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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

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ABSTRACT

CD38 is a multifunctional protein possessing ADP-ribosyl cyclase activity responsible for both the synthesis and the degradation of several Ca^{2+}-mobilizing second messengers. In mammals, CD38 also functions as a receptor. In this study CD38 expression in $CD4^+$, $CD8^+$, or $CD25^+$ T cells was significantly higher in systemic lupus erythematosus (SLE) patients than in Normal controls. Increased CD38 expression in SLE T cells correlated with plasma levels of Th2 (IL-4, IL-10, IL-13) and Th1 (IL-1β, IL-12, IFN-γ, TNF-α) cytokines, and was more prevalent in clinically active SLE patients than in Normal controls. In contrast, elevated anti-CD38 IgG autoantibodies were more frequent in clinically quiescent SLE patients (SLEDAI = 0) than in Normal controls, and correlated with moderate increased plasma levels of IL-10 and IFN-γ. However, clinically active SLE patients were mainly discriminated from quiescent SLE patients by increased levels of IL-10 and anti-dsDNA antibodies, with odds ratios (ORs) of 3.7 and 4.8, respectively. Increased frequency of anti-CD38 autoantibodies showed an inverse relationship with clinical activity (OR = 0.43), and in particular with the frequency of anti-dsDNA autoantibodies (OR = 0.21). Increased cell death occurred in $CD38^+$ Jurkat T cells treated with anti-$CD38^+$ SLE plasmas, and not in these cells treated with anti-$CD38^-$ SLE plasmas, or Normal plasmas. This effect did not occur in CD38-negative Jurkat T cells, suggesting that it could be attributed to anti-CD38 autoantibodies. These results support the hypothesis that anti-CD38 IgG autoantibodies or their associated plasma factors may dampen immune activation by affecting the viability of $CD38^+$ effector T cells and may provide protection from certain clinical SLE features.

1. Introduction

CD38 is a ubiquitous protein expressed in myriad cells and tissues [1–3]. It is a member of a gene family that has the ability to convert NAD into cADPR [4]. In addition to its ADP-ribosyl cyclase enzymatic function, CD38 also catalyzes a transglycosylation reaction exchanging the terminal nicotinamide group of the substrate NADP with nicotinic acid resulting in the production of NAADP [5]. Both cADPR and NAADP are Ca^{2+}-mobilizing messengers thought to be important in various cellular signaling events [6,7].

Increased CD38 expression in different cell types has been associated with a number of human diseases [8,9]. Thus, CD38

Abbreviations: dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

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represents a reliable negative prognostic marker in chronic lymphocytic leukemia (CLL) [10]. However, CD38 expression in CLL B cells also affects expansion and proliferation of the neoplastic clones, and it is, therefore, considered as part of a network sustaining growth and survival of CLL cells [11].

Increased percentages of B cells positive for CD38 have been consistently shown in SLE patients by different groups [12–14]. Active-SLE patients have circulating $CD38^{bright}$ Ig-secreting cells that are not found in Normal individuals [12]. This plasma cell subset disappears from circulation during treatment with humanized anti-CD154 mAb, and it is associated with decreases in anti-double-stranded DNA (anti-dsDNA) Ab levels, proteinuria, and SLE disease activity index [12]. In this sense, oxytocin at physiological concentrations influences the distribution of surface CD38 and increases the growth rate of plasma cells [15]. Moreover, in SLE patients with active disease, B cells expressing high levels of CD38 spontaneously produce IgG class anti-dsDNA *in vitro*, whereas persistence of $CD38^{+}$ B cells during periods with clinically quiescent disease seems to underlie hypergammaglobulinaemia but not anti-dsDNA production [14]. Likewise, T cells from active SLE patients over-express CD38 [16–18], which might be playing a role in modulating TCR signaling [18].

It has been postulated that CD38 may play a role in the pathogenesis of diabetes mellitus, which is likely related to increased anti-CD38 autoantibodies detected in Japanese type 2 [19], as well as Caucasian type 1 and 2 diabetic patients [20]. Autoantibodies to CD38 are also detected in patients with chronic autoimmune thyroiditis and Graves' disease [21]. However, no similar studies have been carried out in other autoimmune diseases, such as SLE.

The aim of the present study is to define the prevalence of autoantibodies to CD38 in SLE subjects, and to explore whether there is an association of these autoantibodies with cytokine plasma levels, clinical activity, and CD38 surface expression in T cells.

2. Materials and methods

2.1. Patients and controls

A total of 69 SLE consecutive patients (63 female, 6 male) attending the outpatient clinic were selected. No exclusion criteria were used for the selection of patients in order to obtain a heterogeneous sample representative of a broad spectrum of clinical and laboratory phenotypes. All SLE patients fulfilled the American College of Rheumatology criteria for SLE [22]. Disease activity was measured using the SLE Disease Activity Index (SLEDAI) [22]. The SLE patients had a median SLEDAI of 2 (range: 0–20). A total of 71 Normal control subjects were selected at the local blood bank (43 female, 28 male). All patients and Normal controls were Caucasians. The study protocol was approved by the Hospital Clínico San Cecilio, and CSIC Review Board and Ethics Committees. Written informed consent was obtained from all participating patients and volunteers according to the Declaration of Helsinki.

2.2. Blood plasma samples

Blood was collected by the BD Vacutainer system into K_2-EDTA tubes (BD Diagnostics, NJ, USA) and plasma was separated from cells by density gradient centrifugation over HISTOPAQUE®-1077 (Sigma–Aldrich, St. Louis, MO). The supernatant was collected, checked for the absence of cells by light microscopy, and fractionated in aliquots that were stored at −80 °C [18].

2.3. Recombinant CD38 proteins

The recombinant His-tagged GST-CD38 fusion protein used as the target antigen in the ELISA and Western blots experiments was kindly obtained from Prof. C. F. Chang at the National University of Singapore [23]. The other recombinant CD38 protein used in Western blot experiments (rCD38) lacks the four putative N-linked glycosylation sites and the intracellular and transmembrane spanning regions and was produced as a soluble, enzymatically active CD38 in yeast [24].

2.4. Analysis of anti-CD38 autoantibodies by enzymatic immunoassay

Anti-CD38 IgG and IgM concentrations were measured by ELISA. Briefly, the His-tagged GST-CD38 fusion protein (Fr #20) was added to 96-well polystyrene plates (Nunc, Roskilde, Denmark) in coating buffer (0.05 M Na_2CO_3/$NaHCO_3$, pH 9.6) and incubated for 16 h at 4 °C. Plates were then washed twice with phosphate buffered-saline (PBS) containing 0.1% Tween 20 (PBS/Tween) and blocked for 2 h with 5% BSA in PBS. Plasma samples were diluted 1:4 in PBS/Tween and 100 μl was added to each well and incubated for 2 h at 37 °C. Following four washings with PBS/Tween, horseradish peroxidase-conjugated goat anti-human IgG (Sigma–Aldrich, St. Louis, MO) anti-serum was added and incubated for 1 h. Plates were then washed 6 times and developed with 100 μl o-phenylenediamine dihydrochloride (OPD) (Sigma–Aldrich, St. Louis, MO). Substrate color development was stopped by 0.5 M sulfuric acid and absorbance was measured at 450 nm using a VersaMax tunable microplate reader (Molecular Devices, Chicago, IL) with the results expressed in optical density units (ODU). For IgM measurement, plasma samples were incubated as before and detected with purified goat anti-human IgM (Sigma–Aldrich, St. Louis, MO) followed by HRP-conjugated rabbit anti-goat IgG (Sigma–Aldrich, St. Louis, MO). The development was as described above. Validation of both assays was done in-house. Control plasma derived from two Normal controls and two SLE patients were added to each assay to determine inter-assay variation, which was always less than 15%. The intra-assay variation of triplicates was always less than 5%. A positive control was performed using the rabbit polyclonal IgG against human CD38 (H-170) (Santa Cruz Biotechnology Inc., Santa Cruz, CA) raised against an epitope corresponding to amino acids 1–170 mapping at the amino terminus of human CD38. This was followed with washing and incubation with the secondary antibody (goat anti-rabbit IgG) conjugated with horseradish peroxidase (Promega Co., Madison, WI). Other antibodies used were HB136, OKT10, and OKT3 mAbs.

2.5. Identification of GST-CD38 by mass spectrometry

Samples were analyzed by LC-MS/MS as described [25] (see Supplemental material and methods)

2.6. Cytokine assay

The Bio-Plex Precision Pro Human Cytokine 10-Plex kit assay (Bio-Rad, Hercules, CA) was used to simultaneously test 10 cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), IL-13, IFN-γ and TNF-α. Assays were performed according with manufacturers' protocol. Analyses of experimental data were performed using five-parameter logistic curve fitting to standard analyte values.

2.7. Ca^{2+} analysis

Changes in intracellular calcium concentrations in Jurkat T cells were measured as described [26].

2.8. CD38 expression assay and assessment of plasma-induced cell death

The percentage of apoptotic cells was determined by flow cytometry analysis by evaluating the modification of scatter