Mechanisms of CD23 Hyperexpression on B Cells from Patients with Rheumatoid Arthritis

SONIA DE MIGUEL, BEGOÑA GALOCHA, JUAN A. JOVER, ANTONIO BAÑARES, CÉSAR HERNÁNDEZ-GARCÍA, JOSE A. GARCÍA-ASENJO, and BENJAMÍN FERNÁNDEZ-GUTIÉRREZ

ABSTRACT. ***Objective.*** To analyze the mechanisms involved in the characteristic hyperexpression of CD23 on peripheral blood B cells from patients with rheumatoid arthritis (RA).

Methods. Peripheral blood mononuclear cells (PBMC) were obtained from patients with active disease and activated during 18 h with an anti-CD3 monoclonal antibody in the presence or absence of blocking antibodies to CD154 or CD40. PBMC were further purified by rosetting and CD23 expression was assessed on B cells by flow cytometry after double staining (CD19/CD23). Lymphocytes were also isolated from synovial fluid (SF). CD154 expression was analyzed on PB or SF CD4+ T cells after double staining (CD4/CD154) by flow cytometry at basal conditions and after different stimuli [anti-CD3 or phorbol myristic acetate (PMA) plus ionomycin]. Co-culture experiments between SF and PB cells were performed to analyze the involvement of the CD40-CD154 interaction on CD23 expression. CD154 and CD23 expression was also analyzed on synovial membrane by immunohistochemical techniques.

Results. A high proportion of activated CD23 B cells was detected in patients with RA. Blocking experiments with both anti-CD40 and anti-CD154 Mab showed a significant reduction in the proportion of PB B cells expressing CD23. Following activation with anti-CD3 Mab or PMA plus ionomycin, CD154 expression was mainly induced on PB CD4+ T cells. In co-culture experiments, SF T cells were more efficient than PB T cells in inducing CD40 dependent CD23 expression on PB B cells. In addition, CD4+ T cells from synovial membrane clearly expressed CD154.

Conclusion. Our results establish a link between CD154-CD40 pathway and CD23 expression on PB B cells from patients with RA. T cells from the synovial microenvironment were active participants in this CD23 expression, presumably in the context of cell recirculation. (J Rheumatol 2001;28:1222–8)

Key Indexing Terms:
RHEUMATOID ARTHRITIS T CELLS B CELLS IMMUNE REGULATION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by a marked mononuclear cell infiltration of synovial tissue and synovial fluid (SF). T lymphocytes predominate in these infiltrates, which contain a population of CD4+ cells that is mostly CD45R0+ (memory T cells), a phenotype that has shown an enhanced capacity for cellular migration[1,2].

Previous results show that T dependent B cell activation (measured as CD23 expression)[3,4] and T dependent B cell differentiation (isotype-specific immunoglobulin production)[5,6] are particularly efficient in RA. Both phenomena are, to some extent, mediated by the T cell–B cell activating molecule CD40L (CD154).

From the Services of Rheumatology and Pathology, Hospital Clínico San Carlos, Madrid, Spain.

Supported in part by a grant from Fondo de Investigación Sanitaria (PI FIS 97/0430).

S. De Miguel, MD; B. Galocha, MD, PhD; J.A. Jover, MD, PhD; A. Bañares, MD, PhD; C. Hernández-García, MD, PhD; J.A. García-Asenjo, MD, PhD; B. Fernández-Gutiérrez, MD, PhD.

Address reprint requests to Dr. B. Fernandez-Gutierrez, Service of Rheumatology, Hospital Clinico San Carlos, 28040 Madrid, Spain.

Submitted June 6, 2000 revision accepted December 14, 2000.

CD154 is a 33 kDa glycoprotein that belongs to the tumor necrosis factor family of proteins. It is rapidly and transiently expressed on the surface of activated CD4+ T cells, and has been shown to be biologically active. CD154 interacts with CD40 on B cells and delivers critical signals promoting B cell activation (CD23 expression), differentiation, and Ig class switching[7-9].

The CD23 molecule, a member of the C-type animal lectin superfamily of signal transmitting receptors, is usually absent or expressed in a small percentage of normal peripheral blood mononuclear cells (PBMC) and is induced mainly on B cells after their culture with interleukin 4 or by cognate T help. CD23 is expressed in its membrane bound form only 4 h after lymphocyte activation, with a peak of expression at 16–24 h, being shed into the medium by proteolytic cleavage as a biologically active soluble form, and downmodulated from the cell surface at 72 h[10-13]. CD23 is the low affinity receptor for IgE, and has a second ligand (CD21) expressed by B cells, some T cells, and follicular dendritic cells[14]. Reports describe a specific regulation of this antigen in autoimmune rheumatic diseases like systemic lupus erythematosus and RA[3,4,15-17].

We wanted to characterize the mechanisms involved in

the hyperexpression of CD23 observed in PB B cells from patients with RA.

MATERIALS AND METHODS

Patients. Patients included in our study fulfilled the American College of Rheumatology revised criteria for RA with active disease[18]. Active disease was defined by at least 3 of the 4 following criteria: morning stiffness > 45 min, erythrocyte sedimentation rate (ESR) > 28 mm/h, and presence of > 3 swollen joints or > 6 painful or tender joints upon motion. Most patients were in the first 2 months of treatment with a second-line drug, and none was taking more than 10 mg/day of prednisone. Healthy volunteers made up the control group. Blood samples were collected into heparinized syringes. Paired SF samples were obtained by aseptic aspiration when clinically indicated and collected into heparinized syringes. Synovial tissue was obtained from 9 patients while undergoing joint replacement surgery. Informed consent was obtained from each patient enrolled in the study.

Monoclonal antibodies (Mab). The following Mab were used: anti-CD3 Mab 454, which has been used at mitogenic concentrations (1/10 supernatant)[3,4,10,12,17] and anti-CD23 Mab $EBVCS_2$ (Dr. S. Friedman, Hospital for Special Surgery, New York, NY, USA); anti-CD154 Mab hCD40-Hm (fusion protein; Dr. P. Lane, Basel Institute for Immunology, Basel, Switzerland)[19]; anti-CD154 TRAP-1 (Dako Corp., Denmark); anti-CD40 Mab 89 (Immunotech, Marseille, France); anti-CD45 Mab D3/9 (control antibody) and anti-CD4 Mab OKT4 (Dr. F. Sanchez-Madrid, Hospital de La Princesa, Madrid, Spain); phycoerythrin (PE) conjugated anti-CD23 and anti-CD4 and fluorescein isothiocyanate (FITC) conjugated anti-CD19 (Coulter Immunology, Hialeah, FL, USA); and anti-CD20 Mab L26 (Dako).

Cell preparation and culture conditions. Mononuclear cells were isolated by density gradient centrifugation on Ficoll-Hypaque (Pharmacia, Sweden) and resuspended in RPMI-1640 medium (Flow Laboratories, Scotland) supplemented with 10% fetal calf serum (Gibco BRL, Paisley, Scotland), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine.

CD23 induction assay. PBMC were cultured in 10 ml round-bottom tubes (Soria-Greiner, Madrid, Spain) at a density of 5×10^6 cells/0.5 ml of either RPMI-1640 medium alone, stimulated with the 454 Mab (anti-CD3, 1/10 dilution of hybridoma supernatant, saturating concentration) or with the 454 Mab in combination with different blocking antibodies (control antibody, anti-CD154 Mab hCD40-Hm, or anti-CD40 Mab 89) to inhibit CD23 expression. These inhibition experiments were performed in RPMI-1640 with AB serum to avoid Fc receptor contribution.

Cells were incubated 18 h at 37ºC in a 5% CO_2 atmosphere. Following extensive washing with Hanks' solution (Gibco BRL), mononuclear cells were further purified by rosetting for 1 h at 4ºC with neuraminidase treated sheep red blood cells (N-SRBC) as described[20]. The rosetting (E+) and nonrosetting (E–) populations were then separated by Ficoll-Hypaque gradient centrifugation.

When indicated, SF or PB T cells previously purified by rosetting (> 90% CD3+) were co-cultured with their paired PB E cells.

Following 18 h of culture, CD23 expression was analyzed on PB B cells by flow cytometry. The percentage of inhibition of CD23 expression was calculated according to the formula:

$$x = 100 - \frac{\text{CD23 expression in the presence of anti-CD3 Mab and blocking antibodies}}{\text{CD23 expression following anti-CD3 Mab}} \times 100$$

CD154 induction assay. For stimulation of T cells, PBMC were cultured in 10 ml round-bottom tubes (Soria-Greiner) at a density of 5×10^6 cells/0.5 ml either in RPMI-1640 medium alone or stimulated with plastic coated 454 Mab (anti-CD3, 1/10 dilution of hybridoma supernatant, saturating concentration). For stimulation with phorbol myristic acetate (PMA) and ionomycin, PBMC were cultured in 10 ml round-bottom tubes (Soria-Greiner) at a density of 5×10^6 cells/0.5 ml with PMA (10 ng/ml) and ionomycin (1 μg/ml). After 6 h incubation, cells were washed once with phosphate buffered saline (PBS), and CD154 expression was analyzed by flow cytometry.

Cell staining and flow cytometry analysis. Following extensive washing in PBS plus 1% bovine serum albumin, cells were stained for 30 min at 4ºC.

To analyze CD23 expression on B cells, the E– population was double stained with PE conjugated anti-CD23 and FITC conjugated anti-CD19 Mab. The results were expressed as the percentage of double positive cells for CD19 and CD23. CD154 expression was analyzed by flow cytometry and labelling with a hCD40-Hm Mab followed by FITC conjugated goat anti-human IgM $F(ab)_2$ (Sigma, St. Louis, MO, USA). To measure CD154 expression on CD4+ cells, PBMC or SF cells were double stained for CD154 and CD4. The results were expressed as the percentage of double positive cells for CD154 and CD4.

Cells were analyzed using a flow cytometer (Epics Profile II; Coulter) to gate the lymphocyte population. A total of 5×10^3 events were analyzed, measuring the mean fluorescence intensity on a logarithmic scale. Appropriate isotype matched control antibodies were used in all cases.

Immunohistochemistry. Five micrometer synovial membrane sections were stained to reveal CD23 and CD154 expression. Double staining using an immunoalkaline phosphatase/diaminobenzidine staining procedure (Histostatin DS™ kit; Zymed, Burlingame, CA, USA) was used to localize CD154 on CD4+ cells and CD23 on B cells. Briefly, sections were blocked for 10 min in nonimmune serum (goat), followed by a 30 to 60 min incubation with primary antibody 1 (anti-CD4 or anti-CD20). Sections were then incubated for 10 min with a biotinylated goat anti-mouse IgG, which was visualized using the avidin biotin method with streptavidin alkaline phosphatase followed by vector blue (Fast blue). Subsequently, sections were counterstained with a double staining enhancer and incubated 10 min with nonimmune serum (goat), and primary antibody 2 (anti-CD154 Mab TRAP-1 or anti-CD23 Mab $EBVCS_2$) was added for 30 to 60 min. Primary antibody 2 was visualized with the same avidin biotin method followed by vector brown (diaminobenzidine). All incubations were at room temperature. Sections were washed with PBS with 0.05% Tween 20 between each incubation. For control staining, irrelevant isotype matched antibodies were applied. Sections were visualized using a Nikon Eclipse E400 microscope.

Statistical analysis. The normality of variables was tested with the Saphiro-Wilk test. Data are reported either as mean ± SD or as median (quantiles 25–75) if the variables do not fit a Gaussian distribution. Normal variables were compared using Student's t test and non-normal variables using the median test.

RESULTS

Analysis of basal CD23 expression. The leukocyte antigen CD23 is expressed by B lymphocytes following activation by a number of different stimuli. To study the differential state of B cell activation in patients with RA, we analyzed CD23 expression on PB B lymphocytes from patients and controls. Cells were rosetted and CD23 expression was analyzed on the E– population at Time 0 (data not shown) and after 18 h of culture, and no differences were observed. Figure 1 shows the percentage of double positive cells for CD19 and CD23. CD23 was expressed on a high proportion of PB B cells from patients with RA when compared with control PB B cells [20.5 (18–26.2) vs 14% (11–22.7); p = 0.04], although no difference in expression of CD23 level was observed between RA and control PB B cells [mean fluorescence intensity 13 (10–21.5) vs 14 (12–18.5); p = 1]. These results indicate that elevated numbers of activated CD23+ circulating B cells are detected in patients with RA.

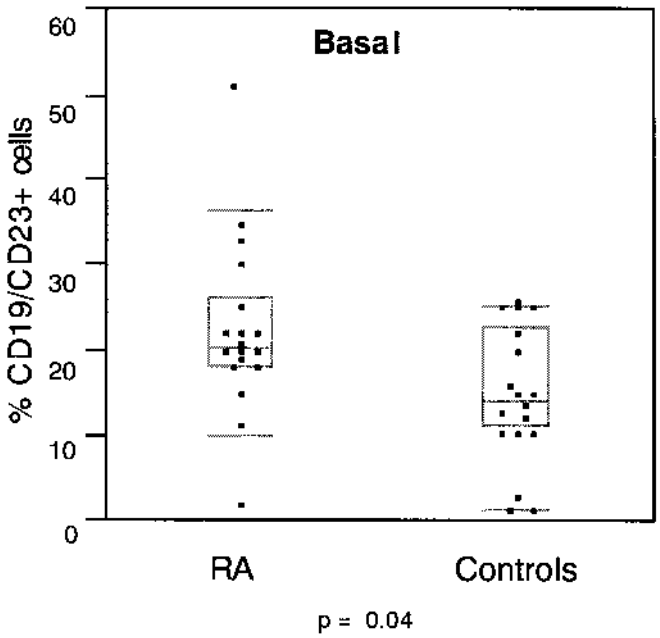


Figure 1. Basal CD23 expression by PB B lymphocytes from patients with RA and controls. The nonrosetting population (E– cells) was stained with anti-CD19 and anti-CD23 Mab and analyzed by flow cytometry. Results are expressed as percentage of CD19 positive cells (B cells) expressing CD23. Boxes represent the median and the 25th and 75th percentiles, and horizontal bars 10th and 90th percentiles.

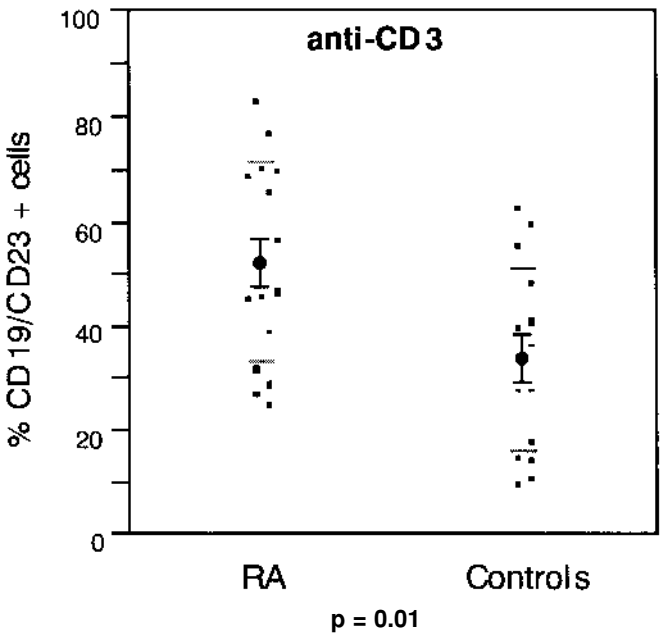


Figure 2. CD23 expression by PB B lymphocytes from patients with RA and controls after T cell activation (p = 0.01). The nonrosetting population (E– cells) was stained with anti-CD19 and anti-CD23 Mab and analyzed by flow cytometry. Results are expressed as the percentage of CD19 positive cells (B cells) expressing CD23. Means dots and error bars are shown; lines correspond to standard deviations.

CD23 expression on PB B cells through T cell signals. To study whether there were differences on CD23 expression on PB B cells from patients with RA compared with PB B cells from controls, we analyzed CD23 expression following T cell activation. PBMC were stimulated with anti-CD3 Mab for 18 h, then cells were rosetted and CD23 expression was analyzed on the E– population. Figure 2 shows the percentage of double positive cells for CD19 and CD23. The proportion of PB B cells expressing CD23 was higher in cells from patients with RA than in the controls (52 ± 19% vs 33 ± 17%; p = 0.01), although no change in the level of CD23 expression (mean fluorescence intensity 48 ± 24 vs 45 ± 22; p = 0.8) was observed comparing RA with control PB B cells.

Mechanisms involved in PB B CD23 expression by T cell signals in RA. To analyze the mechanisms responsible for the increased expression of CD23 on PB B cells from patients with RA, we investigated the role of CD40-CD154 interaction on CD23 expression. PBMC were stimulated with an anti-CD3 Mab in combination with different blocking antibodies to inhibit CD23 expression. Figure 3A shows the percentage of CD19/CD23 PB B positive cells when an anti-CD40 blocking Mab was added to the culture (RA vs controls: 34 ± 17% vs 31 ± 14%, p = 0.6; mean fluorescence intensity 18 ± 10 vs 20 ± 11, p = 0.3). When an anti-CD154 blocking Mab was added (Figure 3B) the percentage of CD19/CD23 PB B positive cells in RA vs controls was: 39 ± 17% vs 28 ± 15%, p = 0.08; mean fluorescence intensity 24 (14–56) vs 28 (11–47), p = 0.7.

Figure 4 shows the percentage of inhibition of CD23 expression. CD23 expression induced by anti-CD3 in PB B cells from patients with RA was inhibited by 35 ± 9% with an anti-CD40 Mab and an inhibition of 25 ± 5% was reached when an anti-CD154 Mab was used. Percentage of inhibition of CD23 in controls was 7 ± 6% and 16 ± 4% with the anti-CD40 and anti-CD154, respectively. For anti-CD154 and anti-CD40 Mab, there was a significant difference between cells from patients with RA vs controls: p = 0.01 and 0.0007, respectively.

CD154 expression on CD4+ T cells from RA and controls. CD154, which is critical to regulation of humoral and cellular immune responses, is transiently expressed on the surface of activated CD4+ T cells. To evaluate the role of CD154 in RA pathogenesis, CD154 expression was investigated on PB CD4+ T cells from patients with RA and controls and also on SF CD4+ T cells from patients with RA. The results are expressed as the percentage of double positive cells for CD4 and CD154. Although few CD4+ T cells expressed CD154 in basal conditions, the proportion of CD154+ cells was slightly higher in the SF CD4+ T cell population (SF cells 2 ± 1%, RA PBMC 1.5 ± 0.9%, and control PBMC 1 ± 0.8%). Control PBMC vs RA PBMC, p = 0.5; RA PBMC vs RA SF cells, p = 0.3; control PBMC vs RA SF cells, p = 0.05 (Figure 5, upper panel).

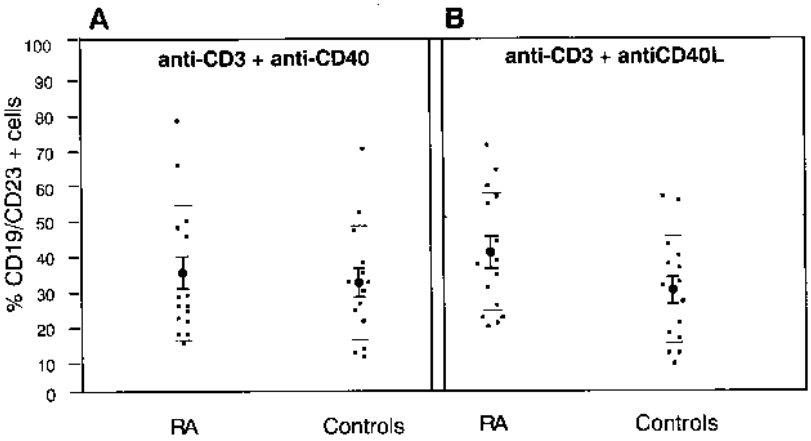


Figure 3. Role of CD40-CD154 interaction on CD23 expression by PB B lymphocytes from patients with RA and controls after T cell activation. After culture for 18 h in the presence of saturating concentrations of anti-CD3 Mab (454 hybridoma supernatant) together with anti-CD40 Mab 89 or anti-CD154 Mab (hCD40-Hm), PB B lymphocytes were prepared from patients with RA and controls by rosetting 5×10^6 PBMC with neuraminidase treated SRBC. The nonrosetting population (E– cells) was stained with anti-CD19 and anti-CD23 Mab and analyzed by flow cytometry. Results are expressed as percentage of CD19 positive cells (B cells) expressing CD23 in the presence of anti-CD40 (A) or anti-CD154 (B). Means dots and error bars are shown; lines correspond to standard deviations. A: p = 0.65, B: p = 0.08.

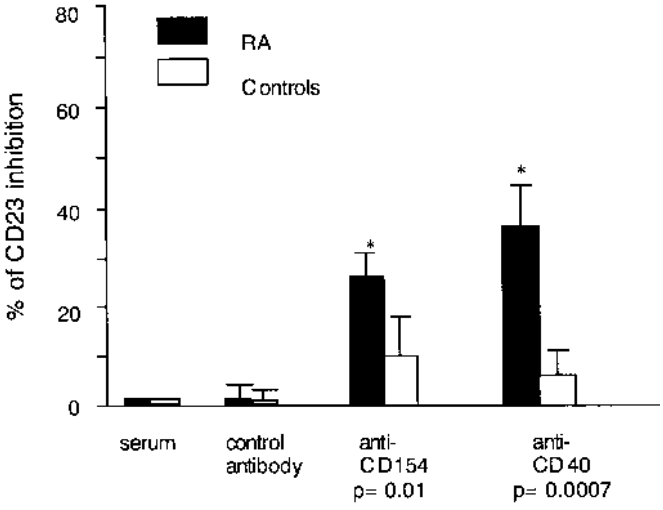
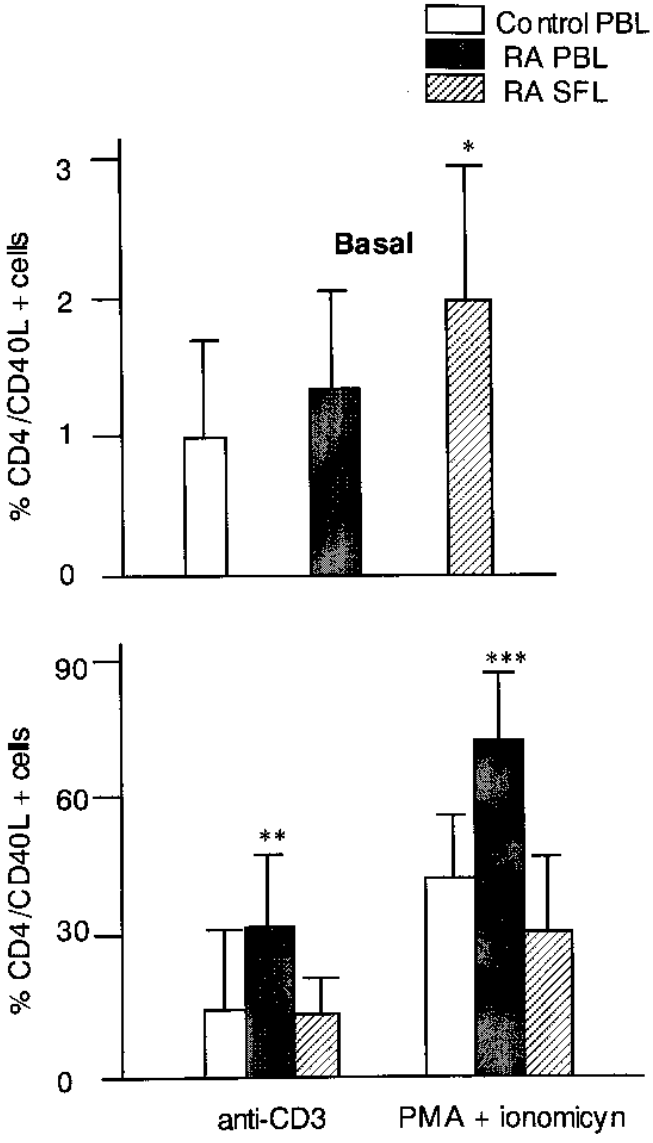


Figure 4. Effects of blocking CD40-CD154 interaction on CD23 expression by PB B lymphocytes from patients with RA and controls after T cell activation. After culture for 18 h in the presence of saturating concentrations of anti-CD3 Mab (454 hybridoma supernatant) together with an anti-CD40 Mab 89 or an anti-CD154 Mab (hCD40-Hm), PB B lymphocytes were prepared from patients with RA and controls by rosetting 5×10^6 PBMC with neuraminidase treated SRBC. The nonrosetting population (E– cells) was stained with anti-CD19 and anti-CD23 Mab and analyzed by flow cytometry. Results are expressed as percentage of inhibition of CD23 expression (mean ± SD).



→

Figure 5. CD154 expression by CD4+ T lymphocytes from patients with RA and controls. PBMC from RA patients and controls and SF lymphocytes from RA patients were cultured for 6 h with medium (upper panel), plastic coated anti-CD3 Mab (454 hybridoma supernatant at saturating concentrations) or PMA (10 ng/ml) plus ionomycin (1 μg/ml) (lower panel). Cells were stained with anti-CD4 and anti-CD154 Mab and analyzed by flow cytometry. Results are expressed as percentage of CD4 positive cells expressing CD154 (mean ± SD). *RA SF cells vs control PBMC, p = 0.05; **RA vs control PBMC, p = 0.04; RA PBMC vs RA SF cells, p = 0.03; ***RA vs control PBMC, p = 0.02; RA PBMC vs RA SF cells, p = 0.001.

To study the effects of T cell activation on CD154 expression, PBMC were stimulated with either plastic coated anti-CD3 Mab or PMA plus ionomycin. Figure 5 shows the percentage of CD4+ cells expressing CD154. Following activation with both stimuli, a higher proportion of double positive cells was found in the RA PB CD4+ T cell population (31 ± 18% for anti-CD3 and 64 ± 19% for PMA plus ionomycin) compared with their paired RA SF CD4+ T cells (8.5 ± 5% for anti-CD3 and 33 ± 24% for PMA plus ionomycin); p = 0.03 for anti-CD3 and p = 0.001 for PMA plus ionomycin. CD154 expression in control PB CD4+ T cells was 10 ± 2.5% for anti-CD3 and 40 ± 16% for PMA plus ionomycin. Control PBMC vs RA PBMC, p = 0.04 for anti-CD3 and p = 0.02 for PMA plus ionomicyn; control PBMC vs RA SF cells, p = 0.5 for anti-CD3 and p = 0.15 for PMA plus ionomycin.

CD154 induced expression was considerably higher on PB CD4+ T cells from patients with RA, suggesting that the potential to respond to different stimuli is increased in these populations of presumably recirculating cells.

CD40-CD154 interaction is required for CD23 hyperexpression on PB B cells from patients with RA. CD40-CD154 interaction is a critical step in B cell–T cell interactions. CD154 is a key molecule for the transmission of helper signals necessary for B cell activation, mainly through the engagement of CD40 on B lymphocytes. We therefore analyzed how CD40-CD154 interaction regulates CD23 expression on PB B cells from patients with RA. PB B cells were separated by rosetting into E+ and E– populations. The E– cells were cultured with their paired PB T cells or their paired SF T cells and with medium alone as a control. The highest CD23 induction was achieved when PB B cells were cultured with their paired SF T cells (7.4 ± 2%; p = 0.04). When cultured with their paired PB T cells or only with medium, CD23 induction was 2.4 ± 2% (p = 0.5) and 0.0 ± 0.5%, respectively (Figure 6).

To further analyze the role of CD40-CD154 interaction with CD23 hyperexpression, we co-cultured the PB E– cells with SF T cells in the presence of anti-CD40 or anti-CD154 blocking Mab. Figure 6 shows that blocking CD40-CD154 interaction substantially diminished the percentage of PB B cells expressing CD23, returning the proportion of positive cells to basal levels. These results indicate that cognate mechanisms involving mainly CD40-CD154 interactions are crucial for the induction of CD23 expression on PB B cells from patients with RA.

Immunohistochemical analysis of synovial tissue from patients with RA. We tried to identify CD23 and CD154 positive cells in the synovial tissue by using a double staining method (CD4/CD154 or CD20/CD23). Using an anti-CD154 Mab, immunohistochemical analysis showed that CD154 was clearly expressed by almost half of the CD4+ T cells located predominantly around blood vessels (Figure 7). A small percentage of B cells (CD20+ cells) was present in

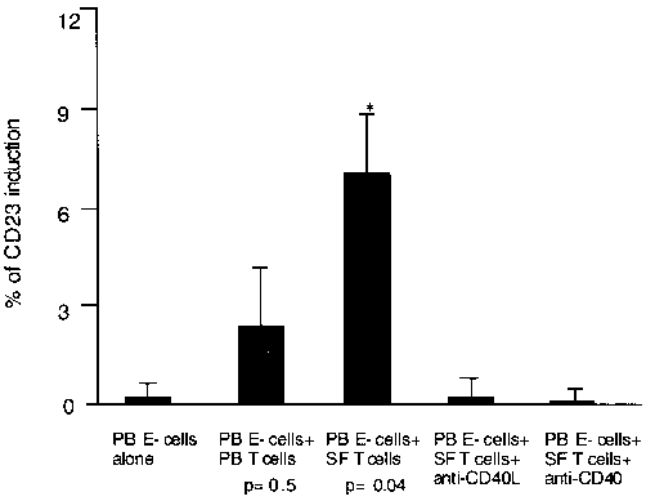


Figure 6. Induction of CD23 expression by CD40-CD154 interactions. PBMC and SF cells were separated into E+ (> 90% CD3+ T cells) and E– populations by rosetting; 5×10^5 PB E– cells were cultured with either medium, their paired PB T cells (5×10^5), or their paired SF T cells (5×10^5). When indicated, PB E– cells and SF T cells were cultured in the presence of anti-CD40 or anti-CD154 Mab at saturating concentrations. After 18 h culture, cells were again rosetted and CD19 and CD23 expression was analyzed by flow cytometry on the nonrosetting population (E– cells). Results are expressed as percentage of CD19 positive cells (B cells) expressing CD23 (mean ± SD of 5 independent experiments). The percentage of PB B CD19+/CD23+ cells was 24 ± 5% when cultured with their paired SF T cells, 20 ± 7% cultured with their paired PB T cells, and 18 ± 9% cultured with medium alone.

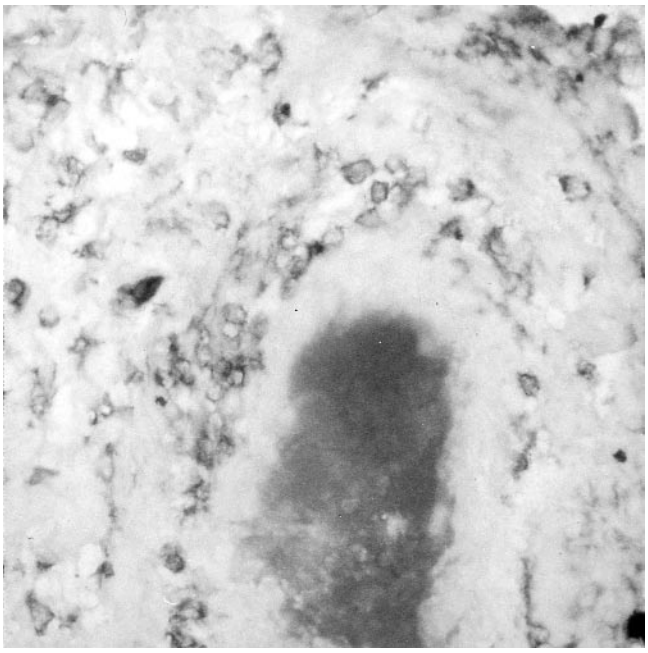


Figure 7. Immunohistochemical analysis of CD154 in synovial tissues from patients with RA. A double staining method with immunoalkaline phosphatase/diaminobenzidine was used to visualized CD154 on CD4+ cells in synovial tissues. CD154 is expressed by almost 50% of the CD4+ cells. CD4+/CD154+ cells were mostly located around blood vessels. This is a representative staining out of 9 performed (magnification ×40).

these samples and they did not express CD23 (data not shown). The results were reproducible in all samples from 9 patients with RA who underwent joint replacement surgery.

DISCUSSION

RA is characterized by a hyperexpression of CD23 on PB B cells[3,4,15,16] and direct cell to cell contact between T and B cells is required for that hyperexpression[10,12,13]. In addition, SF T cells from patients with RA are predominantly memory T cells (CD45R0/CD45RB^{dim}), displaying a phenotype of recent activation (CD69 expression)[5,21] and an enhanced capacity to migrate (expression of integrins)[2,22]. Therefore, it is of particular interest to study the mechanisms involved in CD23 expression on PB B cells from patients with RA, and since the CD40-CD154 interaction is essential for B cell activation, we investigated the involvement of the CD40-CD154 pathway in CD23 expression on PB B cells.

Our results indicate that an elevated number of activated CD23+ circulating B cells are detected in patients with RA. Blocking experiments with both anti-CD40 and anti-CD154 Mab show a significant reduction in the proportion of PB B cells expressing CD23. Interestingly, anti-CD40 Mab always reduce to a greater extent the percentage of cells expressing CD23 than the anti-CD154 Mab. This divergence could be accounted for by the fact that CD40 is generally constitutively expressed on B cells, and only low levels of CD154 by either PB or SF T cells from patients with RA are observed before activation. It is possible that upon activation, the intracellular CD154 store within the CD4+ memory T cells[6] provides a rapid pool of CD154 for cell surface expression that is difficult to block, and it is more feasible to block CD40 expression.

Basal CD154 expression on CD4+ T cells was scarce but always higher on SF T cells. Further, SF T cells showed enhanced CD154/CD40 mediated capacity to interact with and activate PB B cells. These results indicate that even this low level of CD154 expression by SF CD4+ T cells could be physiologically relevant, and may be responsible for the prestimulated phenotype shown by PB B cells in RA[23]. In addition, following activation with anti-CD3 Mab or PMA plus ionomycin, a clearly lower CD154 expression is observed on RA SF CD4+ T cells compared with PB CD4+ T cells. Different laboratories have shown that SF T cells, in contrast to PB T cells from patients with RA, react poorly to stimuli, perhaps in relation to the special synovial microenvironment to which SF T cells are subjected[24,25]. The vigorous PB T cell response to stimuli could be accounted for by the fact that T cells are continuously recirculating between synovial and extrasynovial compartments[26], and when T cells leave the synovial microenvironment, they could react vigorously in relation to their memory phenotype[2,5,21,22,27]. This physiologically relevant expression of CD154 could not only activate B cells but also rescue potential self-reactive B cells from apoptosis[28]. This hypothesis is supported by the fact that synovial membrane staining showed CD154 expression on CD4+ T cells, but no CD23 expression on B cells. In addition, we have previously shown that SF B cells, as well as synovial membrane B cells, from patients with RA are scarce and CD23 expression on SF B cells is very low[3]. In other words, synovial, recirculating T cells from patients with RA seemed to play a predominantly systemic role in relation to the characteristic CD23 hyperexpression on PB B cells.

We have shown that T cells, mostly from the synovial microenvironment, trigger a signal to B cells that is mainly contact dependent/CD40 dependent. We have clearly demonstrated that both T cell and B cell collaboration is essential for B lymphocyte activation in RA, and that SF T cells are particularly efficient in the induction of CD154/CD40 dependent CD23 expression on PB B cells from patients with RA. Since Mab to CD154 and CD23 have been successfully used in the treatment of collagen induced arthritis, the murine model of RA[29,30], a complete understanding of the different steps involved in the regulation of CD154 and CD23 in RA could indicate new therapeutic approaches to this disease.

ACKNOWLEDGMENT

We thank Dr. Isabelle Runkle de la Vega for critical review of the manuscript and M. García for superb secretarial assistance.

REFERENCES

1. Panayi GS, Lanchbury JS, Kingly GH. The importance of T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arthritis Rheum 1992;35:729-35.
2. Cush JJ, Pietschmann P, Oppenheimer-Marks N, Lipsky PE. The intrinsic migratory capacity of memory T cells contributes to their accumulation in rheumatoid synovium. Arthritis Rheum 1992;35:1434-44.
3. Fernandez-Gutierrez B, Hernandez-Garcia C, Bañares A, Jover JA. CD23 hyperexpression in rheumatoid arthritis: evidence for a B cell hyperresponsiveness to cognate and non-cognate T-cell signals. Clin Immunol Immunopathol 1994;72:321-7.
4. Morado-Quiñoa I, Fernandez-Gutierrez B, Hernandez-Garcia C, et al. Rheumatoid arthritis: mechanisms involved in the abnormal T/B cognate interactions [abstract]. Arthritis Rheum 1996;39 Suppl:S61.
5. Thomas R, McIlraith M, Davis LS, Lipsky PE. Rheumatoid synovium is enriched in CD45RB^{dim} mature memory cells that are potent helpers for B cell differentiation. Arthritis Rheum 1992;35:1455-65.
6. MacDonald KPA, Nishioka Y, Lipsky PE, Thomas R. Functional CD40 ligand is expressed by T cells in rheumatoid arthritis. J Clin Invest 1997;100:2404-14.
7. Lederman SM, Yellin MJ, Krichevsky A, Belko J, Lee JJ, Chess L. Identification of a novel surface protein on activated CD4+ T cells that induces contact dependent B cell differentiation (help). J Exp Med 1992;175:1091-101.
8. Lederman S, Yellin MJ, Inghirami G, Lee JJ, Knowles DM, Chess L. Molecular interactions mediating T-B lymphocyte collaboration in human lymphoid follicles. J Immunol 1992;149:3817-26.
9. Aruffo A, Farrington M, Hollenbaugh D, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell 1993;72:291-300.
10. Crow MK, Jover JA, Friedman S. Direct T helper-B cell interactions

induce an early B cell activation antigen. J Exp Med 1986;164:1760-72.
11. De France T, Aubry JP, Rousset F, et al. Human recombinant interleukin 4 induces Fc receptors (CD23) on normal human B lymphocytes. J Exp Med 1987;165:1459-67.
12. Crow MK, Kushner B, Jover JA, Friedman S, Mechanic SE, Stohl W. Human peripheral blood T helper cell-induced B cell activation results in B cell surface expression of the CD23 (BLAST-2) antigen. Cell Immunol 1989;121:99-112.
13. Mossalayi MD, Arock M, Debre P. CD23/Fc epsilon RII: signaling and clinical implication. Int Rev Immunol 1997;16:129-46.
14. Aubry JP, Pochon S, Graber P, Jansen KU, Bonnefoy JY. CD21 is a ligand for CD23 and regulates IgE production. Nature 1992;358:505-7.
15. Kumagai S, Ishida H, Iwai K, et al. Possible different mechanisms of B cell activation in systemic lupus erythematosus and rheumatoid arthritis: opposite expression of low-affinity receptors for IgE (CD23) on their peripheral B cells. Clin Exp Immunol 1989; 78:348-53.
16. Chomarat P, Briolay J, Banchereau J, Miossec P. Increased production of soluble CD23 in rheumatoid arthritis, and its regulation by interleukin-4. Arthritis Rheum 1993;36:234-42.
17. Fernandez-Gutierrez B, de Miguel S, Morado C, Hernandez-Garcia C, Bañares A, Jover JA. Defective early T and T-dependent B cell activation in systemic lupus erythematosus. Lupus 1998;7:314-22.
18. Arnet FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
19. Lane P, Traunnecker A, Hubele S, Inui S, Lanzavecchia A, Gray D. Activated human T cells express a ligand for the human B-cell associated antigen CD40 which participates in T-cell dependent activation of B lymphocytes. Eur J Immunol 1992;22:2573-8.
20. Gottlieb AB, Fu SM, Yu DTY, Wang CY, Halper JP, Kunkel HG. The nature of the stimulatory cell in human allogeneic and autologous MLC reactions; role of isolated IgM-bearing B cells. J Immunol 1979;123:1497-503.
21. Fernandez-Gutierrez B, Hernandez-Garcia C, Bañares AA, Jover JA. Characterization and regulation of CD69 expression on rheumatoid arthritis synovial fluid T cells. J Rheumatol 1995;22:413-20.
22. el-Gabalawy H, Canvin J, Ma GM, et al. Synovial distribution of alpha d/CD18, a novel leukointegrin. Comparison with other integrins and their ligands. Arthritis Rheum 1996;39:1913-21.
23. Poudrier J, Owens T. Co-stimulation by anti-immunoglobulin is required for B cell activation by CD40L low T cells. Eur J Immunol 1994;24:2993-9.
24. Hernandez-Garcia C, Fernandez-Gutierrez B, Morado IC, Bañares AA, Jover JA. The CD69 activation pathway in rheumatoid arthritis synovial fluid T cells. Arthritis Rheum 1996;39:1277-86.
25. Lotz M, Tsoukas CD, Robinson CA, Dinarello CA, Carson DA, Vaughan JH. Basis for the defective responses of rheumatoid arthritis synovial fluid lymphocytes to anti-CD3 (T3) antibody. J Clin Invest 1986;78:713-21.
26. Elewaut D, De Keyser F, De Wever N, et al. A comparative phenotypical analysis of rheumatoid nodules and rheumatoid synovium with special reference to adhesion molecules and activation markers. Ann Rheum Dis 1998;57:480-6.
27. Leitenberg D, Novack TJ, Farber D, Smith BR, Bottomly K. The extracellular domain of CD45 controls association with the CD4-T cell receptor complex and the response to antigen-specific stimulation. J Exp Med 1996;183:249-59.
28. Cook MC, Basten A, Fazekas de St. Groth B. Rescue of self-reactive B cells by provision of T cell help in vivo. Eur J Immunol 1998;28:2549-58.
29. Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ. Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. Science 1993;261:1328-30.
30. Plater-Zyberk C, Bonnefoy JY. Marked amelioration of established collagen-induced arthritis by treatment with antibodies to CD23 in vivo. Nature Med 1995;1:781-5.