng, Q., Pelz, A., Taddeo, A., Khodadadi, L., Klotsche, J., Hoyer, B.F., Alexander, T., Thiel, A., Burmester, G.R., Radbruch, A. & Hiepe, F. Selective depletion of plasma cells in vivo based on the specificity of their secreted antibodies. *Eur. J. Immunol.* **50**, 284–291 (2020).

63. Perez-Diez, A., Wong, C.S., Liu, X., Mystakelis, H., Song, J., Lu, Y., Sheikh, V., Bourgeois, J.S., Lisco, A., Laidlaw, E., Cudrici, C., Zhu, C., Li, Q.Z., Freeman, A.F., Williamson, P.R., Anderson, M., Roby, G., Tsang, J.S., Siegel, R. & Sereti, I. Prevalence and pathogenicity of autoantibodies in patients with idiopathic CD4 lymphopenia. *J. Clin. Invest.* **130**, 5326–5337 (2020).

Statutory Declaration

“I, Lennard Ostendorf, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “CD38 als neues therapeutisches Target bei Systemischem Lupus Erythematodes – CD38 as a novel therapeutic treatment target for Systemic Lupus Erythematosus”, independently and without the support of third parties, and that I used no other sources and aids than those stated. All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

Declaration of your own contribution to the publications

Lennard Ostendorf contributed the following to the below listed publications:

Publication 1: **Ostendorf, L**., Burns, M., Durek, P., Heinz, G.A., Heinrich, F., Garantziotis, P., Enghard, P., Richter, U., Biesen, R., Schneider, U., Knebel, F., Burmester, G., Radbruch, A., Mei, H.E., Mashreghi, M.-F., Hiepe, F., Alexander, T., Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus. New England Journal of Medice, 2020.
Contribution:
Collected data for Figure 1 and 2 and Supplemental Figure 1, performed experiments for Supplemental Figures 2-7 and analysed the data for Supplemental Figures 2, 6, 7 and provided input for the bioinformatical analysis for Supplemental Figures 3-5. Co-wrote manuscript and designed figures with Tobias Alexander, revised the manuscript upon reviewer comments.

Publication 2: Scheibe, F., **Ostendorf, L**., Reincke, S.M., Prüss, H., von Brünneck, A.-C., Köhnlein, M., Alexander, T., Meisel, C., Meisel, A., Daratumumab treatment for therapy-refractory anti-CASPR2 encephalitis. Journal of Neurology, 2019.
Contribution: Performed experiments and analysed data for Figure 3, contributed to the writing of the manuscript and its revision.

Publication 3: Burns, M.*, **Ostendorf, L.***, Biesen, R., Grützkau, A., Hiepe, F., Mei, H.E., Alexander, T., 2021. Dysregulated CD38 Expression on Peripheral Blood Immune Cell Subsets in SLE. International Journal of Molecular Sciences, 2021. *** shared first authorship**
Contribution: Performed experiments, collected data and analysed data for Table 1, Figure 4, Figure 5C. Wrote large parts of the background and discussion of the manuscript.

_____
Signature, date and stamp of first supervising university professor / lecturer

_____
Signature of doctoral candidate

Publikation 1: Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI
Selected Categories: **"MEDICINE, GENERAL and INTERNAL"**
Selected Category Scheme: WoS
Gesamtanzahl: 160 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NEW ENGLAND JOURNAL OF MEDICINE	344,581	70.670	0.686700
2	LANCET	247,292	59.102	0.427870
3	JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION	156,350	51.273	0.300810
4	Nature Reviews Disease Primers	4,339	32.274	0.019740
5	BMJ-British Medical Journal	112,901	27.604	0.152760
6	JAMA Internal Medicine	15,215	20.768	0.095580
7	ANNALS OF INTERNAL MEDICINE	57,057	19.315	0.096020
8	PLOS MEDICINE	30,689	11.048	0.071200
9	Journal of Cachexia Sarcopenia and Muscle	2,799	10.754	0.005870
10	BMC Medicine	13,630	8.285	0.045220
11	Cochrane Database of Systematic Reviews	67,607	7.755	0.158690
12	MAYO CLINIC PROCEEDINGS	14,695	7.091	0.025750
13	CANADIAN MEDICAL ASSOCIATION JOURNAL	15,351	6.938	0.016500
14	JOURNAL OF INTERNAL MEDICINE	10,547	6.051	0.015700
15	Journal of Clinical Medicine	2,315	5.688	0.007210
16	MEDICAL JOURNAL OF AUSTRALIA	11,134	5.332	0.012600
17	PALLIATIVE MEDICINE	5,682	4.956	0.009860
18	AMYLOID-JOURNAL OF PROTEIN FOLDING DISORDERS	1,335	4.919	0.003270

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

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Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

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Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

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Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Reference [50]: https://doi.org/10.1056/NEJMoa2023325

Publikation 2: Daratumumab treatment for therapy-refractory anti-CASPR2 encephalitis

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"CLINICAL NEUROLOGY"** Selected Category Scheme: WoS
Gesamtanzahl: 197 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	LANCET NEUROLOGY	28,671	27.138	0.069040
2	Nature Reviews Neurology	8,095	19.819	0.028090
3	ACTA NEUROPATHOLOGICA	18,783	15.872	0.041490
4	Alzheimers & Dementia	10,423	12.740	0.030040
5	JAMA Neurology	6,885	11.460	0.035270
6	BRAIN	52,061	10.840	0.075170
7	SLEEP MEDICINE REVIEWS	6,080	10.602	0.010720
8	ANNALS OF NEUROLOGY	37,251	10.244	0.053390
9	NEURO-ONCOLOGY	10,930	9.384	0.030350
10	Epilepsy Currents	790	9.333	0.001600
11	MOVEMENT DISORDERS	26,511	8.324	0.037980
12	Translational Stroke Research	2,202	8.266	0.005260
13	NEUROLOGY	88,493	7.609	0.115530
14	NEUROSCIENTIST	4,738	7.461	0.008730
15	JOURNAL OF NEUROLOGY NEUROSURGERY AND PSYCHIATRY	29,695	7.144	0.032980
16	STROKE	65,854	6.239	0.088520
17	BRAIN PATHOLOGY	4,952	6.187	0.007750
18	Brain Stimulation	4,263	6.120	0.014510
19	NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY	3,654	6.059	0.006350
20	Neurotherapeutics	3,973	5.719	0.008980
21	PAIN	36,132	5.559	0.038000
22	Multiple Sclerosis Journal	10,675	5.280	0.021890
23	SLEEP	20,547	5.135	0.025870
24	EPILEPSIA	26,301	5.067	0.032490
25	Alzheimers Research & Therapy	2,192	5.015	0.008470
26	JOURNAL OF NEUROTRAUMA	14,508	5.002	0.021130
27	JOURNAL OF PAIN	9,264	4.859	0.016890
28	Journal of Stroke	694	4.750	0.002880
28	Therapeutic Advances in Neurological Disorders	1,004	4.750	0.002800
30	JOURNAL OF PSYCHOPHARMACOLOGY	5,808	4.738	0.010900
31	PARKINSONISM & RELATED DISORDERS	8,967	4.721	0.019910
32	NEUROREHABILITATION AND NEURAL REPAIR	5,032	4.711	0.009850
33	Annals of Clinical and Translational Neurology	1,377	4.649	0.006450
34	EUROPEAN JOURNAL OF NEUROLOGY	10,206	4.621	0.019350
35	BIPOLAR DISORDERS	5,070	4.490	0.007870

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
36	NEUROSURGERY	28,592	4.475	0.025930
37	JOURNAL OF NEUROSURGERY	34,561	4.318	0.030750
38	CNS DRUGS	4,364	4.206	0.007540
39	PROGRESS IN NEURO-PSYCHOPHARMACOLOGY & BIOLOGICAL PSYCHIATRY	9,823	4.185	0.013170
40	EUROPEAN NEUROPSYCHOPHARMACOLOGY	6,920	4.129	0.015110
41	CURRENT OPINION IN NEUROLOGY	5,344	4.010	0.010200
42	INTERNATIONAL JOURNAL OF NEUROPSYCHOPHARMACOLOGY	6,259	3.981	0.014550
43	CEPHALALGIA	8,721	3.882	0.013940
44	International Journal of Stroke	3,825	3.859	0.014880
45	NEUROGASTROENTEROLOGY AND MOTILITY	7,401	3.842	0.014960
46	JOURNAL OF AFFECTIVE DISORDERS	26,957	3.786	0.053380
47	JOURNAL OF NEUROLOGY	14,359	3.783	0.025160
48	NEUROEPIDEMIOLOGY	3,261	3.697	0.005640
49	Expert Review of Neurotherapeutics	3,888	3.692	0.006910
50	AMERICAN JOURNAL OF NEURORADIOLOGY	22,667	3.653	0.029840
51	Journal of Neurologic Physical Therapy	964	3.633	0.001530
52	EUROPEAN ARCHIVES OF PSYCHIATRY AND CLINICAL NEUROSCIENCE	3,837	3.617	0.005400
53	CLINICAL NEUROPHYSIOLOGY	18,399	3.614	0.023070
54	Frontiers in Neurology	4,272	3.508	0.015580
55	CNS SPECTRUMS	2,200	3.504	0.003180
56	Journal of Neurodevelopmental Disorders	1,106	3.500	0.003410
57	JOURNAL OF NEUROPATHOLOGY AND EXPERIMENTAL NEUROLOGY	9,252	3.490	0.008680
58	Current Neurology and Neuroscience Reports	2,770	3.478	0.007410
59	Journal of Neurogastroenterology and Motility	1,207	3.438	0.002930
60	JOURNAL OF SLEEP RESEARCH	5,092	3.433	0.007460
61	JOURNAL OF HEAD TRAUMA REHABILITATION	4,282	3.406	0.005540
62	JOURNAL OF HEADACHE AND PAIN	2,624	3.403	0.005510
63	Journal of Clinical Sleep Medicine	5,329	3.396	0.011800
64	SLEEP MEDICINE	9,130	3.395	0.016270
65	Current Alzheimer Research	3,740	3.289	0.007910
65	DEVELOPMENTAL MEDICINE AND CHILD NEUROLOGY	11,671	3.289	0.013680
67	JOURNAL OF PAIN AND SYMPTOM MANAGEMENT	9,734	3.249	0.013980

Selected JCR Year: 2017; Selected Categories: "CLINICAL NEUROLOGY"

Reference [51]: https://doi.org/10.1007/s00415-019-09585-6

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Reference [51]: https://doi.org/10.1007/s00415-019-09585-6

Reference [51]: https://doi.org/10.1007/s00415-019-09585-6

Publikation 3: Dysregulated CD38 Expression on Peripheral Blood Immune Cell Subsets in SLE

Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI Selected Categories: **"BIOCHEMISTRY and MOLECULAR BIOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 297 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	CELL	258,178	38.637	0.564970
2	NATURE MEDICINE	85,220	36.130	0.168730
3	Annual Review of Biochemistry	20,499	25.787	0.024820
4	MOLECULAR CELL	69,148	15.584	0.166260
5	Molecular Cancer	15,448	15.302	0.023990
6	PROGRESS IN LIPID RESEARCH	6,139	15.083	0.005730
7	TRENDS IN BIOCHEMICAL SCIENCES	18,416	14.732	0.032060
8	TRENDS IN MICROBIOLOGY	13,604	13.546	0.022780
9	Signal Transduction and Targeted Therapy	1,182	13.493	0.003380
10	Nature Chemical Biology	22,084	12.587	0.060130
11	MOLECULAR PSYCHIATRY	22,227	12.384	0.054730
12	Molecular Plant	11,432	12.084	0.028530
13	NATURAL PRODUCT REPORTS	11,239	12.000	0.013610
14	NATURE STRUCTURAL & MOLECULAR BIOLOGY	27,178	11.980	0.056800
15	NUCLEIC ACIDS RESEARCH	201,649	11.501	0.403470
16	TRENDS IN MOLECULAR MEDICINE	10,618	11.099	0.018720
17	GENOME RESEARCH	41,755	11.093	0.076940
18	MOLECULAR BIOLOGY AND EVOLUTION	50,486	11.062	0.084810
19	CELL DEATH AND DIFFERENTIATION	21,095	10.717	0.029600
20	Redox Biology	10,157	9.986	0.023810

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
21	EMBO JOURNAL	64,724	9.889	0.059690
22	CURRENT OPINION IN CHEMICAL BIOLOGY	10,968	9.689	0.017770
23	PLANT CELL	54,927	9.618	0.048640
24	CURRENT BIOLOGY	63,256	9.601	0.133170
25	MOLECULAR ASPECTS OF MEDICINE	6,207	9.577	0.005750
26	Molecular Systems Biology	8,914	8.991	0.017390
27	Cell Systems	3,822	8.673	0.029290
28	MATRIX BIOLOGY	6,878	8.572	0.011920
29	ONCOGENE	66,303	7.971	0.068320
30	Cell Chemical Biology	3,326	7.739	0.015770
31	CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY	3,675	7.634	0.006380
32	EMBO REPORTS	14,976	7.497	0.030290
33	BIOCHIMICA ET BIOPHYSICA ACTA-REVIEWS ON CANCER	5,650	7.365	0.007800
34	PLOS BIOLOGY	31,650	7.076	0.060300
35	Essays in Biochemistry	2,383	6.966	0.005060
36	CURRENT OPINION IN STRUCTURAL BIOLOGY	11,035	6.908	0.021890
37	CELLULAR AND MOLECULAR LIFE SCIENCES	26,128	6.496	0.037010
38	Science Signaling	12,736	6.467	0.026590
39	ANTIOXIDANTS & REDOX SIGNALING	21,119	6.323	0.024660
40	Molecular Ecology Resources	10,868	6.286	0.019630
41	FREE RADICAL BIOLOGY AND MEDICINE	42,665	6.170	0.036960
42	BIOMACROMOLECULES	38,863	6.092	0.031320

Selected JCR Year: 2019; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
43	Computational and Structural Biotechnology Journal	1,954	6.018	0.004980
44	CYTOKINE & GROWTH FACTOR REVIEWS	5,935	5.982	0.007380
45	Advances in Carbohydrate Chemistry and Biochemistry	634	5.800	0.000340
46	EXPERIMENTAL AND MOLECULAR MEDICINE	5,536	5.418	0.010300
47	AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY	12,243	5.373	0.016040
48	RNA Biology	6,589	5.350	0.015820
49	Acta Crystallographica Section D-Structural Biology	21,750	5.266	0.018220
50	MOLECULAR ECOLOGY	38,951	5.163	0.050800
51	INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES	47,121	5.162	0.057240
52	BIOCHEMICAL SOCIETY TRANSACTIONS	12,651	5.160	0.016140
53	HUMAN MOLECULAR GENETICS	39,652	5.100	0.064170
54	Journal of Genetics and Genomics	2,271	5.065	0.004310
55	Cell and Bioscience	1,898	5.026	0.004210
56	Antioxidants	2,568	5.014	0.004170
57	FASEB JOURNAL	43,126	4.966	0.043730
58	International Review of Cell and Molecular Biology	2,167	4.934	0.004350
59	Open Biology	2,886	4.931	0.009590
60	Journal of Integrative Plant Biology	5,005	4.885	0.006830
61	Advances in Microbial Physiology	1,227	4.875	0.000960
61	Nucleic Acid Therapeutics	1,030	4.875	0.003610

Selected JCR Year: 2019; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
63	JOURNAL OF NUTRITIONAL BIOCHEMISTRY	11,460	4.873	0.011150
64	STRUCTURE	15,145	4.862	0.026940
65	International Journal of Biological Sciences	6,262	4.858	0.009710
66	BIOORGANIC CHEMISTRY	5,712	4.831	0.006730
67	Genes & Diseases	1,081	4.803	0.003310
68	JOURNAL OF MOLECULAR BIOLOGY	56,952	4.760	0.040330
69	BIOFACTORS	3,769	4.734	0.002930
70	BIOELECTROCHEMISTRY	4,944	4.722	0.004950
71	Reviews of Physiology Biochemistry and Pharmacology	805	4.700	0.000670
72	JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY	5,415	4.673	0.005420
73	BIOESSAYS	10,189	4.627	0.016560
74	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES	77,286	4.556	0.143760
75	APOPTOSIS	6,539	4.543	0.005880
76	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR AND CELL BIOLOGY OF LIPIDS	10,266	4.519	0.016350
77	ACS Chemical Neuroscience	6,881	4.486	0.015300
78	JOURNAL OF LIPID RESEARCH	24,223	4.483	0.022420
79	ACS Chemical Biology	12,884	4.434	0.035490
80	FEBS Journal	18,845	4.392	0.025250
81	JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B-BIOLOGY	12,794	4.383	0.013640
82	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE	15,965	4.352	0.024200

Selected JCR Year: 2019; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

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Dysregulated CD38 Expression on Peripheral Blood Immune Cell Subsets in SLE

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Abstract: Given its uniformly high expression on plasma cells, CD38 has been considered as a therapeutic target in patients with systemic lupus erythematosus (SLE). Herein, we investigate the distribution of CD38 expression by peripheral blood leukocyte lineages to evaluate the potential therapeutic effect of CD38-targeting antibodies on these immune cell subsets and to delineate the use of CD38 as a biomarker in SLE. We analyzed the expression of CD38 on peripheral blood leukocyte subsets by flow and mass cytometry in two different cohorts, comprising a total of 56 SLE patients. The CD38 expression levels were subsequently correlated across immune cell lineages and subsets, and with clinical and serologic disease parameters of SLE. Compared to healthy controls (HC), CD38 expression levels in SLE were significantly increased on circulating plasmacytoid dendritic cells, $CD14^{++}CD16^{+}$ monocytes, $CD56^{+}$ $CD16^{dim}$ natural killer cells, marginal zone-like $IgD^{+}CD27^{+}$ B cells, and on $CD4^{+}$ and $CD8^{+}$ memory T cells. Correlation analyses revealed coordinated CD38 expression between individual innate and memory T cell subsets in SLE but not HC. However, CD38 expression levels were heterogeneous across patients, and no correlation was found between CD38 expression on immune cell subsets and the disease activity index SLEDAI-2K or established serologic and immunological markers of disease activity. In conclusion, we identified widespread changes in CD38 expression on SLE immune cells that highly correlated over different leukocyte subsets within individual patients, but was heterogenous within the population of SLE patients, regardless of disease severity or clinical manifestations. As anti-CD38 treatment is being investigated in SLE, our results may have important implications for the personalized targeting of pathogenic leukocytes by anti-CD38 monoclonal antibodies.

Keywords: CD38; SLE; immune profiling

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1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by immune responses against nuclear antigens. The immunopathogenesis of the disease is complex and involves genetic, environmental, hormonal, epigenetic, and immunoregulatory factors that may act either sequentially or simultaneously on the immune system [1]. It is assumed that a defect in the clearance of apoptotic cells with accumulation of undigested apoptotic remnants may provoke the first hit in the break of self-tolerance by activating otherwise quiescent autoreactive lymphocytes. The presence of extracellular DNA additionally stimulates plasmacytoid dendritic cells (pDC) via Toll-like receptors, resulting in

the production of type I interferons (IFN-I), some of the hallmark cytokines in SLE [1–3]. Persistently activated IFN-I signaling pathways, mimicking sustained anti-virus responses, may contribute to SLE pathogenesis by amplifying antigen-specific adaptive autoimmune responses. Upon receiving T cell help, autoreactive B cells are activated, increase their specificity towards nuclear antigens during affinity maturation, and differentiate into class-switched memory B cells and antibody-secreting plasma cells (PC). The newly generated plasma cell precursors detectable in peripheral blood, so-called plasmablasts (PB), migrate to the bone marrow and inflamed organs, where they eventually become long-lived once seeded in dedicated niches and continuously secrete pathogenic autoantibodies for months or even years, thereby contributing to the chronicity of SLE [4].

Many of the immune cells that contribute to SLE pathogenesis may express the multifunctional cell-surface protein CD38, either constitutionally or upon stimulation. As an enzyme, CD38 degrades nicotinamide nucleotides like NAD^+ and both synthesizes and hydrolyzes the second messenger cADPR (cyclic ADP ribose), although the relative contributions of these functions are controversially discussed [5]. While commonly considered an ectoenzyme, CD38 is also detectable within the nucleus and the mitochondrial membrane and as a soluble molecule in the blood [5]. At the same time, CD38 also functions as an activatory receptor on immune cells in the context of cell adhesion, migration, and cytokine secretion [6]. Calcium influx induced by CD38 ligation is also required for dendritic cells and neutrophils to follow chemokine gradients into the lymph node and migrate into inflamed organs [7,8].

Previous studies analyzing the expression of CD38 on leukocytes in SLE indicated increased CD38 expression on different leukocyte subsets compared to healthy controls, such as $CD4^+$ and $CD8^+$ memory T cells [9–12] and monocytes [13]. Research in $CD38^{-/-}$ mice found that CD38 deficiency ameliorated the course of pristane-induced lupus [14]. Because CD38 is expressed constitutively at high levels on human antibody-secreting plasmablasts and plasma cells and is inducible on several immune cell subsets upon activation, CD38 has been considered as a potential therapeutic target in autoantibody-driven diseases such as SLE [15], autoimmune encephalitis [16,17], and autoimmune hemolysis [18]. We recently treated two patients with refractory, life-threatening SLE with the anti-CD38 antibody daratumumab, which resulted in substantial clinical and immunological efficacy [11]. Although the CD38-mediated targeting of plasma cells was presumably the most important factor for the observed clinical responses, it remains unclear if and how daratumumab exerts therapeutically relevant effects on other CD38 expressing immune cell subsets. While there are reports of increased CD38 expression in different peripheral immune cell subsets in SLE [9,10,12,13,15], a comprehensive analysis of CD38 expression in the immune cell compartment in SLE is lacking.

Here, we report the results of a global expression analysis of CD38 on blood leukocytes from two cohorts of SLE patients, using mass and flow cytometry, that confirm and expand previous results, and demonstrate that increased CD38 expression in subsets of innate and adaptive immune cells is a stable and reproducible feature of SLE, largely independent of disease phenotype and severity.

2. Results

2.1. Increased CD38 Expression in Major SLE Leukocyte Subsets

To characterize CD38 expression across peripheral blood leukocyte subsets and identify potential dysregulation of CD38 in SLE, we analyzed leukocytes isolated from cryopreserved whole blood samples of 20 SLE patients and 20 age- and gender-matched healthy controls (HC). Patient demographics are shown in Table 1. Cell samples were analyzed by mass cytometry, using an antibody panel suitable for analyzing the expression of CD38 in major peripheral blood leukocyte subsets (Supplementary Table S1). Data of $CD45^+$ leukocytes were subjected to dimension reduction by opt-SNE [19], and major cell subsets were annotated according to the expression of lineage-defining cell-surface markers on the resulting t-SNE map (Figure 1A and Supplementary Figure S1). CD38 expression was

projected onto the t-SNE maps of concatenated data of SLE or HC samples (Figure 1B). Highest CD38 expression was found on PB/PC (mean signal intensity HC, 572; SLE, 586), followed by NK cells (HC, 79; SLE, 132) and basophils (HC, 89; SLE, 119), monocytes (HC, 38; SLE, 35), and plasmacytoid and myeloid dendritic cells (pDC and mDC) (HC, 30; SLE, 52, and HC 40; SLE 38, respectively). On average levels, CD38 was absent from or weakly expressed by most T and B cells, eosinophils, and neutrophils. Based on the mean CD38 expression of each subset, we found significantly increased CD38 expression in SLE samples on NK cells, pDC, and $CD8^+$ T cells (1.7-, 1.7-, and 2.5-fold increase, respectively, Figure 1C). Since subset-specific CD38 expression varied among both SLE patients and HC, we determined the coefficient of variation (CV) for CD38 expression levels in the leukocyte subsets identified in Figure 1A. Highest CV differences between SLE and HC were identified for $CD8^+$ T cells (mean CV, HC, 61%; SLE, 131%), B cells (HC, 44%; SLE, 95%), eosinophils (HC, 41%; SLE 84%), $CD4^+$ T cells (HC, 60%; SLE 94%), and mDC (HC, 35%; SLE, 68%). In addition, we found evidence of varying CD38 expression on the single-cell level in almost all leukocytes subsets (Figure 1B,D), prompting us to analyze the variability of CD38 expression in greater detail.

Table 1. Patient characteristics.

	Mass Cytometry Cohort			Flow Cytometry Cohort		
	Healthy Controls	**SLE Patients**	***p* Value**	**Healthy Controls**	**SLE Patients**	***p* Value**
Number	20	20		19	36	
Age (median, IQR)	39 (30–46)	39 (30–47)	0.732	28 (24–30)	40 (31–46)	0.001
Sex (*n*% female)	18 (90.0%)	18 (90.0%)	1.00	11 (57.9%)	33 (91.7%)	0.003
SLEDAI-2K (median, range)	–	8 (2–14)		–	4.5 (0–14)	
Clinically active SLE (clinical SLEDAI-2K > 0)	–	17 (85.0%)		–	24 (66.7%)	
Serologically active SLE (serological SLEDAI-2K > 0)	–	18 (90%)		–	29 (80.6%)	
Prednisolone Dose (mg/d; median, range)	–	5.0 (0–20.0)		–	5.0 (0–25.0)	
Other Immunosuppressive Medication (*n*)	–	HCQ: 17 (85.0%) AZA: 4 (20.0%) MTX: 3 (15.0%) BEL: 3 (15.0%) MMF: 1 (5.0%)		–	HCQ: 24 (66.7%) MMF: 9 (25.0%) AZA: 9 (25.0%) CsA: 4 (11.1%) BEL: 4 (11.1%) RTX: 3 (8.3%) UST: 1 (2.8%)	

Abbreviations: Hydroxychloroquine (HCQ), Azathioprine (AZA), Methotrexate (MTX), Belimumab (BEL), Mycophenolate Mofetil (MMF), Ciclosporin A (CsA), Rituximab (RTX), Ustekinumab (UST). Statistical analysis comparing differences in age was performed using the Mann–Whitney test, sex differences with the chi-square test.

2.2. CD38 Expression by NK Cells and Myeloid Immune Cells Is Associated with an Activated Phenotype

Based on our initial finding of increased expression of CD38 in SLE NK cells, but heterogeneous detection of CD38 on NK cells at the single-cell level, we analyzed whether NK cell CD38 expression levels were associated with other phenotypical features of NK cells. Among NK cells, high expression of CD38 was associated with increased expression of CD11c, and $CD38^{++}$ NK cells comprised most HLA-DR-expressing NK cells in HC and SLE patients (Supplementary Figure S2). Together, these data suggest that high CD38 expression could be a feature of activated NK cells, and the increased CD38 expression by NK cells may indicate enhanced activation of circulating NK cells in SLE. Similar observations were made for basophils, mDC, pDC, and monocytes. For example, $CD38^+$ mDC co-expressed Syk, HLA-DR, and CD11c at higher levels, and $CD38^+$ pDC co-expressed increased levels of Syk and HLA-DR compared to their $CD38^{-/low}$ counterparts (Supplementary Figure S2). In monocytes, CD38 expression was significantly associated with expression of SIGLEC-1 ($p < 0.0001$), a marker associated with IFN-I activity [3,20].

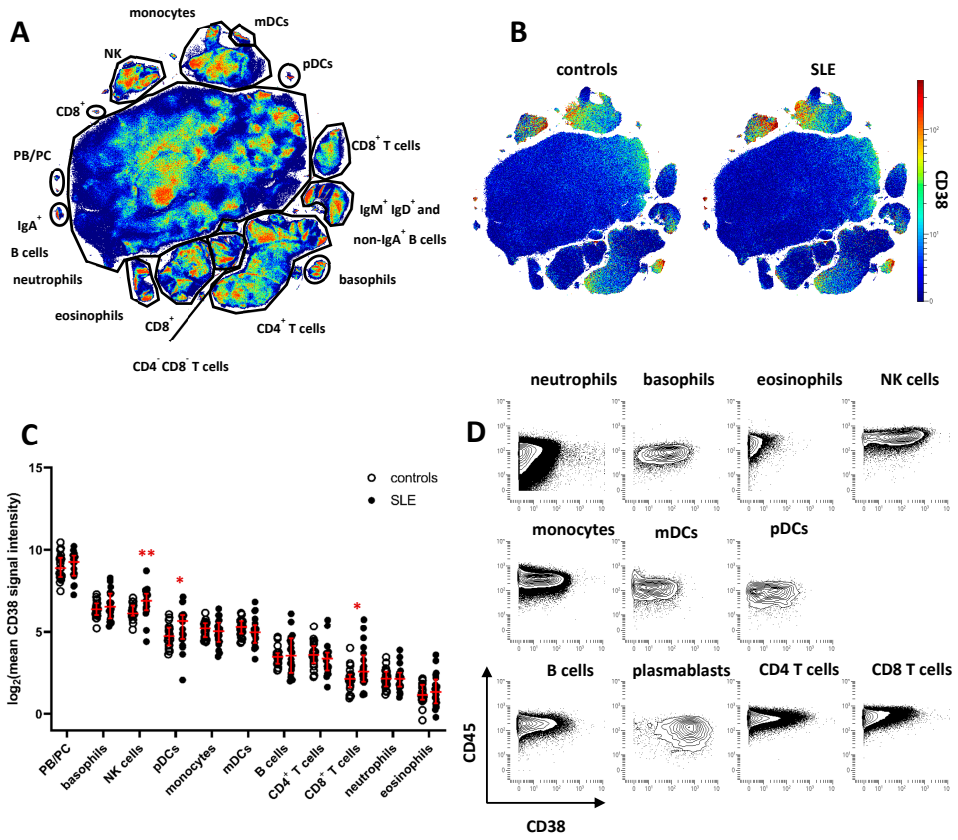

Figure 1. Cell type-specific dysregulation of CD38 expression in patients with SLE. Peripheral blood leukocytes from 20 SLE patients and 20 healthy controls were analyzed by mass cytometry. (**A**) t-SNE map showing all leukocytes analyzed in the study (7.6×10^6 cells). Major cell subsets were annotated. Color indicates cell density. (**B**) CD38 expression across major leukocyte subsets in SLE patients and controls. Concatenated data is shown for both groups. (**C**) Comparison of CD38 expression across major subsets gated in (**A**). Each dot represents the $\log_2$ of mean CD38 signal intensity (SI) of the indicated subset of one donor. Red lines indicate median values and interquartile range. * indicate significantly different CD38 expression in SLE patients vs. controls revealed by Mann–Whitney testing (* $p < 0.05$; ** $p < 0.01$) (**D**) Contour plot representation of CD38 expression of the indicated leukocyte subsets. Concatenated data of 20 healthy controls are shown.

2.3. Increased Expression of CD38 on Distinct Subsets of Peripheral Blood B Cells in SLE

Next, we analyzed the mass cytometry data of CD19$^+$ B cells, including HLA-DR^{high} plasmablasts and HLA-DR^{low} plasma cells [21], by FlowSOM clustering and subsequent hierarchical metaclustering, based on markers expressed by B cells and omitting CD38 (Supplementary Table S1). We obtained ten individual B cell clusters, including two IgD$^+$IgM$^+$ naive B cell clusters, IgA$^+$ and IgA$^-$ memory B cells, CD11c$^+$ B cells, CD27$^+$IgD$^+$IgM$^+$CD1c$^+$ marginal zone-like B cells, CD27$^-$IgD$^-$ B cells, and three clusters of PB/PC distinguished by differential expression of IgA and HLA-DR (Supplementary Figure S3A,B). Naive B cell clusters (c1, c3) and clusters comprising PB/PC (c8, c9, c10) were merged for downstream analyses (Figure 2A, Supplementary Figure S3A). Confirming our results from the global analysis, PB/PC showed the highest expression of CD38 among B cells, followed by naive and memory B cell clusters showing overall lower average expression of CD38 (Figure 2A,B). The lowest mean CD38 expression in the B cell lineage was detected on CD11c$^+$ B cells, which are linked to chronic inflammation [22,23]. We again tested for

differences in the expression of CD38 in SLE vs. HC and detected an increased mean CD38 expression on $CD27^{-}/IgD^{-}$ B cells (2.2-fold increase in SLE) and marginal zone-like B cells (1.6-fold increase), the latter showing the p value between patients and controls. Consistently, we detected significantly increased frequencies of $CD38^{hi}$ and $CD38^{int}$ B cells among marginal zone-like B cells (3.3-fold, $p = 0.01$ and 2.0-fold, $p = 0.003$) and of $CD38^{hi}$ cells among $CD27^{-}IgD^{-}$ B cells (2.6-fold, $p = 0.05$) in SLE patients, but not among other B cell clusters (Supplementary Figure S3D,E). Since targeting of PB/PC is one major rationale for CD38-directed treatment in SLE, we analyzed whether subsets of PB/PC expressed similar levels of CD38, and hence stratified IgA^{+} and IgA^{-} PB/PC, and HLA-DR^{high} PB vs. HLA-DR^{low} PC. In all four subsets, we observed the same trend of increased CD38 expression in SLE patients vs. HC detectable in total PB/PC (Figure 1C), yet not associated with statistical significance (Supplementary Figure S3E). When SLE and HC samples were combined, we did, however, find that IgA^{-} PB/PC (that is, IgG^{+} and IgM^{+} PB/PC) expressed higher levels of CD38 compared to their IgA^{+} counterparts (1.2-fold, $p = 0.07$) and that mean CD38 expression levels were higher on HLA-DR^{high} PB compared to HLA-DR^{low} PC (1.4-fold increase, $p = 0.01$).

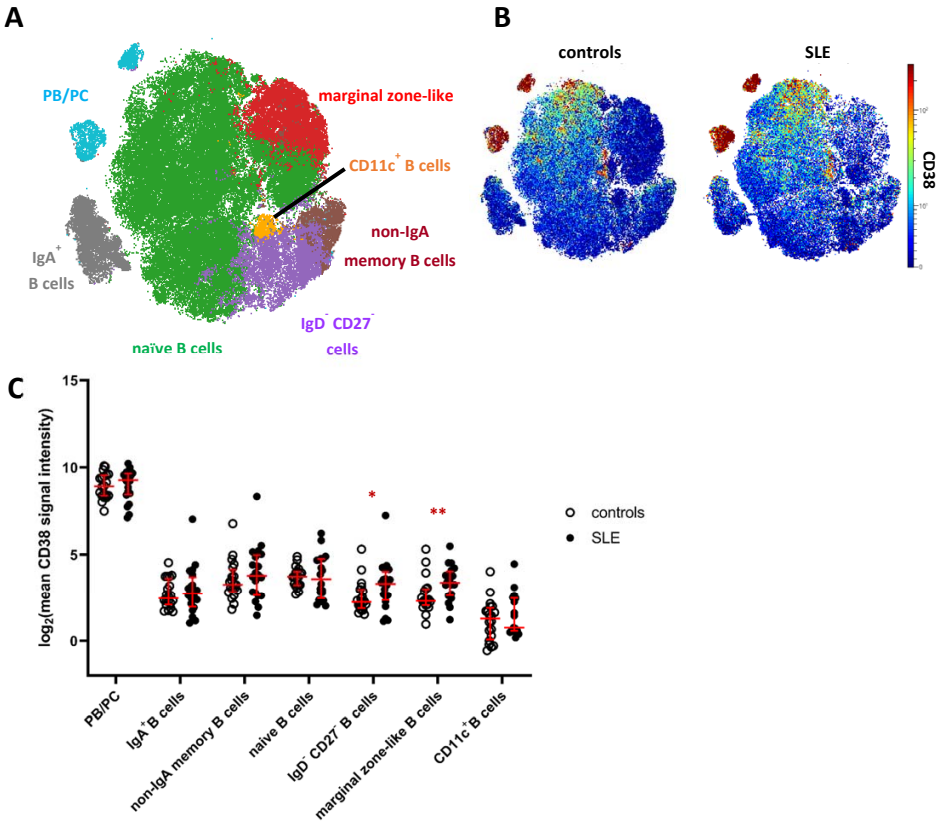

Figure 2. CD38 expression across B cell subsets in patients with SLE. (**A**) t-SNE map showing clusters of $CD19^{+}$ B cells generated by FlowSOM from the mass cytometry dataset. Clusters comprising naive B cells (green) and PB/PC (blue) were merged for further analyses (Supplementary Figure S3). (**B**) CD38 expression across B cells in SLE patients and controls. Both plots show concatenated data of the respective group. (**C**) Comparison of CD38 expression across major clusters as depicted in (**A**). Each dot represents the $\log_2$ of mean CD38 signal intensity (SI) of the indicated subset of one donor. Red lines indicate medians and interquartile ranges. * indicate significantly different CD38 expression in SLE patients vs. controls revealed by Mann–Whitney testing (* $p < 0.05$; ** $p < 0.01$).

2.4. Circulating CD4⁺ and CD8⁺ Memory T Cell Subsets Express Increased Levels of CD38 in SLE

To analyze the CD38 expression pattern and to address potential changes in SLE, we subjected total CD3⁺ T cells (merged from all T cell subsets in Figure 1A) to FlowSOM clustering and subsequent hierarchical metaclustering to obtain fifteen T cell clusters, comprising five CD4⁻ CD8⁻ T cell clusters, three CD8⁺ effector memory (EM) subsets, two CD4⁺ effector memory clusters and clusters representing naïve CD4⁺ and CD8⁺ T cells, regulatory T cells (Treg), as well as CD4⁺ and CD8⁺ central memory (CM) T cells (Figure S4). CD4⁻ CD8⁻ T cell clusters (c6, c8, c9, c10, c11), CD4⁺ effector memory clusters (c4, c5), and CD8⁺ effector memory subsets (c7, c14) were merged for further analysis (Figure 3A). Global CD38 expression for concatenated SLE patient and HC data was projected onto a t-SNE dimension reduction plot (Figure 3B), confirming that CD38 was variably expressed across individual T cells and T cell clusters. In HC, naïve CD4⁺ T cells showed higher average expression of CD38 compared to naïve CD8⁺ T cells ($p < 0.0001$, 2.7-fold) and to CD4⁺ central and effector memory T cell clusters, respectively ($p < 0.001$). The same trend was observed for CD8⁺ effector memory vs. naïve T cells. Of all T cell subsets analyzed in this study, Tregs showed the highest average CD38 expression.

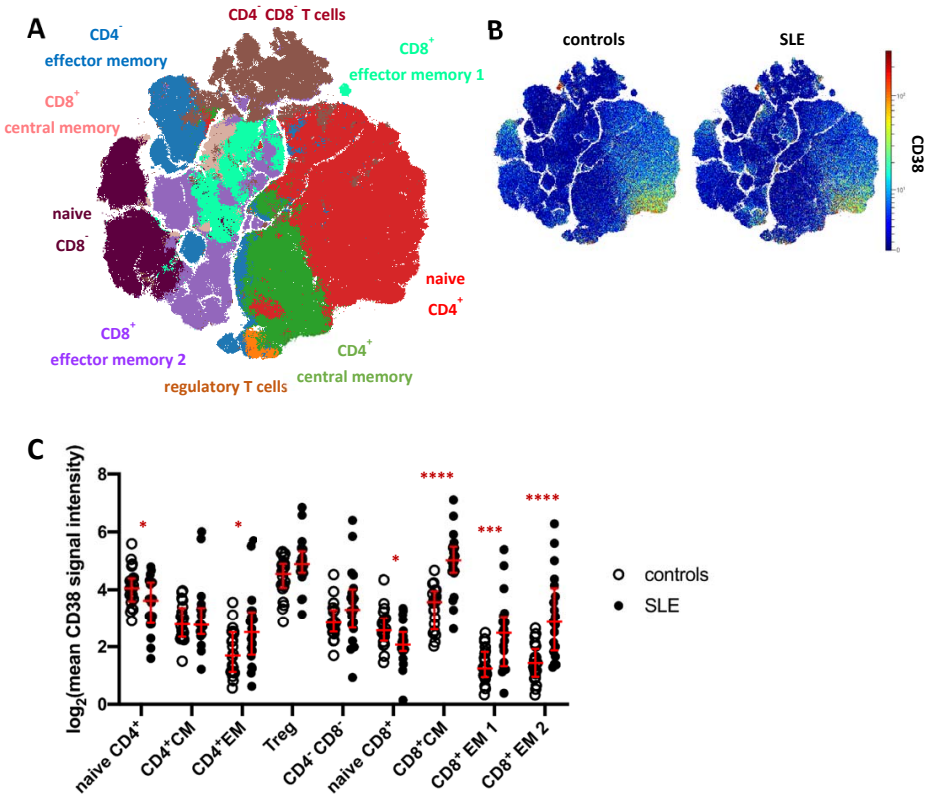

Figure 3. CD38 expression across T cell subsets in patients with SLE. (**A**) t-SNE map showing T cell clusters obtained by FlowSOM clustering from the mass cytometry dataset. Major T cell subsets are annotated. Clusters comprising CD4⁻CD8⁻T cells (brown), CD4⁺ effector memory T cells (blue), and CD8⁺ effector memory subsets (turquoise) were merged for downstream analyses (Supplementary Figure S4A). (**B**) CD38 expression across T cells in SLE patients and controls. (**C**) T cell clusters depicted in (**A**) were analyzed for their CD38 expression. Each dot represents the $\log_2$ of mean CD38 signal intensity (SI) of the indicated subset of one donor. Red lines indicate medians and interquartile ranges. * indicates significantly different CD38 expression in SLE patients vs. controls revealed by Mann–Whitney testing (* $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$).

CD38 expression by T cell clusters was found to be strongly dysregulated in SLE (Figure 3C). In line with increased CD38 expression in B cell and innate immune cell subsets, significantly increased CD38 expression was evident in $CD4^+$ and $CD8^+$ memory T cells. The largest differences were found in $CD8^+$ central memory and effector memory subsets (3.4-fold, 3.1-fold, and 4.9-fold SI, respectively). $CD4^+$ effector memory T cells also showed increased CD38 expression in SLE patients compared to controls (2.4-fold SI). Regulatory T cells showed a tendency towards higher CD38 levels in SLE, which did, however, not yet reach statistical significance. In contrast, naïve $CD4^+$ and $CD8^+$ T cells showed reduced CD38 expression in SLE compared to HC (both 1.4-fold, $p = 0.04$ and $p = 0.02$, respectively).

In accordance with previous works, we observed that CD38 expression was inhomogeneous in clusters of naive and memory T cells (Supplementary Figure S4C), and we determined frequencies of $CD38^{-/low}$, $CD38^{int}$, and $CD38^{hi}$ cells among the different clusters in SLE patients and HC to account for this heterogeneity. Except for naïve $CD4^+$ T cells and Tregs comprising comparably high average frequencies of $CD38^{int}$ and $CD38^{hi}$ cells of 30% and 13%, and 13% and 16%, respectively, all T cell clusters comprised an average of 80% or more $CD38^{-/low}$ cells, indicating that CD38 is selectively expressed by relatively few T cells in peripheral blood. In SLE, we detected significantly increased frequencies of $CD38^{int}$ and $CD38^{hi}$ cells subsets in $CD4^+$ and $CD8^+$ effector memory subsets, $CD8^+$ central memory, Tregs, and $CD4^-CD8^-$ T cells (Supplementary Figure S4). Thus, CD38 was variably expressed by T cells in the peripheral blood, and CD38 expression allowed for the separation of $CD38^{int}$ and $CD38^{hi}$ expressing T cell subsets. Except for naïve $CD4^+$ T cells, CD38 expression marked minor fractions of the different T cell subsets. CD38 expression, the presence of CD38-expressing T cells in the blood, or both, were dysregulated in SLE in naive and memory T helper and cytotoxic T cells, with most striking differences in $CD8^+$ memory T cells.

We noted that some SLE patients, but not HC, showed distinctly high expression of CD38 in memory T cell subsets, indicating that beyond the detection of significantly increased CD38 expression in various memory T cell and other subsets in the entire SLE group, CD38 expression by T cells may strongly vary from patient to patient.

In summary, mass cytometric profiling indicated increased CD38 expression levels in almost all major peripheral blood immune cell lineages in SLE on a global level, which were attributable to increased expression levels on individual, mostly activated immune cell subtypes when analyzed on the single-cell level.

2.5. CD38 Expression Is Increased on Monocyte, NK Cell, and B Cell Subsets in an Independent SLE Cohort Flow Cytometry Cohort

To extend the mass cytometry results, we analyzed the CD38 expression of key leukocyte subsets in a second cohort of 36 SLE patients by multicolor flow cytometry and compared the data to that of 19 HC (Table 1). We analyzed six subsets of $CD19^+$ B cells, in particular $CD24^-CD38^{++}$ PB/PC, $CD24^{++}CD38^{++}$ transitional B cells, and B cells that were neither PB/PC nor transitional B cells, which were further divided according to their expression of IgD and CD27 (Figure 4A). As previously described [24–26], SLE patients showed elevated frequencies of IgD^-CD27^- double-negative B cells, transitional B cells, and PB/PC, but decreased frequencies of IgD^+CD27^- naive and IgD^+CD27^+ unswitched memory B cells. Naive B cells in HC showed intermediate expression of CD38, while most memory and IgD^-CD27^- B cells displayed low if any expression of CD38. Similar to the cohort analyzed by mass cytometry, marginal zone-like IgD^+CD27^+ B cells had significantly higher expression levels of CD38 compared to HC (2.8-fold increase), and CD38 expression on IgD^-CD27^+ class-switched memory B cells was increased in SLE (1.5-fold increase, Figure 4B). While not reaching statistical significance, the relative difference in mean expression of CD38 was comparable and confirmatory to the mass cytometry results.

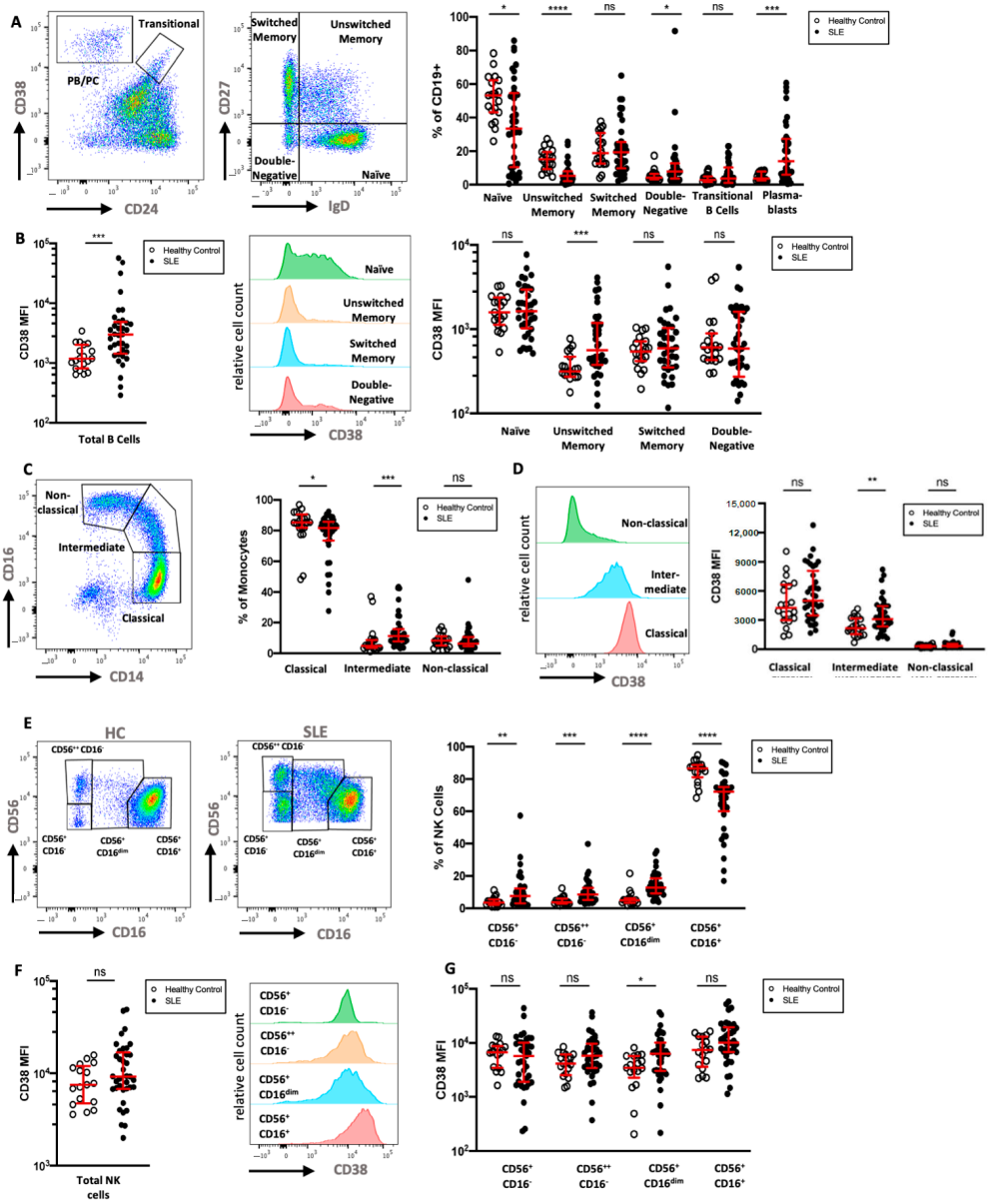

Figure 4. Flow cytometric analysis of CD38 expression in B cells, monocytes, and NK cells (**A**) Gating of CD19$^+$ B cells in a HC, with separation of plasmablasts (CD24$^-$CD38^{++}) and transitional B cells (CD24^{++}CD38^{++}). B cells that were neither plasmablasts nor transitional B cells were divided into naive (IgD$^+$CD27$^-$), unswitched memory (IgD$^+$CD27$^+$), switched memory (IgD$^-$CD27$^+$), and double-negative B cells (IgD$^-$CD27$^-$). Relative frequencies of B cell subsets in 36 SLE patients and 19 HC are shown. (**B**) Comparison of CD38 expression on all B cells and on the previously defined B cell subsets in SLE and HC. (**C**) Gating and relative frequencies of classical (CD14^{++}CD16$^-$), intermediate (CD14^{++}CD16$^+$), and non-classical (CD14$^+$CD16^{++}) monocytes. (**D**) CD38 expression on monocyte subsets in a representative HC and comparison of CD38 expression on monocyte subsets between HC and SLE patients (**E**). Representative gating of CD56$^+$CD3$^-$ NK cell subsets into four subsets in one HC and one SLE patient. (**F**,**G**) Comparison of CD38 expression on all NK cells and on the previously defined NK cell subsets. Mann–Whitney test of 19 HCs and 36 SLE patients. ns, not significant, $p >= 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; MFI: median fluorescence intensity.

Next, we analyzed monocyte subsets in more detail. In particular, classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^{dim}$), and non-classical ($CD14^{dim}CD16^+$) monocytes were investigated (Figure 4C). SLE patients showed higher relative frequencies of intermediate monocytes and lower frequencies of classical monocytes compared to HC. We found that monocytes express intermediate levels of CD38, and that the expression of CD38 was highest on classical monocytes and lower on intermediate and non-classical monocytes (Figure 4D). There was no significant difference in the CD38 expression of total monocytes when comparing SLE patients and HC; however, significantly increased CD38 expression was evident on intermediate monocytes in SLE (1.5-fold increase).

While most circulating NK cells expressed a $CD56^+CD16^{high}$ phenotype in SLE patients, we found increases in the fractions of $CD56^{low}CD16^-$, $CD56^{high}CD16^-$, and especially of the $CD56^+CD16^{dim}$ NK cells, a phenotype that has been observed in NK cells after engagement with target cells [27] (Figure 4E). CD38 expression was uniformly high on NK cells of both patients and HC; however, SLE patients had a larger variation in median CD38 expression (Figure 4F). While three out of four NK cell subsets showed a trend towards higher CD38 expression in SLE patients, only the $CD56^+CD16^{dim}$ subset had significantly increased CD38 levels (2.2-fold increase, Figure 4G).

Given the significant difference in age and sex distribution between HC and SLE patients in these cohorts (Table 1), we analyzed the correlation between age and the CD38 expression levels on immune cell subsets, in which we observed significant differences in CD38 expression. No significant correlation (Spearman correlation, $p > 0.05$, $|r| < 0.4$) was found in this limited sample size, neither for HC nor for SLE patients. Similarly, CD38 expression levels did not significantly differ between male and female donors. In summary, these results expand the mass cytometry results with consistent increases in CD38 expression on subsets of B cells and NK cells in an independent cohort of SLE patients.

2.6. CD38 Expression Correlates between Individual Immune Cell Subsets

To assess the potential correlation of CD38 expression in different immune cell subsets from the mass cytometry dataset, we performed Spearman correlation of mean signal intensity (SI) values across the cell types and subsets identified, separately for controls and SLE patients (Figure 5). We found that CD38 expression levels were either positively or not correlated with each other across the different immune cell types. Inverse correlations were rarely observed and did not reach statistical significance. In HC, we observed 45 positive correlations with $p < 0.001$ (Figure 5A), while we found 56 in SLE (Figure 5B).

In controls, CD38 expression levels on myeloid lineages, except for eosinophils, were widely correlated (Figure 5A). Furthermore, CD38 expression levels on naïve $CD4^+$ and $CD8^+$ T cells, as well as $CD4^-CD8^-$ DN T cells, correlated with those on innate immune cells in many instances, especially with subsets that robustly express CD38, that is, basophils, monocytes, and pDC. Additionally, we observed correlations between CD38 levels of plasmablasts and other B cell subsets, as well as $CD4^+$ and some $CD8^+$ T cell subsets. On the background of overall positively correlated CD38 expression, marginal zone-like B cells were a notable exception, inasmuch as r values were consistently smaller compared to the overall r values observed in this analysis. In SLE patients, we observed a profound reconfiguration in the correlation landscape of CD38 expression (Figure 5B). Different from controls, CD38 expression by pDC did not significantly correlate with CD38 levels expressed by other immune cell subsets, while eosinophil CD38 expression was significantly correlated with CD38 levels of other myeloid cell lineages. Furthermore, NK cell CD38 levels showed multiple positive correlations with all innate cell subsets except DC and with memory T cell subsets. PB/PC CD38 levels were associated with those of neutrophils, basophils, monocytes, and naïve $CD4^+$ T cells, again, only in patients with SLE.

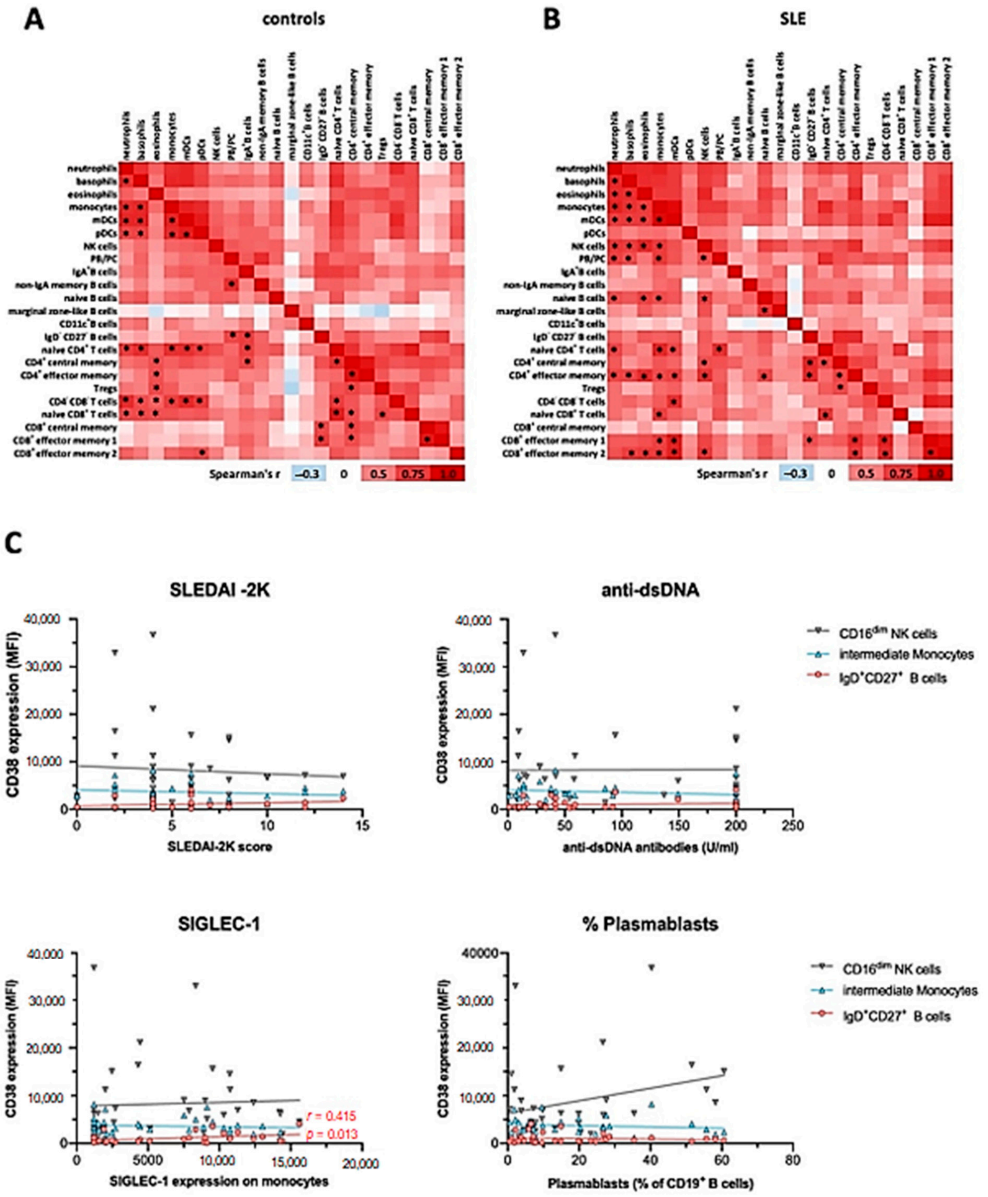

Figure 5. Correlation of CD38 expression levels across major immune cell subsets and with clinical activity. Mean CD38 SI values of immune cell subsets identified in Figures 1–3 were analyzed using Spearman correlation. Red color indicates positive correlation ($r > 0$), white indicates no correlation ($r = 0$), and blue color indicates negative correlation ($r < 0$). * indicates significant correlations with a p value of < 0.001. (**A**) Data of 20 healthy controls. (**B**) Data of 20 SLE patients. (**C**) Spearman correlation of the disease activity score SLEDAI-2K, the immunological disease activity markers dsDNA antibody levels, SIGLEC-1 expression on monocytes, and the percentage of plasmablasts among $CD19^+$ B cells with the CD38 expression on $CD16^{low}$ NK cells, $CD14^{++}CD16^+$ monocytes, and IgD^+CD27^+ B cells, based on the flow cytometric analysis of 36 SLE patients.

Taken together, this analysis suggests that CD38 expression by peripheral blood immune cells is remarkably coordinated, especially across innate cell types and T cells, but also with notable examples spanning different hematopoietic lineages. SLE patients exhibit a reconfigured correlation landscape of CD38 expression, suggesting that CD38 expression, or the abundance of $CD38^+$ cells in different lineages is at least in part regulated by the same or interrelated factors.

Finally, to assess whether SLE patients and HC could be distinguished by their CD38 expression profile, we performed dimension reduction using multidimensional scaling (mds) based on mean CD38 expression of all 40 donors analyzed by mass cytometry across all immune cell types and subsets (Supplementary Figure S5B). SLE patients and controls were indeed distinguishable by their immune cell CD38 expression profiles, with SLE patients and HC occupying mostly distinct areas of the mds plot. SLE patients showed a more diverse pattern of CD38 expression levels than controls, with some patients grouping closer to HC, while others were clearly separated. Notably, SLE outliers (exceeding the value of 5 in mds_1) are the same that showed extraordinarily high CD38 expression in memory T cell subsets, indicating that T cell CD38 expression is a major determinant of patient heterogeneity related to CD38 expression.

In summary, intraindividual correlation of CD38 expression levels by different leukocyte subsets was common in HC, with clusters of the statistically robust correlations between subsets of shared lineages. While there was still a high degree of correlation between the CD38 expression in different immune cell subsets in SLE, some correlations were selectively found in SLE patients, such as among T cell subsets, while on some cell types, such as pDCs, CD38 expression was less often significantly correlated with other subsets.

2.7. CD38 Expression Levels on Immune Cell Subsets Does Not Correlate with Severity or Clinical Manifestations of SLE

Consistently, and although CD38 has been described as an activation marker on immune cells [28], the increased levels of CD38 on the different subsets in SLE did not correlate with clinical activity as measured by the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (Figure 5C). Likewise, we did not observe correlations of CD38 expression patterns with serum levels of anti-double-stranded DNA (dsDNA) antibodies or complement consumption. In addition, we did not find significant correlations of CD38 expression levels with the presence of specific organ manifestations such as nephritis, mucocutaneous, musculoskeletal symptoms, or use of immunosuppressive treatments. Given the potential role of IFN-I in upregulating CD38 [29], we further analyzed the expression of monocytic CD169 (SIGLEC-1), an established surrogate marker for IFN-I activity [20], in the context of the CD38 expression profiles. While SIGLEC-1 expression in monocytes from SLE patients was significantly increased in both SLE cohorts, and $SIGLEC\text{-}1^+$ monocytes robustly expressed CD38 (Supplementary Figure S2B), there was no correlation with average CD38 expression on total monocytes or intermediate monocytes across the patients, indicating that a stimulus independent from IFN-I may have induced the increased CD38 expression in SLE intermediate monocytes. However, the CD38 expression on marginal zone-like B cells mildly correlated with SIGLEC-1 expression on monocytes ($r = 0.415$, $p = 0.013$). Taken together, although the CD38 expression levels in SLE were increased on different cell types, CD38 expression did not correlate with clinical severity, serologic activity, or individual disease manifestations.

3. Discussion

Motivated by the potential therapeutic use of CD38-targeting antibodies for the treatment of SLE, this study dissected the expression profiles of CD38 in peripheral blood immune cells and described dysregulated CD38 expression in SLE patients compared to healthy controls. In accordance with previous reports [9,10,12], we found that CD38 was variably expressed on all immune cell types, with cell-type specific expression levels peaking in PB/PC. CD38 showed inhomogeneous expression profiles in innate and even more so in adaptive immune cell subsets, ranging from lack of expression to very high

levels. This heterogeneity was detectable at levels of low- and high-resolution analyses of different cells types, indicating that the capacity to express CD38 is not restricted to certain immune cell lineages. Initial studies already indicated increased CD38 expression levels on peripheral blood leukocyte cells isolated from SLE patients [9–12,15]. Our data confirm and extend these findings. In particular, we found CD38 expression levels in SLE significantly increased on circulating plasmacytoid dendritic cells, $CD14^{++}CD16^{+}$ intermediate monocytes, $CD56^{+}$ $CD16^{dim}$ natural killer (NK) cells, marginal zone-like $IgD^{+}CD27^{+}$ B cells, and subsets of central and effector memory $CD4^{+}$ and especially $CD8^{+}$ T cells. However, although the CD38 expression on different immune cell subsets showed high intraindividual correlations and leukocyte CD38 expression allowed to segregate most SLE patients from HC, we did not identify significant correlations between CD38 expression levels and disease severity or clinical manifestations, suggesting that increased CD38 expression by immune cells is a static feature of SLE, mechanistically or timely unrelated to acute inflammation and clinical flares. However, CD38 expression profiles, as generated in the present study, integrate potentially superimposed regulation of CD38 expression by individual cells and the emergence and homeostasis of cell subsets expressing different levels of CD38 in the blood. Both potential SLE- or inflammation-related induction of CD38 expression [13,15] and overabundance of constitutively CD38-expressing cells such as PB/PC, being established biomarkers for disease activity in SLE [24], have been described in active SLE and cannot be reliably distinguished from each other. Irrespective of what may cause dysregulation of CD38 in immune cells in SLE, the magnitude of CD38 expression across the different cell types may not be a suitable biomarker candidate of the SLE disease activity and phenotype. Instead, the ability to identify patients with increased CD38 expression or abundance of CD38-expressing cells might be relevant in the context of emerging CD38-directed treatments with approved or preclinical candidate compounds, including CD38 CAR T cells, daratumumab, isatuximab, GBR 1342, TAK169, and TAK079. In that regard, our previous report of two SLE patients who underwent anti-CD38 targeted treatment with the monoclonal antibody daratumumab provided the first insight into consequences of CD38-targeting antibodies on the immune system outside malignant conditions [11]. We observed overall stable counts of the major blood leukocyte subsets, except for NK cells and pDC, both robustly expressing CD38, which transiently decreased in circulation after anti-CD38 treatment. However, more intricate effects of daratumumab treatment may occur, such as interference with B cell and T cell maturation in the bone marrow and thymus, where CD38 is expressed in pro-B cells, pre-B cells, and transitional B cells, as well as $CD4^{+}CD8^{+}$ double positive thymocytes [30–33] in addition to memory plasma cells. In peripheral blood, high expression of CD38 has been suggested as a marker of immunosuppressive, so-called regulatory B cells [34], while pro-inflammatory GM-CSF-producing B cells express low levels of CD38 [35]. CD38 on T cells has been reported to be expressed both on activated, proinflammatory T cells [36] as well as on regulatory T cells [37].

The increased expression of CD38 on SLE marginal zone-like B cells and also $CD27^{+}IgD^{-}$ switched memory B cells was previously unrecognized. This may result from expansions of CD20-expressing plasmablast precursors that already express high levels of CD38 [38]. In fact, a small population of human blood $CD20^{+}CD27^{+}CD43^{+}IgD^{+/-}$ B cells, which includes such PB precursors as well as a unique population exercising function akin to murine B1 cells [38,39], was found to be increased in SLE [40], and may contribute to the increase of median CD38 expression in $CD27^{+}$ B cells in SLE.

Taken together, our data indicate CD38-targeting treatments are expected to have a wide cellular range of action beyond the targeting of PB/PC, preferentially acting on cells expressing high to very high levels of CD38, and exerting immunomodulation by distinct mechanisms, i.e., by depletion of certain cell types, and non-depleting binding to cell-surface CD38 on others. Vice versa, effects of anti-CD38 monoclonal antibodies on $CD38^{-/low}$ cells cannot be excluded, since they might be secondary to the modulation or depletion of primary target cells. Overall, the effects of CD38-targeted therapies on

the immune system will require further investigations, as the effect on the equilibrium of pro- and anti-inflammatory cells is not obvious. Future studies also need to incorporate the distribution of CD38 on immune cells in inflamed tissues, such as skin and kidney in SLE patients.

The individual cell subsets with increased CD38 expression deserve follow up studies to fully explore the expression profile of CD38 across cell subsets, activation, and differentiation states. In particular, it needs to be investigated whether the group of SLE patients characterized by high CD38 immune cell levels (i) maintain this phenotype over the course of disease or (ii) have a different prognosis or long-term outcome compared to those without dysregulated CD38 expression, and (iii) whether this is associated with a certain genetic background. The mechanism of action of CD38-directed therapies on the different cell types beyond depletion requires exploration, including the modulation of its enzymatic activity. As CD38 is a proposed treatment target for SLE, identification of patients with profound increases in CD38 expression pre-treatment could serve as a predictor for treatment responsiveness and thus advance personalized treatments in SLE.

It would be interesting to determine the factors controlling the differential CD38 expression on immune cells and the subset-specific increase of CD38 expression in SLE. Previous reports indicated that a large range of activatory stimuli may modulate the expression of CD38 on different immune cell subsets. Particularly, type I interferons, hallmark cytokines of SLE pathogenesis, were previously shown to induce CD38 [29]. Other known inducers of CD38 expression include interferon gamma [41], tumor necrosis factor (TNF) [42], and LPS [13]. Nevertheless, we found that CD38 expression on different CD38 subsets only poorly correlated with levels of the established interferon surrogate parameter SIGLEC-1 [3], indicating that additional stimuli likely modulate the expression of CD38 in SLE. On pDC, CD38 is inducible by TLR agonists, such as influenza virus, and, when treated with anti-CD38 in vitro, the capacity of pDC to produce TNFa and IFNa is largely abrogated [43]. Other cells, such as antibody-secreting cells (plasmablasts and plasma cells), constitutively express high levels of CD38. The fact that CD38 levels on separate immune cell subsets showed moderate correlations in general but multiple subgroups of high intraindividual correlation, indicates that a combination of different stimuli as well as differential receptor expression may be responsible for the up-regulation of CD38 on certain cell types.

A potential caveat for the interpretation of CD38 expression levels obtained from cytometric assays is the reported presence of anti-CD38 autoantibodies in SLE patients that could potentially downregulate CD38 or inhibit the binding of detection antibodies. Interestingly, a previous study found that endogenous anti-CD38 antibodies negatively correlated with disease activity, which could indicate that endogenous anti-CD38 activity confers a protective effect in SLE [10]. Previous research on the role of NK cells in the pathogenesis of SLE focused on the relative hyporeactivity and impaired cytotoxicity in NK cells [44] and reported the relative increase in $CD56^{bright}$ NK cells [45], which we reproduced in this report. Additionally, we report an increase in the frequency of $CD16^{dim}$ NK cells in SLE, a phenotype that has been linked to a post-activation state of NK cell [27]. The potential role of these cells in the SLE pathogenesis, as well as their value for diagnosis and prognosis, are important aspects for future studies.

In conclusion, we identified a widespread dysregulation of CD38 expression in SLE that was found over a variety of leukocyte subsets in the peripheral blood. CD38 expression highly correlated over different leukocyte subsets within individual patients, but was heterogenous within the population of SLE patients. Future studies will be needed to identify the mechanisms that influence CD38 expression in SLE as well as the pathogenic role of cells with increased CD38 expression, especially in light of anti-CD38 monoclonal antibodies being an emerging plasma cell-targeting therapy in SLE.

4. Materials and Methods

4.1. Patient and Control Blood Samples

Initially, we recruited 20 SLE patients from the Charité–Universitätsmedizin Berlin, Department of Rheumatology and Clinical Immunology. These patients were analyzed using mass cytometry, compared to 20 age- and gender-matched healthy controls. In an additional cohort, 36 SLE patients and 19 healthy controls were included for the flow cytometric analysis. A subset of 10 patients was included in both cohorts. Patient characteristics are summarized in Table 1. SLE patients were diagnosed according to the 2019 EULAR/ACR classification criteria for SLE [46]. Clinical manifestations, the SLEDAI-2K [47], and immunosuppressive medication were recorded by the treating physician. Established markers of immunological activity such as complement factor levels, autoantibody titers, and SIGLEC-1 expression on monocytes [20] were routinely analyzed by the local laboratory (Labor Berlin).

4.2. Cryopreservation of Whole Blood Samples

Heparinized whole blood samples were cryopreserved using Proteomic Stabilizer (Smart Tube Inc., San Carlos, CA, USA) as indicated by the manufacturer within 30 min after phlebotomy and stored at −80 °C. On the day of processing, samples were thawed in a stirred water bath at 10 °C and incubated with 5 mL of thaw-lyse buffer (Smart Tube Inc.) for 10 min at room temperature in 15 mL centrifuge tubes. Cells were centrifuged at 700× *g* for 5 min and the erythrocyte lysis step was repeated with 25 mL of thaw-lyse buffer for 5 min at room temperature in 50 mL centrifuge tubes. Peripheral blood leukocytes were washed twice with cell staining medium (1 × PBS made from 10 × PBS ((Rockland Immunochemicals, Gilbertsville, PA, USA) using MilliQ water, supplemented with 0.5% (*w/v*) BSA (PANBiotech, Aidenbach, Germany) and 0.02% sodium azide (Sigma-Aldrich, St. Louis, MO, USA)). Cell counts were determined volumetrically using a MACSQuant Analyzer 10 (Miltenyi Biotec, Bergisch-Gladbach, Germany). Prior to further processing in 5 mL polystyrene tubes (Corning, Corning, NY, USA), cell counts were adjusted to 1.6×10^6 cells per sample.

4.3. Mass Cytometry

Samples were barcoded for 20 min using a five-choose-two scheme at room temperature using isothiocyano-benzyl-EDTA (ITCB-EDTA) containing isotopically enriched Palladium ions with atomic masses of 102, 104, 106, 108, and 110 Da [48]. Each run contained five patient and five control samples. A reference sample, labelled with mDOTA-Rh103, was included in each run to monitor assay performance. Assignment of patient and control samples to individual barcodes and runs is included in Supplementary Table S1B,C. After barcoding, samples were washed twice with 3 mL cell staining medium and then pooled. Fc receptor blocking was performed for 10 min at room temperature using 0.2 mg/mL Beriglobin (CSL Behring, King of Prussia, PA, USA), followed by a washing step with 2 mL cell staining medium. Cells were then incubated for 15 min in heparin–sodium (Ratiopharm, Ulm, Germany) at a final concentration of 100 U/mL in cell staining medium (heparin-CSM) at room temperature, as described before [49]. Cell-surface staining was performed for 30 min at room temperature in the presence of heparin and stopped by addition of 2 mL cell staining medium. Antibodies are listed in Supplementary Table S1A. Cells were pelleted, and washed once more with 2 mL PBS before fixation with 4% paraformaldehyde solution (Electron Microscopy Sciences, Hatfield, PA, USA) for 10 min at room temperature. After fixation, cells were centrifuged and gently resuspended in 1.6 mL −20 °C cold methanol (Carl Roth, Karlsruhe, Germany). Samples were stored at −80 °C overnight. The following day, cells were washed twice with 4 mL cell staining medium, followed by another 15 min- blocking step with heparin-CSM at room temperature. Antibodies directed against intracellular antigens (Supplementary Table S1A) were added directly to the cell suspension and incubated for 60 min at room temperature. Cells were then washed once with 2 mL cell staining medium and PBS before fixing the cells with 2% paraformaldehyde

for 10 min at room temperature. Next, the samples were incubated at room temperature for 25 min in 2 mL PBS supplemented with 1:500 (*v/v*) 0.125 mM iridium-based DNA intercalator (Fluidigm, South San Francisco, CA, USA). Cells were then washed twice with 2 mL cell staining medium and twice with 2 mL deionized water, before filtering samples through a 30 µm cell strainer (Corning, Corning, NY, USA). Finally, the sample was resuspended at 7.5 × 105 cells/mL, and 10% (*v/v*) EQ Four Element Calibration Beads (Fluidigm, South San Francisco, CA, USA) were added to the sample before acquisition. Cells were acquired on a mass cytometer (Helios, Fluidigm, South San Francisco, CA, USA) at a rate of 250 to 350 events per second. Raw mass cytometry data were converted to Flow Cytometry Standard 3.0 files during acquisition. Data files were normalized using the Helios software version 6.7.1014 based on EQ Four Element Calibration Beads passport P13H2302.

Prior to analysis, all cytometric channels except time, event length, and the Gaussian parameters were arcsinh transformed with a scale argument of 5. Intact, nucleated cells events were identified by exclusion of beads according to their 140Ce signal, and by gating on events stained by iridium intercalator and CD45 antibody. Cells with an event length of >30 were excluded to minimize doublets. Cell samples were debarcoded by Boolean gating [50,51]. Compensation of signal spillover was performed as described [52,53]. On average, 190.921 cells were included from each donor (range, 131.628–200.000 cells), yielding 7.64 × 106 total cells, which were used for downstream analyses.

4.4. PBMC Isolation

For flow cytometry, peripheral blood mononuclear cells (PBMCs) were isolated and stained as previously described [54]. In short, 35 mL of a 1:1 (*v/v*) mix of heparinized blood and phosphate-buffered saline supplemented with 0.2% bovine serum albumin (PBS/BSA) were layered onto 15 mL of Ficoll-Paque PLUS (GE Healthcare, Chicago, IL, USA) in 50 mL centrifuge tubes. After centrifugation (20 min at room temperature), PBMCs were isolated and washed twice in cold PBS/BSA, and were kept on ice for further use.

4.5. Flow Cytometry

Approximately 0.5 × 10^6 cells were stained in 100 µL PBS for 15 min with the antibodies indicated in Supplementary Table S2 as well as the Fixable Viability Dye eFluor™ 780 (ThermoFisher, Waltham, MA, USA) to stain dead cells. The samples were acquired on a FACSCanto flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). All cytometry experiments were performed according to published guidelines [55]. In order to ensure comparability of fluorescence intensities across measurements, daily bead calibration was performed and samples from patients and controls were preferentially measured on the same day. Cytometry data were analyzed using FlowJo v10.6.2 (FlowJo, LLC, Ashland, OR, USA) for Mac.

4.6. Data Analysis

Statistical analyses and visualizations were created using Prism v9.0.0 (GraphPad, San Diego, CA, USA). For comparisons of CD38 expression between healthy controls and SLE patients, the Mann–Whitney test was used. Correlation analysis of CD38 expression between different cell types or subsets was performed using Spearman correlation. Multidimensional scaling (mds) was performed using base R [56], as well as the tidyverse [57] and ggplot2 [58] packages. Dimension reduction by t-SNE was performed using the opt-SNE [19] implementation in OMIQ.ai (Santa Clara, CA, USA) with the default settings. The markers used for opt-SNE calculations are indicated in Supplementary Table S1A. On average, 99% of all events were assigned to one of the major leukocyte lineages shown in Figure 1A, according to their expression of lineage-defining markers (Supplementary Figure S1). The remaining events (not annotated in Figure 1A) were excluded from further analysis. On average, 4939 B cells (range 270–13.608, in total 1.97 × 10^5 cells) and 37.709 T cells per donor (1.147–58.041, in total 1.50 × 10^6 cells) were used for analyses shown in Figures 2 and 3. FlowSOM clustering [59,60] was performed in omiq.ai using the

markers listed in Supplementary Table S1, using a 10 × 10 grid, Euclidean distance, and consensus metaclustering. The final number of metaclusters was set to 10 for B and 15 for T cell analyses.

Supplementary Materials: The following are available online at https://www.mdpi.com/1422-0067/22/5/2424/s1: Supplementary Figure S1: T-SNE map of mass cytometry data from Figure 1A, colored by the expression of lineage-defining cell-surface markers which were used to annotate the major immune cell subsets., Supplementary Figure S2: Analysis of coexpression of CD38 and myeloid markers in innate immune cells and CD38 expression peripheral blood monocytes in the context of SIGLEC-1 expression. Supplementary Figure S3: Analysis of B cell subsets and PB/PC for their CD38 expression in patients with SLE. Supplementary Figure S4: Analysis of T cell subsets for their CD38 expression in patients with SLE. Supplementary Figure S5: Differential correlation of CD38 expression among immune cell subsets between SLE and controls. Supplementary Table S1: Mass cytometry antibodies and details, Supplementary Table S2: Flow Cytometry antibodies.

Author Contributions: Concept: T.A. and H.E.M.; methodology: M.B., H.E.M., and A.G.; experiments and formal analysis: L.O. and M.B.; resources: H.E.M., A.G., T.A., and F.H.; writing—original draft preparation: L.O., M.B., and H.E.M.; writing—review and editing: T.A., H.E.M., F.H., R.B., and A.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Charité–University Medicine Berlin (EA1/104/17 and EA1/132/16).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data from this study are available from the corresponding author upon request.

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References

1. Tsokos, G.C. Systemic Lupus Erythematosus. *N. Engl. J. Med.* **2011**, *365*, 2110–2121. [CrossRef] [PubMed]
2. Lande, R.; Ganguly, D.; Facchinetti, V.; Frasca, L.; Conrad, C.; Gregorio, J.; Meller, S.; Chamilos, G.; Sebasigari, R.; Riccieri, V.; et al. Neutrophils Activate Plasmacytoid Dendritic Cells by Releasing Self-DNA-Peptide Complexes in Systemic Lupus Erythematosus. *Sci. Transl. Med.* **2011**, *3*, 73ra19. [CrossRef] [PubMed]
3. Biesen, R.; Demir, C.; Barkhudarova, F.; Grün, J.R.; Steinbrich-Zöllner, M.; Backhaus, M.; Häupl, T.; Rudwaleit, M.; Riemekasten, G.; Radbruch, A.; et al. Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. *Arthritis Rheum.* **2008**, *58*, 1136–1145. [CrossRef] [PubMed]
4. Hiepe, F.; Radbruch, A. Plasma cells as an innovative target in autoimmune disease with renal manifestations. *Nat. Rev. Nephrol.* **2016**, *12*, 232–240. [CrossRef] [PubMed]
5. Hogan, K.A.; Chini, C.C.S.; Chini, E.N. The Multi-faceted Ecto-enzyme CD38: Roles in Immunomodulation, Cancer, Aging, and Metabolic Diseases. *Front. Immunol.* **2019**, *10*, 1187. [CrossRef] [PubMed]
6. Deaglio, S.; Morra, M.; Mallone, R.; Ausiello, C.M.; Prager, E.; Garbarino, G.; Dianzani, U.; Stockinger, H.; Malavasi, F. Human CD38 (ADP-ribosyl cyclase) is a counter-receptor of CD31, an Ig superfamily member. *J. Immunol.* **1998**, *160*, 395–402.

7. Partida-Sánchez, S.; Cockayne, D.A.; Monard, S.; Jacobson, E.L.; Oppenheimer, N.; Garvy, B.; Kusser, K.; Goodrich, S.; Howard, M.; Harmsen, A.; et al. Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nat. Med.* **2001**, *7*, 1209–1216. [CrossRef]
8. Partida-Sánchez, S.; Goodrich, S.; Kusser, K.; Oppenheimer, N.; Randall, T.D.; Lund, F.E. Regulation of Dendritic Cell Traf-ficking by the ADP-Ribosyl Cyclase CD38. *Immunity* **2004**, *20*, 279–291.
9. Katsuyama, E.; Suarez-Fueyo, A.; Bradley, S.J.; Mizui, M.; Marin, A.V.; Mulki, L.; Krishfield, S.; Malavasi, F.; Yoon, J.; Sui, S.J.H.; et al. The CD38/NAD/SIRTUIN1/EZH2 Axis Mitigates Cytotoxic CD8 T Cell Function and Identifies Patients with SLE Prone to Infections. *Cell Rep.* **2020**, *30*, 112–123.e4. [CrossRef]
10. Pavón, E.J.; Zumaquero, E.; Rosal-Vela, A.; Khoo, K.-M.; Cerezo-Wallis, D.; García-Rodríguez, S.; Carrascal, M.; Abián, J.; Graeff, R.; Callejas-Rubio, J.-L.; et al. Increased CD38 expression in T cells and circulating anti-CD38 IgG autoantibodies differentially correlate with distinct cytokine profiles and disease activity in systemic lupus erythematosus patients. *Cytokine* **2013**, *62*, 232–243. [CrossRef]
11. Ostendorf, L.; Burns, M.; Durek, P.; Heinz, G.A.; Heinrich, F.; Garantziotis, P.; Enghard, P.; Richter, U.; Biesen, R.; Schneider, U.; et al. Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus. *New Engl. J. Med.* **2020**, *383*, 1149–1155. [CrossRef] [PubMed]
12. Perry, D.J.; Titov, A.A.; Sobel, E.S.; Brusko, T.M.; Morel, L. Immunophenotyping reveals distinct subgroups of lupus patients based on their activated T cell subsets. *Clin. Immunol.* **2020**, *221*, 108602. [CrossRef] [PubMed]
13. Amici, S.A.; Young, N.A.; Narvaez-Miranda, J.; Jablonski, K.A.; Arcos, J.; Rosas, L.; Papenfuss, T.L.; Torrelles, J.B.; Jarjour, W.N.; Guerau-De-Arellano, M. CD38 Is Robustly Induced in Human Macrophages and Monocytes in Inflammatory Conditions. *Front. Immunol.* **2018**, *9*, 1593. [CrossRef] [PubMed]
14. Burlock, B.; Richardson, G.; García-Rodríguez, S.; Guerrero, S.; Zubiaur, M.; Sancho, J. The Role of CD38 on the Function of Regulatory B Cells in a Murine Model of Lupus. *Int. J. Mol. Sci.* **2018**, *19*, 2906. [CrossRef] [PubMed]
15. Cole, S.; Walsh, A.; Yin, X.; Wechalekar, M.D.; Smith, M.D.; Proudman, S.M.; Veale, D.J.; Fearon, U.; Pitzalis, C.; Humby, F.; et al. Integrative analysis reveals CD38 as a therapeutic target for plasma cell-rich pre-disease and established rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Res.* **2018**, *20*, 1–14. [CrossRef] [PubMed]
16. Scheibe, F.; Ostendorf, L.; Reincke, S.M.; Prüss, H.; Von Brünneck, A.-C.; Köhnlein, M.; Alexander, T.; Meisel, C.; Meisel, A. Daratumumab treatment for therapy-refractory anti-CASPR2 encephalitis. *J. Neurol.* **2019**, *267*, 317–323. [CrossRef]
17. Ratuszny, D.; Skripuletz, T.; Wegner, F.; Groß, M.; Falk, C.; Jacobs, R.; Ruschulte, H.; Stangel, M.; Sühs, K.-W. Case Report: Daratumumab in a Patient With Severe Refractory Anti-NMDA Receptor Encephalitis. *Front. Neurol.* **2020**, *11*. [CrossRef]
18. Schuetz, C.; Hoenig, M.; Moshous, D.; Weinstock, C.; Castelle, M.; Bendavid, M.; Shimano, K.; Tolbert, V.; Schulz, A.S.; Dvorak, C.C. Daratumumab in life-threatening autoimmune hemolytic anemia following hematopoietic stem cell transplantation. *Blood Adv.* **2018**, *2*, 2550–2553. [CrossRef]
19. Belkina, A.C.; Ciccolella, C.O.; Anno, R.; Halpert, R.; Spidlen, J.; Snyder-Cappione, J.E. Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets. *Nat. Commun.* **2019**, *10*, 1–12. [CrossRef]
20. Rose, T.; Grützkau, A.; Hirseland, H.; Huscher, D.; Dähnrich, C.; Dzionek, A.; Ozimkowski, T.; Schlumberger, W.; Enghard, P.; Radbruch, A.; et al. IFNα and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. *Ann. Rheum. Dis.* **2012**, *72*, 1639–1645. [CrossRef]
21. Odendahl, M.; Mei, H.; Hoyer, B.F.; Jacobi, A.M.; Hansen, A.; Muehlinghaus, G.; Berek, C.; Hiepe, F.; Manz, R.; Radbruch, A.; et al. Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response. *Blood* **2005**, *105*, 1614–1621. [CrossRef]
22. Wang, S.; Autoimmunity Molecular Medicine Team; Wang, J.; Kumar, V.; Karnell, J.L.; Naiman, B.; Gross, P.S.; Rahman, S.; Zerrouki, K.; Hanna, R.; et al. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11chiT-bet+ B cells in SLE. *Nat. Commun.* **2018**, *9*, 1–14. [CrossRef] [PubMed]
23. Karnell, J.L.; Kumar, V.; Wang, J.; Wang, S.; Voynova, E.; Ettinger, R. Role of CD11c + T-bet + B cells in human health and disease. *Cell. Immunol.* **2017**, *321*, 40–45. [CrossRef]
24. Jacobi, A.M.; Mei, H.; Hoyer, B.F.; Mumtaz, I.M.; Thiele, K.; Radbruch, A.; Burmester, G.-R.; Hiepe, F.; Dörner, T. HLA-DRhigh/CD27high plasmablasts indicate active disease in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **2009**, *69*, 305–308. [CrossRef]
25. Mei, H.E.; Hahne, S.; Redlin, A.; Hoyer, B.F.; Wu, K.; Baganz, L.; Lisney, A.R.; Alexander, T.; Rudolph, B.; Dörner, T. Plasmablasts With a Mucosal Phenotype Contribute to Plasmacytosis in Systemic Lupus Erythematosus. *Arthritis Rheumatol.* **2017**, *69*, 2018–2028. [CrossRef]
26. Odendahl, M.; Jacobi, A.; Hansen, A.; Feist, E.; Hiepe, F.; Burmester, G.R.; Lipsky, P.E.; Radbruch, A.; Dörner, T. Disturbed Peripheral B Lymphocyte Homeostasis in Systemic Lupus Erythematosus. *J. Immunol.* **2000**, *165*, 5970–5979. [CrossRef]
27. Grzywacz, B.; Kataria, N.; Verneris, M.R. CD56(dim)CD16(+) NK cells downregulate CD16 following target cell induced activation of matrix metalloproteinases. *Leuk.* **2007**, *21*, 356–359. [CrossRef]
28. Sandoval-Montes, C.; Santos-Argumedo, L. CD38 is expressed selectively during the activation of a subset of mature T cells with reduced proliferation but improved potential to produce cytokines. *J. Leukoc. Biol.* **2004**, *77*, 513–521. [CrossRef]

29. Mihara, K.; Yoshida, T.; Ishida, S.; Takei, Y.; Kitanaka, A.; Shimoda, K.; Morishita, K.; Takihara, Y.; Ichinohe, T. All-trans retinoic acid and interferon-α increase CD38 expression on adult T-cell leukemia cells and sensitize them to T cells bearing anti-CD38 chimeric antigen receptors. *Blood Cancer J.* **2016**, *6*, e421. [CrossRef] [PubMed]
30. Palanichamy, A.; Barnard, J.; Zheng, B.; Owen, T.; Quach, T.; Wei, C.; Looney, R.J.; Sanz, I.; Anolik, J.H. Novel Human Transitional B Cell Populations Revealed by B Cell Depletion Therapy. *J. Immunol.* **2009**, *182*, 5982–5993. [CrossRef]
31. Sims, G.P.; Ettinger, R.; Shirota, Y.; Yarboro, C.H.; Illei, G.G.; Lipsky, P.E. Identification and characterization of circulating human transitional B cells. *Blood* **2005**, *105*, 4390–4398. [CrossRef]
32. Tenca, C.; Merlo, A.; Zarcone, D.; Saverino, D.; Bruno, S.; De Santanna, A.; Ramarli, D.; Fabbi, M.; Pesce, C.; Deaglio, S.; et al. Death of T cell precursors in the human thymus: A role for CD38. *Int. Immunol.* **2003**, *15*, 1105–1116. [CrossRef]
33. Bendall, S.C.; Davis, K.L.; Amir, E.-A.D.; Tadmor, M.D.; Simonds, E.F.; Chen, T.J.; Shenfeld, D.K.; Nolan, G.P.; Pe'Er, D. Single-Cell Trajectory Detection Uncovers Progression and Regulatory Coordination in Human B Cell Development. *Cell* **2014**, *157*, 714–725. [CrossRef]
34. Blair, P.A.; Noreña, L.Y.; Flores-Borja, F.; Rawlings, D.J.; Isenberg, D.A.; Ehrenstein, M.R.; Mauri, C. CD19+CD24hiCD38hi B Cells Exhibit Regulatory Capacity in Healthy Individuals but Are Functionally Impaired in Systemic Lupus Erythematosus Patients. *Immunity* **2010**, *32*, 129–140. [CrossRef]
35. Li, R.; Rezk, A.; Miyazaki, Y.; Hilgenberg, E.; Touil, H.; Shen, P.; Moore, C.S.; Michel, L.; Althekair, F.; Rajasekharan, S.; et al. Proinflammatory GM-CSF–producing B cells in multiple sclerosis and B cell depletion therapy. *Sci. Transl. Med.* **2015**, *7*, 310ra166. [CrossRef]
36. Deterre, P.; Berthelier, V.; Bauvois, B.; Dalloul, A.; Schuber, F.; Lund, F. CD38 in T-and B-cell functions. In *Human CD38 and Related Molecules*; Karger Publishers: Basel, Switzerland, 2000; Volume 75, pp. 146–168.
37. Patton, D.T.; Wilson, M.D.; Rowan, W.C.; Soond, D.R.; Okkenhaug, K. The PI3K p110δ Regulates Expression of CD38 on Regulatory T Cells. *PLoS ONE* **2011**, *6*, e17359. [CrossRef]
38. Quách, T.D.; Rodríguez-Zhurbenko, N.; Hopkins, T.J.; Guo, X.; Hernández, A.M.; Li, W.; Rothstein, T.L. Distinctions among Circulating Antibody-Secreting Cell Populations, Including B-1 Cells, in Human Adult Peripheral Blood. *J. Immunol.* **2016**, *196*, 1060–1069. [CrossRef]
39. Griffin, D.O.; Holodick, N.E.; Rothstein, T.L. Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20+CD27+CD43+CD70−. *J. Exp. Med.* **2011**, *208*, 67–80. [CrossRef]
40. Griffin, D.O.; Rothstein, T.L. A small CD11b+ human B1 cell subpopulation stimulates T cells and is expanded in lupus. *J. Exp. Med.* **2011**, *208*, 2591–2598. [CrossRef]
41. Musso, T.; Deaglio, S.; Franco, L.; Calosso, L.; Badolato, R.; Garbarino, G.; Dianzani, U.; Malavasi, F. CD38 expression and functional activities are up-regulated by IFN-gamma on human monocytes and monocytic cell lines. *J. Leukoc. Biol.* **2001**, *69*, 605–612.
42. Kang, B.; Tirumurugaan, K.G.; Deshpande, D.A.; Amrani, Y.; Panettieri, R.A.; Walseth, T.F.; Kannan, M.S. Transcriptional regulation of CD38 expression by tumor necrosis factor-α in human airway smooth muscle cells: Role of NF-κB and sensitivity to glucocor-ticoids. *FASEB J.* **2006**, *20*, 1000–1002. [CrossRef]
43. Rahil, Z.; Leylek, R.; Schürch, C.M.; Chen, H.; Bjornson-Hooper, Z.; Christensen, S.R.; Gherardini, P.F.; Bhate, S.S.; Spitzer, M.H.; Fragiadakis, G.K.; et al. Landscape of coordinated immune responses to H1N1 challenge in humans. *J. Clin. Investig.* **2020**, *130*, 5800–5816. [CrossRef]
44. Park, Y.-W.; Kee, S.-J.; Cho, Y.-N.; Lee, E.-H.; Lee, H.-Y.; Kim, E.-M.; Shin, M.-H.; Park, J.-J.; Kim, T.-J.; Lee, S.-S.; et al. Impaired differentiation and cytotoxicity of natural killer cells in systemic lupus erythematosus. *Arthritis Rheum.* **2009**, *60*, 1753–1763. [CrossRef]
45. Schepis, D.; Gunnarsson, I.; Eloranta, M.L.; Lampa, J.; Jacobson, S.H.; Kärre, K.; Berg, L. Increased proportion of CD56bright natural killer cells in active and inactive systemic lupus erythematosus. *Immunology* **2009**, *126*, 140–146. [CrossRef]
46. Aringer, M.; Costenbader, K.; Daikh, D.; Brinks, R.; Mosca, M.; Ramsey-Goldman, R.; Smolen, J.S.; Wofsy, D.; Boumpas, D.T.; Kamen, D.L.; et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. *Arthritis Rheumatol.* **2019**, *71*, 1400–1412. [CrossRef]
47. Gladman, D.D.; Ibañez, M.; Urowitz, M.B. Systemic lupus erythematosus disease activity index 2000. *J. Rheumatol.* **2002**, *29*, 288–291.
48. Zunder, E.R.; Finck, R.; Behbehani, G.K.; Amir, E.D.; Krishnaswamy, S.; Gonzalez, V.D.; Lorang, C.G.; Bjornson, Z.; Spitzer, M.H.; Bodenmiller, B.; et al. Palladium-based mass tag cell barcoding with a doublet-filtering scheme and single-cell deconvolution algorithm. *Nat. Protoc.* **2015**, *10*, 316–333. [CrossRef]
49. Rahman, A.H.; Tordesillas, L.; Berin, M.C. Heparin reduces nonspecific eosinophil staining artifacts in mass cytometry experiments. *Cytom. Part A* **2016**, *89*, 601–607. [CrossRef]
50. Mei, H.E.; Leipold, M.D.; Schulz, A.R.; Chester, C.; Maecker, H.T. Barcoding of Live Human Peripheral Blood Mononuclear Cells for Multiplexed Mass Cytometry. *J. Immunol.* **2015**, *194*, 2022–2031. [CrossRef]
51. Olsen, L.R.; Leipold, M.D.; Pedersen, C.B.; Maecker, H.T. The anatomy of single cell mass cytometry data. *Cytom. Part A* **2019**, *95*, 156–172. [CrossRef]
52. Chevrier, S.; Crowell, H.L.; Zanotelli, V.R.; Engler, S.; Robinson, M.D.; Bodenmiller, B. Compensation of Signal Spillover in Suspension and Imaging Mass Cytometry. *Cell Syst.* **2018**, *6*, 612–620.e5. [CrossRef]

53. Budzinski, L.; Schulz, A.R.; Baumgart, S.; Burns, T.; Rose, T.; Hirseland, H.; Mei, H.E. Osmium-Labeled Microspheres for Bead-Based Assays in Mass Cytometry. *J. Immunol.* **2019**, *202*, 3103–3112. [CrossRef]
54. Ostendorf, L.; Mothes, R.; Van Koppen, S.; Lindquist, R.L.; Bellmann-Strobl, J.; Asseyer, S.; Ruprecht, K.; Alexander, T.; Niesner, R.A.; Hauser, A.E.; et al. Low-Density Granulocytes Are a Novel Immunopathological Feature in Both Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorder. *Front. Immunol.* **2019**, *10*, 2725. [CrossRef]
55. Cossarizza, A.; Chang, H.; Radbruch, A.; Acs, A.; Adam, D.; Adam-Klages, S.; Agace, W.W.; Aghaeepour, N.; Akdis, M.; Allez, M.; et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). *Eur. J. Immunol.* **2019**, *49*, 1457–1973. [CrossRef]
56. R Core Team. *R: A Language and Environment for Statistical Computing*, version 3.6; R Foundation for Statistical Computing: Vienna, Austria, 2018; Available online: https://www.R-project.org (accessed on 25 March 2020).
57. Wickham, H.; Averick, M.; Bryan, J.; Chang, W.; McGowan, L.; François, R.; Grolemund, G.; Hayes, A.; Henry, L.; Hester, J.; et al. Welcome to the Tidyverse. *J. Open Source Softw.* **2019**, *4*, 1686. [CrossRef]
58. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2009.
59. Van Gassen, S.; Callebaut, B.; Van Helden, M.J.; Lambrecht, B.N.; Demeester, P.; Dhaene, T.; Saeys, Y. FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. *Cytom. Part A* **2015**, *87*, 636–645. [CrossRef]
60. Nowicka, M.; Krieg, C.; Crowell, H.L.; Weber, L.M.; Hartmann, F.J.; Guglietta, S.; Becher, B.; Levesque, M.P.; Robinson, R.B. CyTOF workflow: Differential discovery in high-throughput high-dimensional cytometry datasets. *F1000Research* **2019**, *6*, 748. [CrossRef]

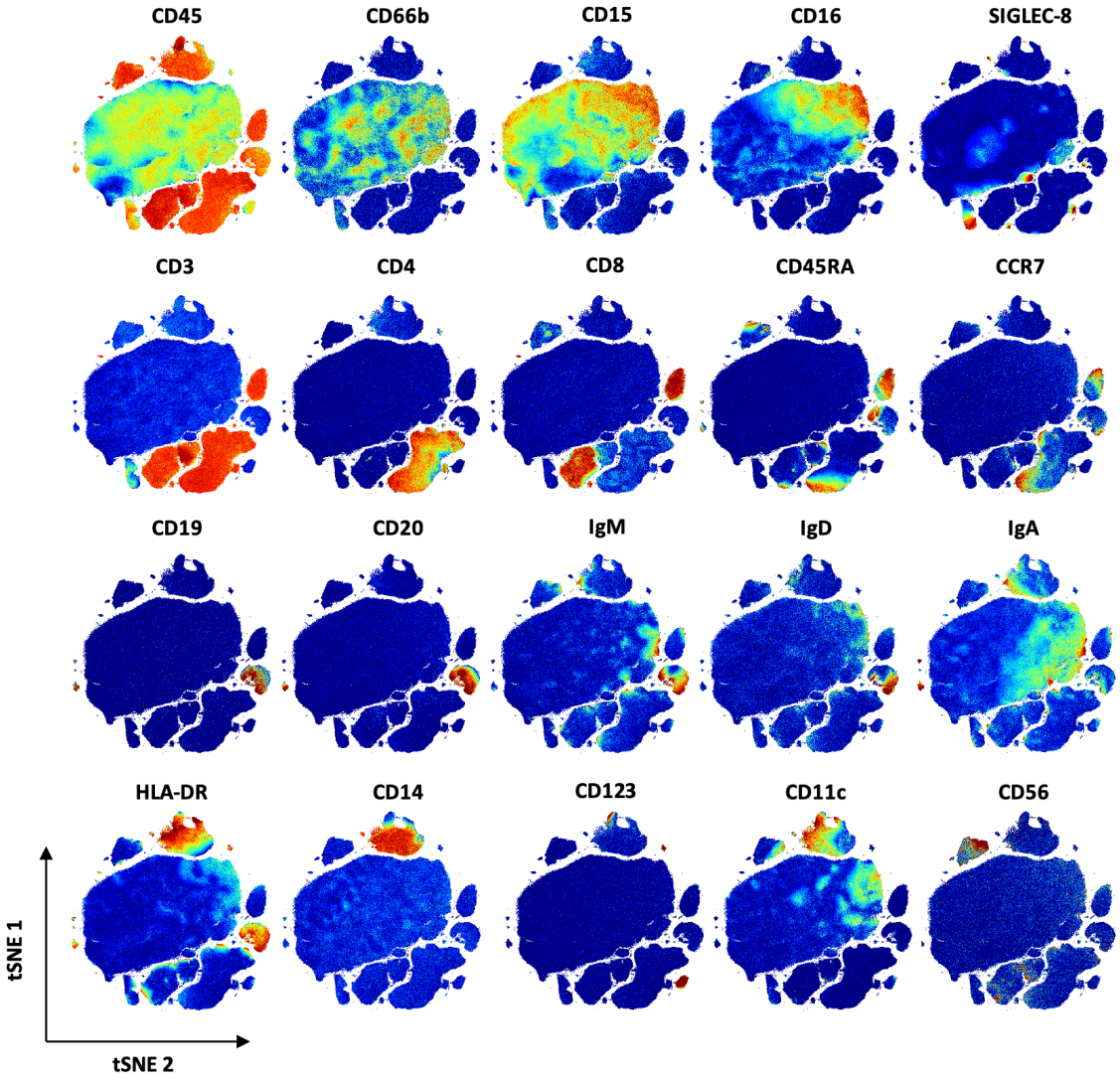

Figure S1. T-SNE map of mass cytometry data from Figure 1A, colored by the expression of cell-surface markers which were used to annotate the major immune cell subsets.

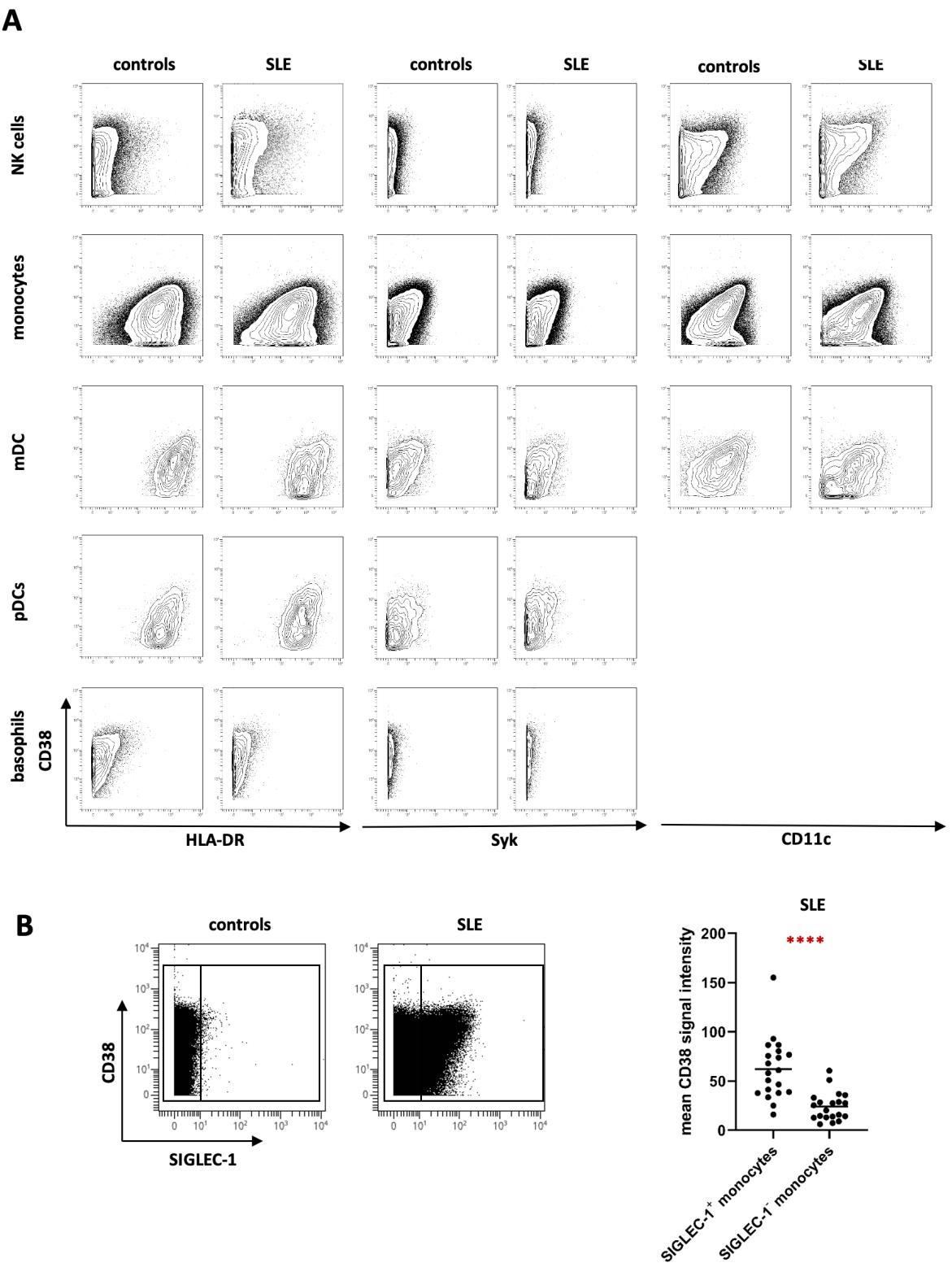

Figure S2. Analysis of co-expression of CD38 and myeloid markers by innate immune cells and CD38 expression by peripheral blood monocytes in the context of SIGLEC-1 expression. (**A**) Contour plots of HLA-DR, Syk and CD11c expression on NK cells, total monocytes, myeloid (mDCs) and plasmacytoid (pDCs) dendritic cells. Concatenated data of 20 healthy controls and 20 SLE patients are shown. (**B**) Total monocytes were gated from the mass cytometry data. Dot plots show the expression of CD38 and SIGLEC-1 in SLE patients and healthy controls. Concatenated data is shown for both groups. For SLE patients, SIGLEC-1^{+} and SIGLEC-1$^{-/low}$ monocytes were gated, and their CD38 expression was compared using the Mann-Whitney test (****, $p < 0.0001$).

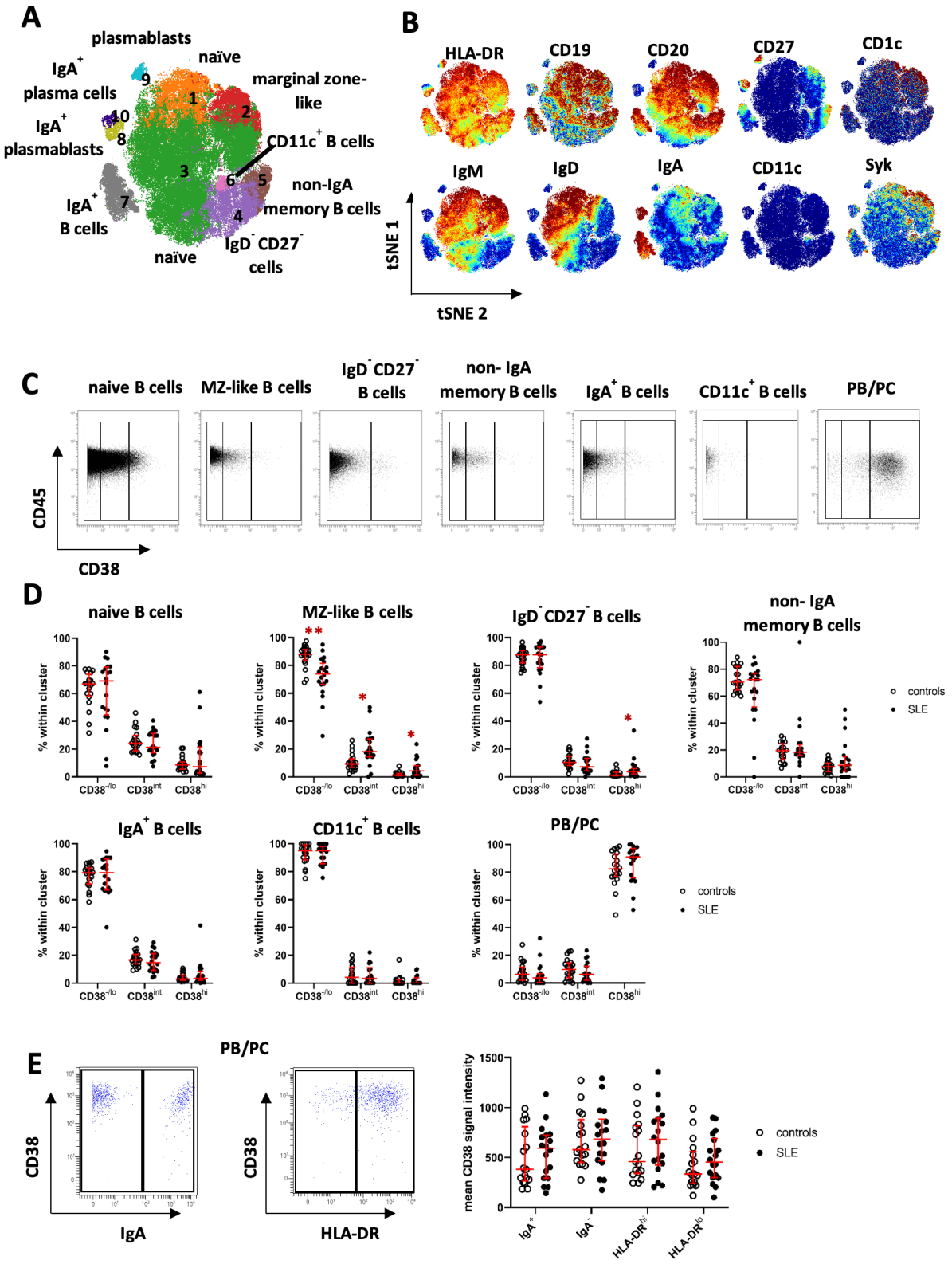

Figure S3. Analysis of B cell subsets and PB/PC for their CD38 expression in patients with SLE This supplementary figure extends Figure 2. (**A**) t-SNE map showing 10 B cell clusters generated by FlowSOM. Clusters comprising naive B cells (c1, c3) and PB/PC (c8, c9, c10) were merged for further analyses in Figure 2. Concatenated data of 20 healthy controls are shown. (**B**) t-SNE map depicted in (**A**) colored by the expression of markers expressed by B cells. (**C**) B cell subsets including PB/PC were analyzed their CD38 expression, by delineating subsets expressing low or no CD38, intermediate levels of CD38, or high levels of CD38 . Concatenated data of 20 healthy controls and 20 SLE patients are shown (**D**) B cell subsets and PB/PC shown in (**C**) were analyzed for differences in abundance of $CD38^{-/low}$, $CD38^{int}$ and $CD38^{hi}$ expressing cells between

controls and SLE patients. Each dot represents the frequency of the indicated subset of one donor. Red lines indicate medians and interquartile ranges. Asterisks indicate significantly different abundances in SLE patients vs controls obtained by Mann-Whitney testing (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) (**E**) Subsets of PB/PC were gated according to the expression of IgA and HLA-DR (left) and analyzed for CD38 expression (right). Gating is shown for one representative healthy control. Data of 3 controls and 2 SLE patients were excluded from this analysis due to PB/PC counts of less than 7 cells in a given subset. In the summary plot, each dot represents the mean CD38 signal intensity (SI) of the indicated subset of one donor. Red lines indicate medians and interquartile ranges.

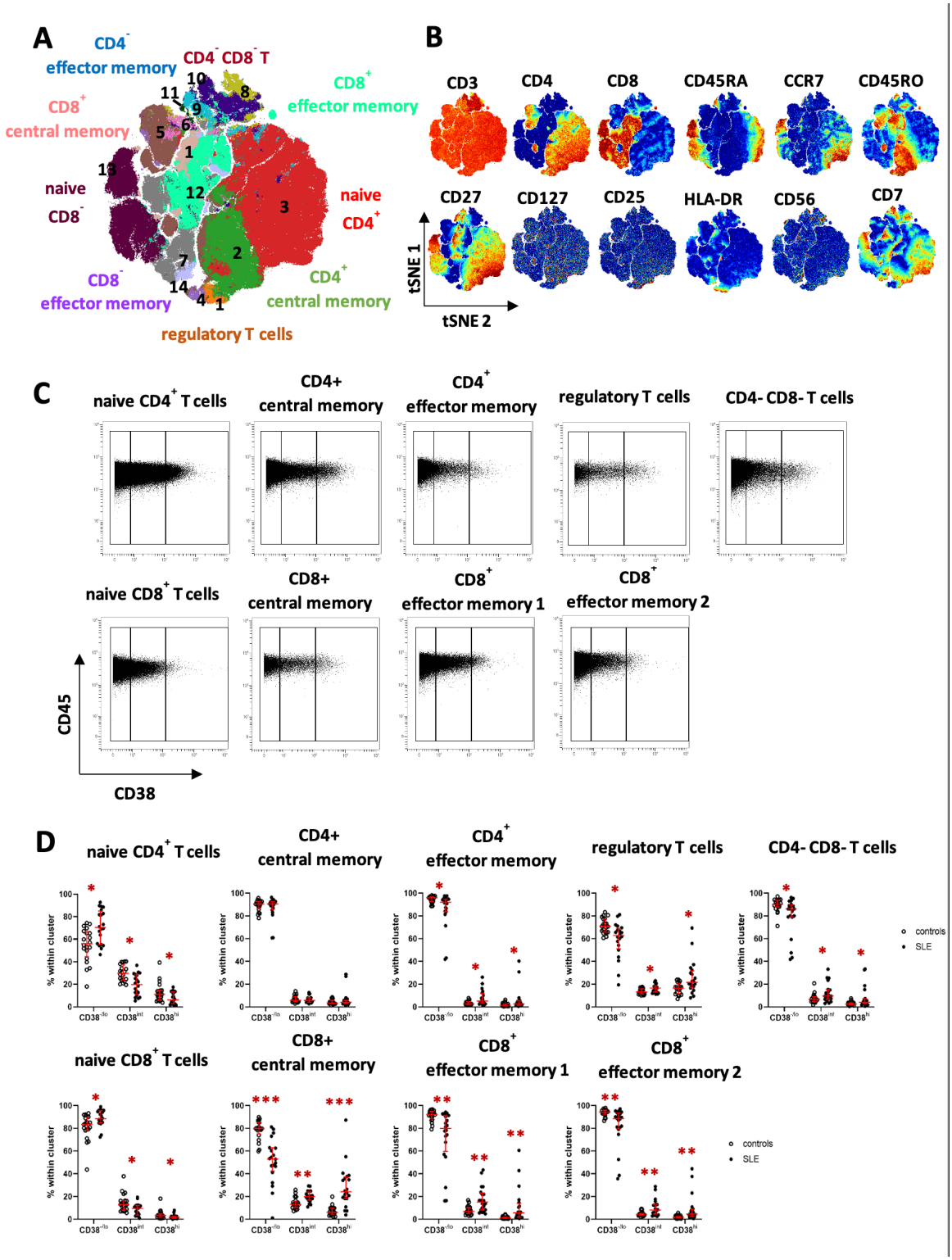

Figure S4. Analysis of T cell subsets for their CD38 expression in patients with SLE and controls. This supplementary Figure extends Figure 3. (**A**) T-SNE map showing 15 T cell clusters generated by FlowSOM. $CD4^-$ $CD8^-$ T cell clusters (c6, c8, c9, c10, c11), $CD4^+$ effector memory clusters (c4, c5) and $CD8^+$ effector memory subsets (c7, c14) were merged for further analysis in Figure 3. Concatenated data of 20 healthy controls and 20 SLE patients are shown (**B**) T-SNE map colored by the expression of markers expressed by T cells. (**C**) T cell subsets were manually gated according to their CD38 expression, subsetting them into $CD38^{-/low}$, $CD38^{int}$ and $CD38^{hi}$ expressing cells. Concatenated data of 20 healthy controls and 20 SLE patients are shown (**D**) The abundance of subsets gated in (**C**) was analyzed in healthy controls and SLE patients. Each dot represents the frequency of the indicated subset of one donor. Red lines indicate medians and interquartile ranges. Asterisks indicate significantly different frequencies in SLE patients vs controls revealed by Mann-Whitney testing (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

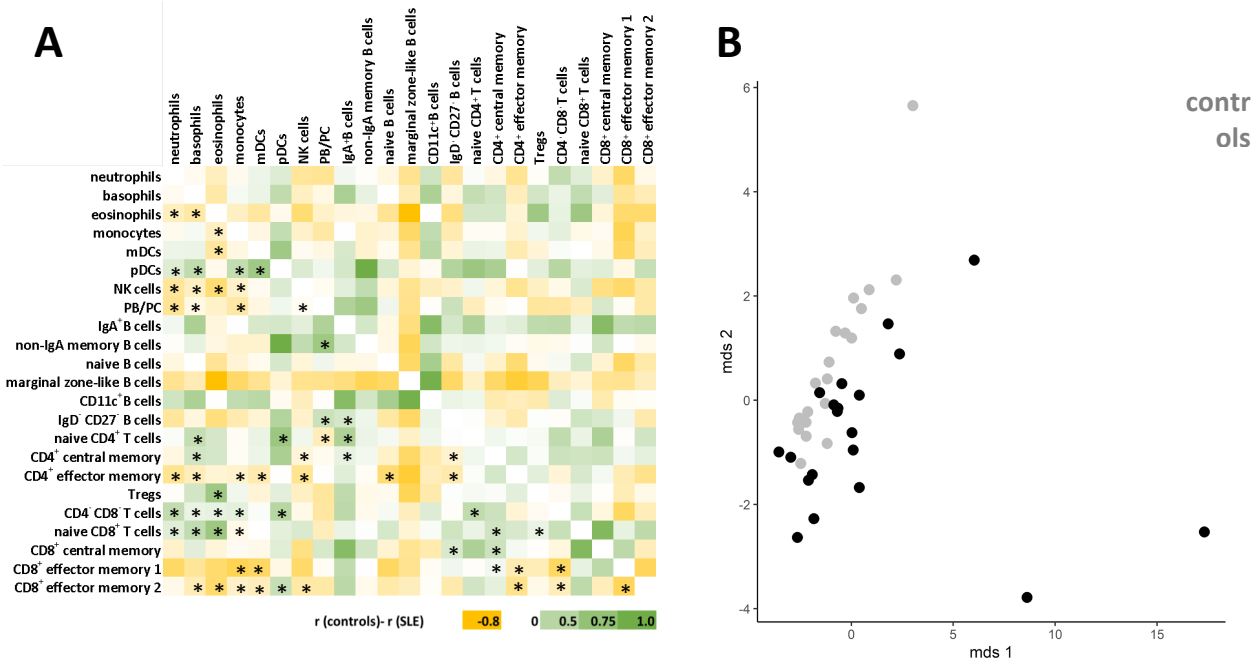

Figure S5. Differential correlation of CD38 expression between immune cell subsets in SLE and controls. This supplementary Figure extends Figure 5. (**A**) Spearman's r values of SLE patients were subtracted from r values of controls (shown in Figure 5A,B) to highlight changes in correlation of CD38 expression levels between leukocyte subsets in the two groups. Asterisks indicate correlations that were considered significant in one group, but not found in the other, based on a p value of < 0.001. Yellow color indicates higher r values in SLE patients, green color indicates higher r values in controls. (**B**) Mean CD38 signal intensity values from all immune cell subsets analyzed in Figure 5 A,B were used to perform dimension reduction by multidimensional scaling.

Table S1. Mass Cytometry Antibodies and Details.

(A) Antibodies

isotope mass	metal	antibody target or reagent function	antibody clone	supplier	Cat no.	RRID	cell-surface (sf) /intracellular (ic)	used for global opt_SNE	used for B cell opt-SNE	used for T cell op t-SNE
89	Y	CD15	W6D3	Biolegend	323002	AB_756008	sf	x		
103	Rh	mDOTA								
104	Pd	barcoding								
105	Pd	barcoding								
106	Pd	barcoding								
108	Pd	barcoding								
110	Pd	barcoding								
113	In	CD66b	REA306	Miltenyi Biotec	130-108-019	AB_2658994	sf	x		
115	In	Siglec 8	#837535	R & D Systems	MAB7975		sf	x		
140	Ce	CD14	RMO52	Beckman Coulter	custom order		sf	x		
141	Pr	CD127	A019D5	Biolegend	351302	AB_10718513	sf	x		x
143	Nd	CD19	BU12	DRFZ	n.a.		sf	x	x	
145	Nd	CD4	RPAT4	Biolegend	300502	AB_314070	sf	x		x
146	Nd	CD45RO	UCHL1	DRFZ	n.a.		sf	x	x	x
147	Sm	CD20	Rituximab	Charité	n.a.		sf	x	x	
148	Nd	IgA	IS11-8E10	Miltenyi Biotec	130-093-073	AB_1036150	sf	x	x	
149	Sm	Syk	4D10.2	Fluidigm	3149020B		ic	x	x	
151	Eu	CD123	6H6	Biolegend	306002	AB_314576	sf	x		
152	Sm	CD45RA	4G11	DRFZ	n.a.		sf	x	x	x
154	Sm	CD1c	AT5-8E7	Miltenyi Biotec	130-108-032	AB_2661165	sf	x	x	x
155	Gd	CD27	2E4	Sanquin	M9185		sf	x	x	x
160	Gd	CD11c	BU15	Biolegend	337202	AB_1236381	sf	x	x	x
161	Dy	CD7	CD7-6B7	Biolegend	343102	AB_1659214	sf	x		x
162	Dy	IgM	MHM-88	Biolegend	314502	AB_493003	sf	x	x	
163	Dy	CD197	G043H7	Biolegend	353202	AB_10945157	sf	x	x	x
167	Er	CD38	HIT2	Biolegend	303502	AB_314354	sf			
168	Er	CD16	3G8	Biolegend	302002	AB_314202	sf	x	x	x
169	Tm	CD25	MA-251	Biolegend	356102	AB_2561752	sf	x	x	x
170	Er	Siglec1	7-239	Miltenyi Biotec	130-108-017	AB_2655556	sf	x		
173	Yb	IgD	IA6-2	Biolegend	348202	AB_10550095	sf	x	x	
174	Yb	HLA-DR	L243	DRFZ	n.a.		sf	x	x	x
176	Yb	CD56	REA196	Miltenyi	130-108-016	AB_2658728	sf	x		
191	Ir	DNA intercalator		Fluidigm	201192A		ic			
193	Ir	DNA intercalator		Fluidigm	201192A		ic			
195	Pt	CD3	UCHT1	DRFZ	n.a.		sf	x		x
196	Pt	CD8	GN11	DRFZ	n.a.		sf	x		x
198	Pt	CD45	Hi30	Biolegend	304002	AB_314390	sf	x	x	x
209	Bi	CD11b	ICRF44	Biolegend	301302	AB_314154	sf	x		?
n.a. - not applicable										

(B) Sample acquisition

Pool 1								
patients					**healthy controls**			
Code no	**gender**	**born**	**barcode**		**Code no**	**gender**	**born**	**barcode**
SLE_004_d0	f	1952	**1**		ND036	f	1950	**6**
SLE_020_d0	f	1960	**2**		ND002	f	1960	**7**
SLE_015	f	1972	**3**		HC_006	f	1972	**8**
SLE_012	f	1975	**4**		HC_033	f	1976	**9**
SLE_009	m	1983	**5**		HC_012	m	1985	**10**
					reference			***mDOTA***
Pool 2								
patients					**healthy controls**			
Code no	**gender**	**born**	**barcode**		**Code no**	**gender**	**born**	**barcode**
SLE_016	f	1965	**1**		ND001	f	1966	**6**
SLE_008	f	1979	**2**		HC013	f	1978	**7**
SLE_007	f	1983	**3**		HC_028	f	1986	**8**
SLE_017	f	1991	**4**		HC_032	f	1993	**9**
SLE_002	m	1993	**5**		HC_017	m	1993	**10**
					reference			***mDOTA***
Pool 3								
patients					**healthy controls**			
Code no	**gender**	**born**	**barcode**		**Code no**	**gender**	**born**	**barcode**
SLE_006	f	1983	**1**		HC_030	f	1986	**6**
SLE_010	f	1988	**2**		HC_020	f	1986	**7**
SLE_011	f	1985	**3**		HC_029	f	1985	**8**
SLE_018	f	1975	**4**		HC009	f	1976	**9**
SLE_021	f	1993	**5**		HC008	f	1993	**10**
					reference			***mDOTA***
Pool 4								
patients					**healthy controls**			
Code no	**gender**	**born**	**barcode**		**Code no**	**gender**	**born**	**barcode**
SLE_022	f	1993	**1**		HC_023	f	1993	**6**
SLE_019_d0	f	1975	**2**		HC_019	f	1976	**7**
SLE_003_d0	f	1967	**3**		ND017	f	1967	**8**
SLE_014_d0	f	1983	**4**		ND025	f	1984	**9**
SLE_001_d0	f	1976	**5**		ND038	f	1971	**10**
					reference			***mDOTA***

(C) Barcoding Scheme

barcode no.	104Pd	105Pd	106Pd	108Pd	110Pd	103Rh
1	x	x				
2	x		x			
3	x			x		
4	x				x	
5		x	x			
6		x		x		
7		x			x	
8			x	x		
9			x		x	
10				x	x	
reference						x

Table S2. Flow Cytometry Antibodies.

Target	Fluorochrome	Clone	Company	Catalogue Number	RRID
IgD	FITC	IA6-2	BD Biosciences	555778	AB_396113
CD38	APC	IB6	Miltenyi Biotec	130-092-261	AB_871666
CD27	PE	O323	Biolegend	302808	AB_314300
CD24	PerCP/Cy5.5	ML5	Biolegend	311116	AB_10960741
HLA-DR	V500	G46-6	BD Biosciences	561224	AB_10563765
CD3	APC/Cy7	SK7	Biolegend	344818	AB_10645474
CD14	APC/Cy7	63D3	Biolegend	367108	AB_2566710
CD19	APC/Cy7	HIB19	Biolegend	302218	AB_314248
CD19	V450	HIB19	Biolegend	560353	AB_1645564
CD14	FITC	63D3	Biolegend	325604	AB_830677
CD16	FITC	3G8	Biolegend	302006	AB_314206
CD16	Pacific Blue	3G8	Biolegend	302032	AB_2104003
CD56	PE/Cy7	HCD56	BD Biosciences	318318	AB_604107
SIGLEC1	PE	7-239	Biolegend	346004	AB_2189029

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publikationsliste

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Lennard Ostendorf, Philipp Dittert, Robert Biesen, Ankelien Duchow, Victoria Stiglbauer, Klemens Ruprecht, Dominik Seelow, Werner Stenzel, Raluca A. Niesner, Anja E. Hauser, Friedemann Paul, Helena Radbruch „*SIGLEC1 (CD169): a marker of active neuroinflammation in the brain but not in the blood of multiple sclerosis patients“* Sci Rep *2021 (IF: 3.99)*

Marta Ferreira-Gomes, Andrey Kruglov, Pawel Durek, Frederik Heinrich, Caroline Tizian, Gitta Anne Heinz, Anna Pascual-Reguant, Weijie Du, Ronja Mothes, Chaofan Fan, Stefan Frischbutter, Katharina Habenicht, Lisa Budzinski, Justus Ninnemann, Peter K. Jani, Gabriela Maria Guerra, Katrin Lehmann, Mareen Matz, **Lennard Ostendorf**, Lukas Heiberger, Hyun-Dong Chang, Sandy Bauherr, Marcus Maurer, Günther Schönrich, Martin Raftery, Tilmann Kallinich, Marcus Alexander Mall, Stefan Angermair, Sascha Treskatsch, Thomas Dörner, Victor Max Corman, Andreas Diefenbach, Hans-Dieter Volk, Sefer Elezkurtaj, Thomas H. Winkler, Jun Dong, Anja Erika Hauser, Helena Radbruch, Mario Witkowski, Fritz Melchers, Andreas Radbruch, Mir-Farzin Mashreghi „*SARS-CoV-2 in severe COVID-19 induces a TGF-β-dominated chronic immune response that does not target itself*“ Nat Comms 2021 (IF: 12.12)

Marie Burns*, **Lennard Ostendorf***, Robert Biesen, Andreas Grützkau, Falk Hiepe, Henrik E. Mei, Tobias Alexander “*Dysregulated CD38 Expression of Peripheral Blood Immune Cell Subsets in SLE*” IJMS 2021 *: equally contributing (IF: 4.56)

Lennard Ostendorf, Marie Burns, Pawel Durek, Gitta Anne Heinz, Frederik Heinrich, Panagiotis Garantziotis, Philipp Enghard, Ulrich Richter, Robert Biesen, Udo Schneider, Fabian Knebel, Gerd Burmester, Andreas Radbruch, Henrik E. Mei, Mir-Farzin Mashreghi, Falk Hiepe, Tobias Alexander „*Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus*“ New Engl J Med 2020 (IF: 74.69)

Lennard Ostendorf, Ronja Mothes, Sofie van Koppen, Randall L. Lindquist, Judith-Bellmann-Strobl, Susanna Asseyer, Klemens Ruprecht, Tobias Alexander, Raluca A. Niesner, Anja E. Hauser, Friedemann Paul, Helena Radbruch „*Low-Density Granulocytes Are a Novel Immunopathological Feature in Both Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorder*“ Front. Immunol. 2019 (IF: 6.43)

Franziska Scheibe, **Lennard Ostendorf,** S. Momsen Reincke, Harald Prüss, Ann-Christin von Brünneck, Martin Köhnlein, Tobias Alexander, Chrstian Meisel, Andreas Meisel „*Daratumumab treatment for therapy-refractory anti-CASPR2 encephalitis*“ Journal of Neurology 2019 (IF: 3.78)

Ruth Leben, **Lennard Ostendorf,** Sofie van Koppen, Asylkhan Rakhymzhan, Anja E. Hauser, Helena Radbruch and Raluca A. Niesner „*Phasor-Based Endogenous NAD(P)H Fluorescence Lifetime Imaging Unravels Specific Enzymatic Activity of Neutrophil Granulocytes Preceding NETosis*“ IJMS 2018 (IF: 4.56)

Janina R. Behrens, Julia Wanner, Joseph Kuchling, **Lennard Ostendorf**, Lutz Harms, Klemens Ruprecht, Thoralf Niendorf, Sven Jarius, Brigitte Wildemann, René M. Gieß, Michael Scheel, Judith Bellmann-Strobl, Jens Wuerfel, Friedemann Paul „*7 Tesla MRI of Balo's concentric sclerosis versus multiple sclerosis lesions*“ Annals of Clinical and Translational Neurology 2018 (IF: 3.66)

Helena Radbruch, Ronja Mothes, Daniel Bremer, Stefanie Seifert, Ralf Köhler, Julian Pohlan, **Lennard Ostendorf**, Robert Günther, Ruth Leben, Werner Stenzel, Raluca Aura Niesner, Anja E. Hauser „*Analyzing Nicotinamide Adenine Dinucleotide Phosphate Oxidase Activation in Aging and Vascular Amyloid Pathology*“ Front. Immunol. 2017 (IF: 6.43)

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