Complement receptor type 1 (CR1, CD35) is a potent inhibitor of B-cell functions in rheumatoid arthritis patients

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Abstract

The involvement of B cells, complement activation and subsequent immune complex deposition has all been implicated in the pathogenesis of rheumatoid arthritis (RA). Although the reduced expression of complement receptor 1 (CR1, CD35) and 2 (CR2, CD21) on the B cells of RA patients has been known for a long time, their exact role in B-cell tolerance and autoimmunity is not yet fully understood. To get a deeper insight into the possible mechanisms, we studied the expression and function of CR1 and CR2 on various subsets of B cells of healthy donors and RA patients at various stages of the disease by FACS analysis, 3H-thymidine incorporation and ELISA. We found that CD19+CD27− naive B cells up-regulate the expression of the inhibitory CR1 during differentiation to CD19−CD27+ memory B cells both in healthy donors and in RA patients, whereas the expression of the activatory CR2 is down-regulated. This clearly demonstrates that the expression of these two antagonistic complement receptors is regulated differentially during the development of human B cells, a phenomenon which may influence the maintenance of peripheral B-cell tolerance. Our functional studies show that after clustering CR1 both by its natural ligand and To5 mAb, the inhibitory function of CD35 is maintained in RA patients, despite its significantly reduced expression compared with healthy individuals. Besides blocking B-cell receptor-induced proliferation, CR1 inhibits the differentiation of B cells to plasmablasts and their immunoglobulin production. Since the reduced expression of CR1 in RA patients does not affect its inhibitory function, this receptor might serve as a new target for therapeutical interventions.

Keywords: autoimmunity, B-cell subpopulations, expression of CR1 and CR2, inhibition of B-cell function

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by the presence of autoantibodies, joint inflammation and subsequent destruction of cartilage and bone (1). Although the pathogenesis of RA is not fully understood, the involvement of B-cell activation, inappropriate stimulation and regulation of complement has all been implicated (2). Complement components are actively synthesized in the synovium (3) and consumption of various components has been demonstrated in the inflamed joint (4). Moreover, autoantibody-containing immune complexes can activate the complement cascade via both the classical and alternative pathways, rendering B-cells active participants in the pathogenesis of RA (5). These data suggest that complement activation is a main event in the inflammatory process leading to the pathogenesis of RA. Based on experimental data obtained in animal models, it is well accepted that antigen-specific B lymphocytes are required both as antigen-presenting and autoantibody-producing cells for the induction of severe autoimmune arthritis (6, 7). B cells can proliferate and undergo antigen-driven selection in structures similar to germinal centres formed in the synovium (8). These cells show phenotypes that highlight a terminal differentiation process toward plasma cells and memory B cells, producing high-affinity antibodies against auto-antigens, including rheumatoid factors, antibodies to collagen type II and citrullinated proteins, which can form immune complexes (9).
Immune complexes generated under healthy and autoimmune conditions contain activated fragments of the central complement component C3. These complexes have the capacity to bind to the surface of B cells through complement receptor 1 (CR1), complement receptor 2 (CR2) and the inhibitory Fc receptor, CD32b, and the crosstalk between the B-cell receptor (BCR) and these structures strongly influences humoral immunity (10–12). CR1 (CD35) is a type-I membrane glycoprotein expressed by several cell types, including erythrocytes, B cells, thymocytes, monocytes, macrophages, neutrophil and eosinophil granulocytes, follicular dendritic cells (FDCs), Kupffer cells and podocytes (13). Ligands of CR1 are C3b, iC3b and C4b fragments of C3 and C4 proteins. CR1 functions also as regulator of complement activation, acting as a cofactor for the Factor I–mediated cleavage of C3b and C4b and as an inhibitor of both the classical and alternative pathway convertases (14).

In addition, as we have demonstrated earlier, aggregated C3, the C3b-like ligand of CR1, inhibits the proliferation and Ca2+ response of BCR-activated human tonsil B cells (15). CR1 is also indispensable during the development of memory B lymphocytes by forming a linkage between immune complexes trapped by FDCs and germinal center B cells (16).

The human CR2 is expressed by B cells, FDCs, activated T cells, mast cells and basophil granulocytes (17). Ligands of CD21 are C3d/g, iC3b (18), Epstein-Barr virus (19) and CD23 (20). CR2 on the surface of B cells appears in a trimolecular complex of CD81/CD19/CR2. When it is coligated with the BCR, it mediates activation, proliferation and differentiation and lowers the threshold of antigen sensitivity of B cells (21). Like CR1, expression of CR2 on FDCs is also essential for developing an effective memory response (16).

The importance of CR1, CR2 and FcγRIIb (CD32b) has been demonstrated in several autoimmune diseases, both in human (22, 23) and animal studies (24–26). The expression and function of the inhibitory FcγRIIb have been studied extensively (22, 27, 28) both under physiological and autoimmune conditions [RA and systemic lupus erythematosus (SLE)], demonstrating a reduced inhibitory function of the receptor in the latter cases. In RA, altered and reduced expression both of CR1 and CR2 (28, 29) has been proven on B cells. Prokopiec et al. (28) showed that B cells of RA patients express lower levels of CR1 and CR2 than healthy individuals, but they could not find any correlation between disease activity and low complement-receptor expression by B cells. The functional role of CR1 and CR2 was investigated in several animal models of collagen-induced arthritis, and a prominent role of complement receptors in the regulation of human B-cell functions has been demonstrated (24–26). It is important to emphasize, however, that the results obtained from experiments with mice can be adapted to human systems only with great care. Although in the mouse CR1 and CR2 are alternatively spliced products of the same gene (Cv2), in human two different genes encode these cell-membrane molecules. Human CR1 and CR2 differ not only in their ligand binding but also in their cellular distribution and function (30). Therefore, the physiological and pathological function of CR1—such as its inhibitory effect—cannot be revealed and examined in mice.

Our aim is to unravel how the expression and function of CR1 and CR2 on different human B-cell subsets, namely on CD19+CD27− naive, CD19+CD27+ memory B cells and CD19intCD27high plasmablasts are regulated under physiological and autoimmune conditions. Our results clearly show that the expression of the inhibitory CR1 and the activatory CR2 are regulated differentially during the development of human B cells. To get a deeper insight into the CR1-mediated regulatory function in autoimmune diseases, the role of CR1 on B cells of RA patients was also studied. Using well-defined experimental conditions, we re-examined the CR1-mediated inhibition of proliferation and antibody production by human B cells, since data found in the literature are controversial (31, 32). We show that CR1 clustering both by its natural ligand and the receptor-specific To5 mAb results in a strong inhibition of proliferation, differentiation to plasmablasts and antibody production not only in healthy individuals but also in RA patients. Importantly, the reduced level of CR1 on the B cells of RA patients does not affect its inhibitory function, thus CR1 can be considered a promising therapeutic target in rheumatoid arthritis.

Methods

Patients and controls

Blood samples were obtained from 23 healthy individuals, aged 43 ± 15 (18 women, 5 men). From RA patients, aged 60 ± 10, blood of 26 active, 11 moderately active and 10 inactive patients (42 women, 5 men) were obtained from the Institute of Rheumatology and Physiotherapy, Hungary. All of the patients fulfilled the criteria of a definitive diagnosis of RA, suggested by the American College of Rheumatology (33). Patients were grouped based on the disease activity score for 28 joints (DAS28) (34). They were considered active with DAS28 > 5.1, moderately active with 3.2 < DAS28 < 5.1 and inactive with DAS28 < 3.2. The current treatment regimens included non-steroid anti-inflammatory drugs, steroid anti-inflammatory drugs (prednisolone, methylprednisolone) and disease-modifying anti-rheumatoid drugs (methotrexate, leflunomide, chloroquine, sulfasalazine). No patients underwent biological therapy during the period of the experiments. For the functional assays, only B cells of patients with active disease were used (average DAS28: 6.17). The study was approved by the local ethical committee (Institutional Review Board of National Institute of Rheumatology and Physiotherapy) and written informed consent was obtained from all participating subjects.

Flow cytometry

Immunofluorescence measurements were performed using a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA, USA) and the FCSExpress software, version 3.0. PBMCs were isolated from the heparinized blood of healthy individuals and RA patients by Ficoll Hypaque (GE Healthcare, Uppsala, Sweden) density-gradient centrifugation and washed in PBS. PBMCs (4 × 103) were stained with the following antibodies: allophycocyanin (APC)-conjugated anti-human CD19 (Immunotools, Friesoythe, Germany), PE-conjugated anti-human CD27 (Caltag Laboratories, Bangkok, Thailand), FITC-conjugated anti-human CR1 (Clone: E11, BDPharmingen, Heidelberg, Germany) and FITC-conjugated anti-human CR2 (Clone: LT21, Immunotools). Isotype-matched FITC-
PE- and APC-conjugated mouse immunoglobulins were used as negative controls for non-specific staining. On the basis of forward and side scattering, lymphocytes were counted and dead cells were excluded from the measurements. CD19+ B cells were further identified as CD19-CD27- naive, CD19-CD27+ memory B cells and CD19+CD27+ plasmablasts. After incubation with the appropriate antibodies on ice for 30 min, cells were washed and re-suspended in 200 µl PBS containing 1% FCS and 0.1% NaN3. Data from 400 000 cells were collected and analysed.

Isolation and aggregation of human C3; characterization by transmission electron microscopy

Human C3 was isolated from pooled normal human serum by fast protein liquid chromatography as described by Basta and Hammer (35). Purified C3 was collected, concentrated and dialysed against PBS. To minimize IgG contamination, C3 solution was incubated with Protein G beads (ThermoScientific, Rockford). The purity of C3 was checked by SDS-PAGE and Coomassie blue staining. C3 fractions were stored at −20°C until use. Aggregated C3 was generated by incubating isolated human C3 at 63°C for 20 min.

For the characterization of aggregated C3 by transmission electron microscope (TEM) 10 µl aliquots of the protein solution were placed on formvar-carbon-coated 400-mesh copper grids (Electron Microscopy Sciences, Washington, PA, USA) and stained with uranyl acetate. The aggregates were characterized by TEM on a Philips CM 10 TEM (FEI Company, Hillsboro, OR, USA), operating at 100 kV. Images were taken with a Megaview II Soft Imaging System, routinely at magnifications of ×46000 and ×64000 and analysed using an ITEM® 5.2 software package (Olympus Soft Imaging System GmbH, Münster, Germany). These studies reveal that the heat treatment of the isolated human C3 caused considerable changes both in the size and in the structure and confirms earlier results proving the appearance of ‘C3b-like’ epitopes on the aggregated protein, which mediate CR1 binding (15).

B-cell proliferation assay

Peripheral B cells were isolated from healthy individuals and patients with active RA with RosetteSep B-cell isolation Kit (StemCell Technologies, Vancouver, Canada) through negative selection. Cells were cultured at 2 × 105 cells/well in 100 µl RPMI-1640 medium (SigmaAldrich) supplemented with 10% FCS and gentamycin in 96-well microtitre U-bottom plates (Costar) at 37°C in a humidified atmosphere containing 5% CO2. B cells isolated from healthy individuals and patients with active RA were activated with 5 µg/ml anti-human IgG/M/A (Jackson) in the presence or absence of 50ng/ml rhIL-2 (Immunotools), 50ng/ml rhIL-10 (Immunotools), 100ng/ml sCD40L (Immunotools) and various amounts of aggregated C3 were added to the wells. As controls, we employed cells cultured in medium, cytokines or heat-aggregated C3 only. On Day 4, cells were washed and rhIL-2 and rhIL-10 were added. After 8 days of culture, the cells were used to determine the number of CD20+CD19+CD27+ plasmablasts, and culture supernatants were collected to analyse the amount of secreted IgM and IgG by ELISA. Briefly, cells were washed twice in ice-cold PBS then stained with the following antibodies: FITC-conjugated anti-human CD20 (Immunotools), PE-conjugated anti-human CD27 (Caltag Laboratories) and APC-conjugated anti-human CD19 (Immunotools). Isotype-matched FITC-, PE- and APC-conjugated mouse immunoglobulins were used to assess non-specific staining. After incubation with the appropriate antibodies on ice for 30 min, cells were washed and re-suspended in 200 µl PBS containing 1% FCS and 0.1% NaN3. Measurements were performed using a FACSCalibur flow cytometer (BD Biosciences). Data of 100 000 cells were collected and the percentage of plasmablasts was counted in the living B-cell population. Data presented show mean percentage of plasmablasts ± SD of duplicate samples of one representative experiment or mean percentage of inhibition ± SEM of four independent experiments.

Assay for immunoglobulin production

IgM and IgG production of isolated human B cells was measured by ELISA. Briefly, duplicate wells of microtitre plates (Costar) were coated with 3 µg/ml anti-human IgM or IgG (BD Pharmingen) at 4°C, overnight. After washing four times with PBS containing 0.05% Tween20, the plates were incubated with the cell-culture supernatants for 2h at 37°C. As standard, human IgM or IgG isolated from human serum was used at different concentrations. After washing, the plates were incubated with HRP-conjugated anti-human IgM (1:1000) purchased from DAKO (Glostrup, Denmark) or anti-human IgG (1:1000) obtained from BD Pharmingen. For visualization, tetramethylbenzidine (Sigma Aldrich) was used as a chromogen, Optical Density (OD) values were measured at 450nm. The amount of the secreted immunoglobulins was determined using the OD values of known concentrations of standard human IgM and IgG. Data are shown as mean ng/ml secreted antibody ± SD of duplicate samples of one representative experiment or mean percentage of inhibition ± SEM of four independent experiments.
Statistical analysis

Statistical analysis on the distribution of B-cell subpopulations and receptor expression was performed using the permutation test. In the case of the functional studies, the results of one representative experiment of four independent assays with similar results are shown. Data were analysed using PrismSoftware, version 4.0 (GraphPad Software).

Results

RA patients at different stages of the disease have similar frequencies of B-cell subsets as healthy controls

Catalán et al. (27) recently showed that the frequency of B cells in the blood of healthy individuals and RA patients is similar by examining the percentages of human B-cell subsets of healthy and autoimmune individuals. In the present study, we aimed to clarify whether there is any difference regarding the percentage of various B-cell subsets in RA patients in the inactive, moderately active and active states. As seen in Fig. 1, the percentage of CD19<sup>+</sup>CD27<sup>-</sup> naive and CD19<sup>+</sup>CD27<sup>+</sup> memory B cells is highly dispersed in each group, and there is no significant difference regarding the frequency of the distinct B-cell subsets (P < 0.05) when various groups of RA patients and control subjects are compared. Furthermore, there is no difference in the appearance of CD19<sup>low</sup>CD27<sup>high</sup> plasmablasts in the different groups investigated here.

Naive B cells up-regulate CR1 and down-regulate CR2 expression during differentiation to memory cells both in healthy donors and RA patients

Earlier studies showed that the expression of CR1 and CR2 is lower in the B cells of RA patients (28), but no data are available so far regarding the distribution of these complement receptors on different B-cell subpopulations either in healthy individuals or in autoimmune patients. To examine this, we analysed the expression of CR1 and CR2 in the following B-cell subsets: CD19<sup>+</sup>CD27<sup>-</sup> naive, CD19<sup>+</sup>CD27<sup>+</sup> memory B cells and CD19<sup>low</sup>CD27<sup>high</sup> plasmablasts. We found that the memory B cells of patients with active RA and healthy individuals express significantly higher levels of CR1 than naive B cells (P < 0.05) (Fig. 2A), whereas in the case of RA patients with disease at a moderately active or inactive stage, this elevation is not significant (P > 0.05). As demonstrated in Fig. 1, CR1 expression on plasmablasts is significantly lower in each group when compared with naive and memory cells (P < 0.001). Expression of CR2, however, changes in the opposite way; it decreases during differentiation to memory B cells (Fig. 2B). The reduction is significant (P < 0.05) in healthy donors, and in patients with active or inactive RA. This result demonstrates that the expression of these two antagonistic complement receptors is regulated differentially during the development of human B cells. CR2 expression on plasmablasts is very low in contrast to the other B-cell subsets (P < 0.001), confirming earlier results (22, 37).

CR1 inhibits the proliferation of B cells derived from patients with active RA

It has been described earlier that clustering CR1 via its ligand inhibits BCR-induced activation of B cells obtained from healthy individuals (15). Since the expression of this complement receptor is significantly reduced on the B cells of RA patients, it was important to investigate whether the inhibitory function of CR1 is still exerted. Therefore, separated B cells of patients with active RA were activated via their BCR in the presence of aggregated C3, the natural multimeric C3b-like ligand of CR1, and <sup>3</sup>H-thymidine incorporation was measured. As seen in Fig. 3A and B, the C3b-like ligand exerted a strong and dose-dependent inhibition on the BCR-induced proliferation. It is important to note that CR1-ligation alone had no effect at all. To further confirm the role of CR1, the experiments were carried out using the CR1-specific monoclonal antibody To5, clustered by anti-mouse IgG. As seen in Fig. 3C and 3D, the antibody-mediated
cross-linking of CR1 had a similar effect as the multimeric ligand, namely it caused a strong inhibition of B-cell proliferation of both healthy donors and patients with active RA. It should be mentioned that we did not see any difference between the proliferative capacity of B cells derived from healthy donors and patients with active RA—confirming the results of Prokopec et al. (28).

**CR1 clustering prevents B-cell differentiation to plasmablasts**

In the next step, we set out to investigate, whether CR1 cross-linking also affects plasmablast formation. To this end, freshly isolated B cells were cultured in the presence of anti-human IgG/M/A antibody, IL-2, IL-10 and sCD40L and aggregated C3—as detailed in Fig. 4. After 8 days, cells were washed and stained to monitor the expression of CD20 and CD27. Among the CD19+ cells, plasmablasts were identified by their CD20 and CD27 expression. As shown in Fig. 4, cells of healthy donors and patients with active RA—confirming the results of Prokopec et al. (28).

**CR1 mediates inhibition of immunoglobulin production by B cells of healthy donors and patients with active RA**

Since we found that B-cell differentiation to plasmablasts is strongly inhibited by CR1-clustering, next we set out to investigate whether the major B-cell function, namely immunoglobulin production, is also affected by the complement-derived ligand. To define this, supernatants of B cells cultured as described in the previous paragraph were collected at Day 8 and measured for the amount of secreted IgM and IgG. As illustrated in Fig. 5, B cells of healthy donors produce little amounts of IgM (Fig. 5A) and IgG (Fig. 5B) without BCR stimulation or in the presence of various cytokines. The highest amount of antibody was produced when cells were cultured in the presence of anti-human IgG/M/A and IL-2, IL-10 and sCD40L. A similar effect was observed in the case of B cells isolated from patients with active RA (Fig. 5C and D). CR1-clustering inhibited antibody secretion strongly in each case, and the effect of the complement protein was dose dependent. In the case of patients with active RA, the amount of secreted antibodies (both IgG and IgM) was lower; however, the inhibitory effect of the aggregated C3 was still significant. Interestingly, in the patient’s B cells, the BCR stimulus alone also induced antibody production, which might be due to the altered signalling threshold of autoimmune B cells (39).

**Discussion**

The fate of B lymphocytes is mediated by several cell-surface receptors, such as BCR, CR1, CR2, and FcγRIIb, which are able to interact with immune complexes of various compositions (40). In the pathogenesis of RA, the involvement of B cells, complement activation and subsequent immune-complex formation has been
implicated. It is well accepted that in animal models, the dysfunction of the immune-complex binding receptors—such as CR1, CR2 and FcγRIIb—induces and increases the severity of collagen-induced arthritis (25–27, 41), a model for human RA. The expression and function of FcγRIIb are well established both under physiological and autoimmune conditions, highlighting an altered and reduced inhibitory potential of the receptor under pathological conditions (27, 41). The role of complement receptors is less well known, and most of the results derive from animal studies. It is important to emphasize, however, that results related to the expression and function of the immune-complex binding receptors obtained from studies with mice can be adapted to humans with great care only, since the cellular distribution, specificity and function of these cell-membrane structures are not identical in these species. The receptors for CR1 and CR2 in mice are encoded by one gene (Cr2) in contrast to humans, where two separate genes code for these cell-membrane proteins, underlying their different functions. Namely, in humans CR1 is an inhibitory receptor (15)—in contrast to CR2, which has similar functions in mice and men. In addition, B cells are increasingly recognized as key cell types in the pathogenesis of RA, since they can function as antigen-presenting-, autoantibody- and cytokine-producing cells, as well. Non-depleting antibodies that target autoreactive B cells and modulate their functions by binding to receptor complexes, including CR1 or CR2, may also be an effective therapy.

In earlier studies, a lower level of CR1 and CR2 on B cells of RA patients was reported by Prokopec et al. (28), whereas the

**Fig. 3.** CR1 clustering inhibits BCR-induced proliferation of B cells of active RA patients and healthy donors. Isolated B cells were activated with the F(ab’), fragment of anti-human IgG/M/A antibody (5 µg/ml) in the presence of different concentrations of heat-aggregated C3 (A/1, A/2, B/1 and B/2) and by the CR1-specific antibody To5 (C/1, C/2, D/1 and D/2). As control, cells were cultured in medium, in the presence of heat-aggregated C3 only or an isotype-matched control mouse Ig. Cells were harvested after pulsing with 1 µCi/well ^3^H-thymidine for the last 18 h of culture. In A/1, B/1, C/1 and D/1 results (mean ± SD cpm of triplicate samples) of one representative of five independent experiments with similar results are shown. In the inserted panels A/2, B/2, C/2 and D/2 results of five independent experiments are summarized (mean percentage of inhibition ± SEM).
frequency of B cells in RA patients and healthy individuals was found similar (27). To get a better understanding of the interplay between B cells and the complement system in RA, we studied the expression and function of CR1 and CR2 on various B-cell populations. We analyzed the naive, memory and plasmablast pools of B cells. In the case of healthy donors, we found that the expression of CR1 is up-regulated during the differentiation of B lymphocytes toward memory cells. In contrast to this, the appearance of CR2 decreased, proving that these two receptors are regulated in a different manner on human B cells. Confirming earlier results (22, 37), we also detected a significant reduction in the level of complement receptors on human plasmablasts. Although in RA patients the expression level of both CR1 and CR2 was lower on all B-cell subsets than in healthy controls, during differentiation there was a similar change. We have clearly demonstrated that the difference between the distinct B-cell subsets in healthy individuals and in RA patients was not caused by the different frequencies of the subpopulations. This finding again emphasizes that these complement receptors are regulated differentially in human B lymphocytes. Taking into consideration that CR1 and CR2 have opposite functions in human B cells (15, 21), a delicate regulation of B-cell development may take place, depending on the composition of the immune complexes. The higher expression of CR1 on memory B cells could function as a final barrier to reduce the opportunity of autoreactive memory B cells to differentiate into autoantibody-producing plasma cells. This hypothesis is supported by our results showing lower frequencies of CD27^{high}/CD20^{−}/CD19^{−} plasmablasts in B-cell cultures where CR1 is clustered by its natural ligand. The lower expression of CR2 on peripheral memory B cells has a similar effect, since it elevates the activation threshold of effector memory B cells. In the case of RA patients, all B-cell subpopulations reduced the expression of complement receptors, but the change in the expression pattern during the differentiation of B lymphocytes has been maintained. This finding points to a strict regulation of complement receptors, which seems to be missing in other B-cell-mediated autoimmune diseases, such as SLE (22, 23). Our experiments show that the imbalance in the regulation of B-cell development is due to the reduced expression of complement receptors on the B cells of RA patients and not to the differences between distinct B-cell subsets. Earlier studies demonstrated that dysfunction of FcγRIIb, the other inhibitory immune-complex-binding receptor on human B cells, induces over-activation and differentiation of effector memory B cells, breaking down peripheral tolerance (12, 28, 42). This finding together with ours highlights the complex regulation of B-cell tolerance, which can be broken down by changes in the expression and function of the different immune-complex-binding receptors and also by changes in the actual composition of the immune complexes.

In autoimmune conditions, even a small alteration in the expression of various receptors may be sufficient to cause imbalance in the regulation of B cells leading to autoimmunity. In spite of the significant decrease of CR1 expression in RA patients, the inhibitory capacity of this complement receptor is preserved, and its ligation results in a significant inhibition of B-cell proliferation and antibody production—similarly to that found in the case of healthy individuals. Clustering CR1 by its natural ligand caused a dose-dependent inhibition of proliferation, plasmablast differentiation and antibody secretion of B cells from healthy people and patients with active RA. These functional results together with the expression data suggest other roles of CR1 than induction of arthritis, since its inhibitory function is unaffected by the lower expression level on arthritic B cells.

Fig. 4. CR1 clustering inhibits plasmablast differentiation. B cells isolated from healthy individuals (A) and active RA patients (B) were stimulated with the F(\(ab\))\(_2\) fragment of anti-human IgG/M/A (5 µg/ml) in the presence or absence of 50 ng/ml rhIL-2, 50 ng/ml rhIL-10, 100 ng/ml sCD40L and various concentrations of aggregated C3. As control, cells cultured in medium, cytokines or heat-aggregated C3 only were used. On Day 4, cells were washed and further cultured with fresh rhIL-2 and rhIL-10. The number of CD20^{−}/CD19^{−}/CD27^{high} plasmablasts was assessed after 8 days by flow cytometry. Frequency of plasmablasts is expressed as mean ± SD of duplicate cultures. In A/1 and B/1, one representative of four independent experiments with similar results is shown. In the inserted panels, A/2 and B/2 results of four independent experiments are summarized (mean percentage of inhibition ± SEM).
The fate of B lymphocytes is determined by signals transduced through several cell-surface receptors, such as BCR, CR1, CR2 and FcγRIIb. We have shown for the first time that CR1 and CR2 are expressed differentially by human B lymphocytes; moreover, their role is antagonistic. Thus our findings suggest that these complement receptors play an important role in the immune-complex-mediated fine-tuning of B cells’ function. Since the reduced expression of CR1 in RA patients does not affect its inhibitory function, this receptor might serve as a new objective for therapeutic interventions, targeting specifically the autoreactive B cells through CR1, such as in SLE (43).

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