Aberrant CD200/CD200R1 expression and its potential role in Th17 cell differentiation, chemotaxis and osteoclastogenesis in rheumatoid arthritis

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Abstract

Objective. CD200/CD200R1 signalling has an immunoregulatory effect on the activation threshold of the inflammatory immune response and maintains immune homeostasis. In this study we evaluated the status of CD200/CD200R1 interaction in patients with RA.

Methods. The expression of CD200 and CD200R1 was examined by immunohistochemistry and flow cytometry and was compared between RA patients and healthy controls (HCs). Sorted CD4+ T cells were stained with carboxyfluorescein succinimidyl ester (CFSE) and annexin V-propidium iodide to evaluate the effect of CD200 on cell proliferation and apoptosis. The effect of CD200 on Th17 differentiation, function and osteoclastogenesis was determined by flow cytometry, transwell migration assay and immunocytochemistry, respectively.

Results. The proportion of CD200+ cells and CD200R1+ cells in peripheral blood mononuclear cells, peripheral CD14+ cells and CD4+ T cells was significantly lower in the RA patients than in HCs, whereas the number of CD200+ cells was higher in synovium from RA patients than in that from HCs. After treatment with infliximab and MTX we found increased expression of peripheral CD200/CD200R1 that correlated with a decrease in the 28-joint DAS. CD200Fc in vitro partially inhibited CD4+ T cell proliferation, promoted CD4+ T cell apoptosis, reduced CD4+ T cell differentiation into Th17 cells and down-regulated CCR6-mediated Th17 chemotaxis in cells from RA patients. In addition, the engagement of the CD200 receptors on CD14+ cells with CD200Fc in vitro reduced osteoclastogenesis and inhibited CD14+ cell-driven Th17 differentiation.

Conclusion. Abnormal CD200/CD200R1 expression in RA may contribute to abnormal Th17 cell differentiation, chemotaxis and osteoclastogenesis.

Key words: CD200/CD200R1, rheumatoid arthritis, Th17 cells, osteoclastogenesis.

Introduction

RA is a chronic inflammatory autoimmune disease characterized by persistent synovitis and progressive bone erosion. Current treatments, such as TNF-α inhibitor therapy, have focused on alleviating synovitis but do not adequately repair bone and cartilage erosion, thereby warranting the development of new treatments that not only inhibit inflammatory synovitis but also effectively stop or reverse bone and cartilage destruction.

CD200 is a type I transmembrane glycoprotein that belongs to the immunoglobulin superfamily and is expressed on a variety of cells, including myeloid-derived cells, activated T cells, B cells and neurons [1, 2]. CD200 consists of
an extracellular, a transmembrane and an intracellular domain. Its intracellular region lacks a signalling motif, therefore signalling from CD200 can be transduced via its receptors [3], including CD200R1-4, of which CD200R1 has the highest binding affinity [4–6]. As CD200 receptors are present mainly on myeloid-derived cells, such as dendritic cells, macrophages and activated T cells [7], the known immunoregulatory roles of the CD200/CD200R1 pathway are focused on the suppression of myeloid-derived cell activation, including negative regulation of macrophage function and repression of the degranulation of mast cells and basophils [8–11]. Moreover, Gorczynski et al. [12] found that CD200-deficient mice had increased susceptibility to developing CIA. Administration of CD200R-Ig to disrupt the CD200–CD200R interaction was also found to increase the susceptibility of mice to CIA [13]. Simelyte et al. [14] reported that CD200Fc can be an effective therapeutic agent for established CIA owing to its targeting of pro-inflammatory cytokine expression in the joint. The authors confirmed that the CD200/CD200R1 pathway in CIA plays a significant physiological role in regulating disease severity [15]. Although the available evidence has highlighted an important role for CD200/CD200R in experimental autoimmune diseases, the role of CD200/CD200R1 in human autoimmune diseases, such as RA, remains unknown. Previously we reported that aberrant CD200/CD200R1 expression and function in SLE contributes to abnormal T cell responsiveness and dendritic cell activity [16]. In this study we explored the expression and function of this pathway in subjects with RA.

Materials and methods

Patients and healthy controls

This study was approved by the Institutional Review Board of Peking Union Medical College Hospital and written informed consent was obtained from each participating patient and healthy control (HC). Altogether, a total of 30 RA patients with active disease and fulfilling the 1987 ACR revised criteria for RA were enrolled in the study (3 men and 27 women), ranging in age from 43 to 67 years. Mean disease duration at the time of evaluation was 8.8 years and none had received non-biologic or biologic DMARDs before entering this study. Clinical disease activity was determined using the 28-joint DAS with CRP (DAS28-CRP).

Thirteen patients were treated with infliximab (anti-TNF-α monoclonal antibody) plus MTX and underwent two MRI assessments. The first was performed at the time of starting treatment and the second at week 30 (after five infusions of infliximab). The MRI images were scored for synovitis and bone oedema by two independent, blinded readers using the OMERACT Rheumatoid Arthritis MRI Scoring System (RAMRIS) [17]. Bone oedema was scored as 0–3 using the following criteria based on the volume of oedema: 0, no oedema; 1, 1–33% bone oedema; 2, 34–66% bone oedema; 3, 67–100% bone oedema. Synovitis was scored as 0–3 based on the volume of contrast-enhanced tissue in the synovium compartment in three wrist regions (A, the distal radioulnar joint; B, the radiocarpal joint; C, the intercarpal and carpometacarpophalangeal joints) and each MCP joint (0: normal; 1: mild; 2: moderate; 3: severe). Five gender- and age-matched patients with OA and 10 gender- and age-matched healthy volunteers were enrolled as patient controls and HCs.

Antibodies and reagents

Monoclonal antibodies targeting the following molecules were used, either unlabelled or as FITC, phycoerythrin (PE), allopophysocyanin (APC) or PE-cyanin7 (PE-Cy7) conjugates: CD4 (RPA-T4), CD14 (1D13), IFN-γ (B27), Foxp3 (PCH101), CD200R (OX-108), CD80 (L307.4), CD25 (BC96), CD200 (OX104), CCR6 (R6H1), CD86 (IT2-2), CD3 (UCHT1), CD28 (CD28.2) and CD200R (OX-108) (BD Pharmingen, San Diego, CA, USA); IL-17 (64DEC17) (ebiScience, San Diego, CA, USA); CD80 (L307.4) and CD86 (IT2-2) (BioLegend, San Diego, CA, USA). In all of the experiments, control mAbs for the respective IgG isotypes were included. The Cell Trace carboxyfluorescein succinimidyl ester (CFSE) cell proliferation kit and the Apoptosis Assay kit #2 were obtained from Invitrogen (Carlsbad, CA, USA). Recombinant IL-2, IL-4, IL-6, IL-1β, IL-23, TGF-β and CCL20 were all purchased from PeproTech (Rocky Hill, NJ, USA). Lipopolysaccharide (LPS), ionomycin and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma-Aldrich (St Louis, MO, USA). Additionally, tartrate-resistant acid phosphatase (TRAP) expression was measured using a commercial kit obtained from Sigma-Aldrich. Recombinant human CD200Fc, M-CSF, receptor activator for nuclear factor κB ligand (RANKL), anti-mouse CD200 antibody (goat IgG) and anti-human CD200 R1 antibody (goat IgG) were purchased from R&D Systems (Minneapolis, MN, USA). Antibodies against DOK2 (ab47507) and phosphorylated DOK2 (pDOK2; phospho Y299) were purchased from Abcam (Cambridge, UK).

Cell separation, culture, stimulation and treatment

Peripheral blood and synovial tissue were collected. Peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll-Hypaque density-gradient centrifugation and synovial cell suspension was prepared by enzyme digestion. CD4+ T cells and CD14+ monocytes were isolated using human CD4+ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany; 130-091-301) and human CD14 MicroBeads (Miltenyi Biotec; 130-050-201). The average purity of the isolated CD4+ T cells and CD14+ monocytes was 99.6% and 99.5%, respectively.

The cells were cultured in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 0.05 mM non-essential amino acids, 2 mM l-glutamine and 10% heat-inactivated fetal calf serum (FCS) in a humidified CO2-containing atmosphere at 37°C. For the cell proliferation assays, sorted CD4+ T cells (1 x 10⁵ cells/ml) were stained with 5 µM CFSE and stimulated with anti-CD3 and
anti-CD28 mAbs at a concentration of 1 μg/ml alone or in the presence of the IgGFc isotype CD200Fc at concentrations of 200 ng/ml unless indicated otherwise. Cell proliferation was measured on day 5 by flow cytometry using an Accuri C6 flow cytometer (Accuri Cytometers, Ann Arbor, MI, USA). Cellular apoptosis and necrosis were also detected by measuring the direct binding of FITC-annexin V and propidium iodide (PI) (Invitrogen) by flow cytometry.

For the Th17 cell differentiation experiments, first CD4+ T cells (1 x 10^6 cells/ml) were co-cultured with anti-CD3 (1 μg/ml, soluble)/anti-CD28 (2 μg/ml, soluble), then with the addition of IL-1β, IL-6, TGF-β and IL-23 at concentrations of 10, 20, 50 and 100 ng/ml or synovial CD14 cells (1 x 10^5 cells/ml) or peripheral CD14 cells (1 x 10^5 cells/ml) for 7 days alone or in the presence of the IgGFc isotype or CD200Fc. PMA, ionomycin and protein transport inhibitor (Golgistop; BD Pharmingen) were added 5 h before the cells were collected and stained for membrane molecules. Intracellular staining of IL-17 was also performed after fixation and permeabilization with fixation/permeabilization buffer and was detected by flow cytometry. Gating strategy to identify CD4+IL-17+ T cells was as follow: initial gating by typical forward and sideways scatter, CD4+ T lymphocytes identified as CD4+ expression; within the CD4+ T cell population, the subset of cells brightly expressing IL-17 was determined as CD4+IL-17+ T cells. Isotype-matched control antibody was used to set up the threshold of positivity for each marker.

Immunohistochemistry

Synovial tissue was collected with arthroscopy. The samples were collected from five untreated active RA patients, five patients with OA and five HCs after obtaining informed consent from each patient and control. Immunohistochemistry was performed using an immunoperoxidase technique with diaminobenzidine to examine the expression of CD200 and CD200R1 in synovium from HCs and OA and RA patients. The specificity of staining was confirmed by using matched-isotype control antibodies. Stained sections were evaluated semi-quantitatively using scoring systems for synovial tissue. All areas of specimens were analysed and a histological score was calculated. A score of 0 represented the lowest and 3 the highest level of CD200/CD200R1 expression [18]. Finally, the slides were photographed using a transmitted light microscope (Eclipse 80i; Nikon, Tokyo, Japan).

Western blotting

CD4+ T cells were cultured with recombinant human CD200Fc at a dosage of 200 ng/ml. The IgGFc isotype at the same dosage was used as control. After 5 days the cells were harvested, washed twice in ice-cold PBS and lysed by incubation for 1 h in a buffer containing 20 mM N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES; pH 7.9), 20% glycerol, 1% Nonidet P-40, 1 mM MgCl2, 0.5 mM EDTA, 0.1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulphonyl fluoride (PMSF) and a proteinase inhibitor cocktail (BD Biosciences, Franklin Lakes, NJ). The lysates were kept on ice and vortexed every 10 min for 1 h before centrifugation at 12 000g at 4 °C. Equal amounts of protein were separated by SDS-PAGE (Invitrogen), transferred to Immobilon polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), blocked with 5% dried milk in PBS containing 0.5% Tween 20, incubated with specific antibodies against DOK2 or pDOK2, then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody and finally developed using Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, Boston, MA, USA).

Transwell migration assay

The induced human Th17 cells were seeded in the upper chambers of the transwell. The lower chambers were filled with CCL20 alone or in the presence of the IgGFc isotype or CD200Fc. After 8 h of incubation, the cells that migrated to the lower chamber were counted.

Generation of monocyte-derived osteoclast and TRAP+ staining

CD14+ monocytes (1 x 10^6 cells/ml) were cultured with M-CSF (30 ng/ml) and RANKL (100 ng/ml) alone or in the presence of the IgGFc isotype or CD200Fc for 14 days. In vitro osteoclast differentiation was analysed by TRAP+ staining according to the manufacturer’s protocol.

Statistical analysis

All data were analysed using SPSS 13.0 software (SPSS, Chicago, IL, USA). The data that passed the Kolmogorov–Smirnov and Shapiro–Wilk tests (P > 0.05) were considered to be a normal distribution. For the data with a normal distribution and homogeneity of variance [mean (s.e.m.)], one-way analysis of variance with adjusted Bonferroni correction was used to assess the differences between the groups. An independent-sample t-test and paired-sample t-test were used to compare the differences between two groups and the differences before and after treatment. For data with a non-normal distribution [median (25th–75th percentiles)], the Mann–Whitney test was used to compare differences between two groups. Correlations between DAS28, DMRI, ΔTh1, ΔTh17 and ΔTreg (CD4+CD25+Foxp3+ T cell) with changes in CD200 and CD200R1 were calculated using Spearman’s rho test. P-values < 0.05 were considered statistically significant.

Results

Aberrant expression of CD200/CD200R1 in human RA

First we examined the expression of CD200 and CD200R1 by various cell subtypes in patients with active RA and found that the proportion of CD200+ cells in the PBMCs, CD14+ cells and CD4+ T cells was significantly lower than in HCs [3.8% (s.e.m. 0.4) vs 14.8% (s.e.m. 1.5), P < 0.0001; 3.6% (s.e.m. 0.2) vs 14.4% (s.e.m. 1.5), P < 0.0001; and 1.9% (s.e.m. 0.3) vs 6.4% (s.e.m. 1.1), P < 0.05, respectively; Fig. 1A, B, D and F]. In addition, the proportion of
FIG. 1 Abnormal expression of CD200/CD200R1 in patients with active RA

(A–C) CD200 and CD200R1 expression by PBMCs in HCs and RA patients. (D and E) CD200 and CD200R1 expression by peripheral CD14⁺ cells in HCs and RA patients. (F and G) CD200 and CD200R1 expression by peripheral CD4⁺ cells in HCs and RA patients. (H and I) Comparison of CD200 and CD200R1 expression in synovium from HCs (n=5), OA patients (n=5) and RA patients (n=5) (white arrow: CD200⁺ cells; yellow arrow: CD200R1⁺ cells; original magnification 100×). Stained sections were evaluated semi-quantitatively using scoring systems for synovial tissue. All areas of specimens were analysed and a histological score was calculated. A score of 0 represented the lowest and 3 the highest level of CD200/CD200R1 expression. (J) Comparison of CD200 and CD200R1 expression by CD4⁺ and CD14⁺ cells in synovial membrane (SM) and peripheral blood (PB) from RA patients (n=5). PBMCs: peripheral blood mononuclear cells; HCs: healthy controls.
CD200R1* cells in PBMCs, CD14* cells and CD4* T cells was also significantly lower than in HCs [8.0% (s.e.m. 1.2) vs 20.6% (s.e.m. 1.3), P < 0.0001; 2.1% (s.e.m. 0.2) vs 9.4% (s.e.m. 1.4), P < 0.05; and 2.4% (s.e.m. 0.3) vs 14.2% (s.e.m. 2.0), P < 0.0001, respectively; Fig. 1A, C, E and G]. No significant difference was found for the levels of CD200/CD200R1 in male compared with female patients (P > 0.05).

CD200 expression in synovium

We next examined CD200/CD200R1 expression in synovium of RA patients and compared it with that of HCs and OA patients. We found that in contrast to CD200* cells in OA synovium, which were mainly identified in the lining layer and to a lesser extent in the sublining layer, CD200* cells in RA synovium also extended into the deeper layer. The proportion of CD200* cells was higher in synovium from RA [2.47 (s.e.m. 0.18)] than from OA patients [1.82 (s.e.m. 0.24), P < 0.05] and from HCs [0.65 (s.e.m. 0.17), P < 0.001] (Fig. 1H and I). Unlike the intensity of CD200, there was no statistically significant difference in expression of CD200R1 in synovium from RA and OA patients and HCs [0.78 (s.e.m. 0.16) vs 0.89 (s.e.m. 0.13) vs 0.68 (s.e.m. 0.19), P > 0.05; Fig. 1H and I].

We compared CD200/CD200R1 expression by CD4* and CD14* cells from RA synovium with that from peripheral blood. In RA patients, FACS analysis demonstrated that the expression of CD200 and CD200R1 was similar on CD4* T cells from synovium compared with peripheral blood [6.0 (s.e.m. 0.5) vs 5.5 (s.e.m. 0.3), P > 0.05 and 2.0 (s.e.m. 0.2) vs 1.8 (s.e.m. 0.4), P > 0.05, respectively]. However, the expression of CD200 and CD200R1 on CD14* monocytes from RA synovium was higher than from peripheral blood [31.2 (s.e.m. 1.7) vs 19.5 (s.e.m. 1.0), P < 0.05 and 25.9 (s.e.m. 2.9) vs 18.9 (s.e.m. 1.3), P < 0.05, respectively; Fig. 1J].

The proportion of CD200* and CD200R1* cells in PBMCs, CD14* monocytes and CD4* T cells from RA patients increased following treatment with infliximab plus MTX and was negatively correlated with ΔDAS28, ΔMRI bone oedema and synovitis scores, ΔTh1 and ΔTh17 and positively correlated with ΔTreg cell numbers (see supplementary Table S1, available at Rheumatology Online).

CD200Fc effects on CD4* T cell proliferation, necrosis and apoptosis

We confirmed that soluble CD200Fc could trigger CD200/CD200R1 signalling by inducing the phosphorylation of DOK2 in CD4* T cells from RA patients (Fig. 2A). In addition, we found that CD200Fc partially inhibited anti-CD3/anti-CD28-stimulated CD4* T cell proliferation in RA patients [54.1% (s.e.m. 4.0) vs 46.9% (s.e.m. 2.4), P < 0.05] but not HCs [55.6% (s.e.m. 5.0) vs 58.2% (s.e.m. 5.4), P = 0.215; Fig. 2B]. Moreover, CD200Fc promoted apoptosis and necrosis in CD4* T cells from RA patients [apoptosis 0.77% (s.e.m. 0.03) vs 1.2% (s.e.m. 0.1), P < 0.001; late apoptosis/necrosis 2.1% (s.e.m. 0.4) vs 15.2% (s.e.m. 0.7), P < 0.0001, respectively] but not from HCs [apoptosis 0.49% (s.e.m. 0.03) vs 0.41% (s.e.m. 0.02), P > 0.05; late apoptosis/necrosis 1.8% (s.e.m. 0.4) vs 2.4% (s.e.m. 0.4), P > 0.05, respectively; Fig. 2C].

CD200Fc effects on Th17 differentiation and chemotactic CCR6 expression

Then we examined whether CD200Fc could affect CD4* T cell differentiation into Th17 cells and found that TGF-β, IL-1β, IL-23 and IL-6 in vitro induced Th17 cell differentiation in active RA patients and HCs, which was inhibited by in vitro CD200Fc (Fig. 3A). Interestingly, Th17 cell differentiation was also reduced in CD4* T cells from RA patients after treatment with DMARDs that increased cell expression of CD200/CD200R1 (Fig. 3B). In addition, we also generated Th17 cells by co-incubation of CD4* T cells from RA patients (HCs as control) with peripheral or synovial CD14* cells isolated from the same RA patients and found that both peripheral and synovial CD14* cells from RA patients increased the generation of Th17 cells and this Th17 generation was significantly inhibited by in vitro CD200Fc (Fig. 3C).

As CCR6 is a critical chemokine involved in Th17 migration to the inflammatory site in RA, we then examined whether CD200Fc could also affect CCR6-mediated Th17 chemotaxis and found that CD200Fc down-regulated CCR6 expression by Th17 cells and inhibited CCL20-mediated Th17 chemotaxis in RA patients, as assessed by transwell experiments (Fig. 3D).

CD200Fc inhibition of peripheral CD14* monocyte-derived osteoclast generation

Given that abnormal CD200/CD200R1 expression by CD4* T cells was corrected after treatment with infliximab plus MTX in RA patients, correlating with less radiographic progression, we explored whether CD200Fc can regulate osteoclast generation in RA patients. We generated osteoclasts by stimulating peripheral blood CD14* monocytes from RA patients with M-CSF and RANKL and found that in vitro CD200Fc reduced osteoclastogenesis as demonstrated by TRAP staining (Fig. 4A). When we stimulated RA peripheral CD14* cells with LPS, the expression of CD80 and CD86 was up-regulated, and this...
up-regulation was inhibited by in vitro CD200Fc in RA patients but not HCs (Fig. 4B).

Discussion

Recent studies have shown that the CD200/CD200R1 pathway has an immunosuppressive effect on the inflammatory cellular immune response and participates in the modulation of the severity of autoimmune diseases, such as CIA and experimental autoimmune uveoretinitis (EAE) [19, 20], in animal models and is therefore a potential target for anti-inflammatory treatment of human autoimmune diseases. However, our knowledge of the role of the CD200/CD200R1 axis in human diseases, especially in RA, is limited. This study demonstrated for the first time that there is aberrant expression of CD200/CD200R1 in cells from both the synovium and peripheral blood of RA patients.

Th17 cells are pro-inflammatory CD4+ T cells characterized by the expression of RAR-related orphan receptor C (RORc) and the production of IL-17A (IL-17), IL-17F and IL-6, and they are thought to be the key effector cells in the pathogenesis of RA [21, 22]. Previous studies have demonstrated increased infiltration of Th17 cells and production of IL-17 in synovium from RA patients [23], which not only up-regulated downstream pro-inflammatory cytokines, including TNF-α and IL-1β, but also synergized with these cytokines, leading to a vicious
FIG. 3 CD200Fc inhibited Th17 differentiation and down-regulated chemotactic CCR6 expression by Th17 cells

(A) TGF-β, IL-1β, IL-23 and IL-6 in vitro induced Th17 cell differentiation in active RA patients and HCs but was inhibited by in vitro CD200Fc. (B) CD200Fc in vitro inhibited CD4+ T cell differentiation into Th17 cells in untreated RA patients, whereas Th17 cell differentiation was also reduced in CD4+ T cells from treated RA patients who had corrected expression of CD200. (C) Both peripheral and synovial CD14+ cells from RA patients increased CD4+ cell differentiation into Th17 cells and this Th17 generation was significantly inhibited by in vitro CD200Fc. Th17 cells were generated by co-incubation of CD4+ cells from RA patients (HCs as controls) with peripheral or synovial CD14+ cells isolated from the same RA patients. (D) CD200Fc down-regulated chemotactic CCR6 expression by Th17 cells and inhibited CCL20-mediated chemotaxis of Th17 in RA patients. Data are depicted as the mean (S.E.M.) of six independent experiments performed on six different HCs and six different RA patients. HCs: healthy controls.
cycle of uncontrolled inflammation. There was also a study demonstrating that CD200+ chronic lymphocytic leukaemia cells suppressed production of the above mentioned pro-inflammatory cytokines and tumour cell lysis in mixed lymphocyte cultures [24] and that this suppression could be reversed by antagonistic CD200 antibodies. Furthermore, knockdown of CD200 in melanoma cells has been shown to enhance T cell responses in vitro. Of note, all the data were based on mixed lymphocyte cultures. It is therefore difficult to determine whether the reported effects are associated with direct inhibition or are transduced via antigen presenting cells (APCs). Our experiment with purified CD4+ T cells found that in vitro CD200Fc could inhibit CD4+ T cell differentiation into Th17 cells in untreated RA patients. Moreover, the addition of CD200Fc also induced DOK2 phosphorylation in CD4+ T cells stimulated with anti-CD3/anti-CD28, which was consistent with a previous report [25], and promoted CD4+ T cell apoptosis and necrosis in RA patients. In addition, CD200Fc down-regulated CCR6 expression and inhibited CCL20/CCR6-driven Th17 cell chemotaxis.

These findings suggest that CD200 can engage CD200R1 on CD4+ T cells, transduce an inhibitory signal and is involved in the fine-tuning and regulation of the immune response of CD4+ T cells, especially Th17 cells, in RA. As for the reason why the effect on proliferation and apoptosis was not observed in CD4+ T cells from HCs, we speculate that in vitro engagement of CD200R1 with...
CD200Fc could induce phosphorylation of DOK2 (Fig. 2A), thereby transducing an inhibitory signal on downstream phosphoinositide 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) pathways, which are pivotal in regulating cell proliferation and apoptosis and are over-activated in RA [1–3, 15, 21, 22].

Monocytes/macrophages are well-described components of synovial membrane inflammation [26, 27] that are characterized by the production of cytokines that perpetuate inflammation and proteases that induce cartilage destruction in RA [28–30].

In our study we found that the abnormal CD200/CD200R1 expression in RA had functional consequences for CD14+ monocytes. As CD200 lacks an intracellular signalling motif, it is presumed that CD200 exerts its immunological function via binding to its receptors. Peripheral CD14+ cells from RA patients cultured in the presence of CD200Fc had reduced osteoclastogenesis, and CD200Fc inhibited synovium and peripheral CD14+ cell-driven Th17 differentiation and down-regulated CD80 and CD86 expression on peripheral CD14+ cells challenged by LPS.

Synovium from RA patients had aberrant expression of CD200 and CD200R1. In contrast to synovium from OA patients (in whom CD200+ cells were mainly identified in the lining layer and, to a lesser extent, in the sublining layer where myeloid-derived cells are located), in synovium from RA patients, CD200+ cell infiltration extended beyond the lining and sublining layer into the deeper layer and adjacent lymphoid microstructures, where activated T and B cells are located. What is the underlying significance of such abnormal cell distribution in RA? Can such architectural disorganization with loss of contact inhibition of myeloid-derived cells contribute to localized onset of inflammation in the joint? We found that in vitro addition of CD200Fc not only reduced osteoclastogenesis by peripheral CD14+ monocytes but also inhibited CD14+ monocyte-like synoviocyte (MLS)-driven Th17 differentiation, thereby maintaining T cell homeostasis by preventing the shift towards inflammation and consequently restraining structural damage. In addition, we found that CD200Fc also down-regulated CD80 and CD86 expression by peripheral CD14+ cells, thereby inhibiting T cell activation and antigen presentation. These findings indicate that CD200/CD200R1 signalling can also exert immunosuppressive functions on myeloid-derived cells by inhibiting their differentiation into osteoclasts and MLS-driven Th17 differentiation as well as down-regulating co-stimulatory molecule expression. These functions restrain the strength of the autoreactive adaptive immune response and maintain the homeostasis involved in osteoimmunology. However, it remains to be explored whether the decreased peripheral CD200/CD200R1 results from cell migration to the synovium in response to inflammation.

Taken together, we demonstrated that there was abnormal expression of CD200 and CD200R1 in RA patients. This abnormal expression was corrected after treatment with a TNF-α antagonist plus MTX. Our functional analysis using in vitro CD200Fc collectively indicates that CD200/CD200R1 exerts anti-inflammatory functions via multiple mechanisms and suggests a potential immunotherapeutic role for targeting CD200/CD200R1 in RA.

**Rheumatology key messages**

- CD200 and CD200R1 expression are abnormal in RA patients.
- Aberrant expression of CD200/CD200R1 may contribute to abnormal Th17 cell differentiation, chemotaxis and osteoclastogenesis in RA patients.

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**Supplementary data**

Supplementary data are available at *Rheumatology* Online.

**References**


