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CD23 Regulates Monocyte Activation Through A Novel Interaction with the Adhesion Molecules CD11b–CD18 and CD11c–CD18

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Summary

CD23 is expressed on a variety of haemopoietic cells and displays pleiotropic activities in vitro. We report that in addition to CD21 and IgE, CD23 interacts specifically with the CD11b and CD11c, the α chains of the β2 integrin adhesion molecule complexes CD11b–CD18 and CD11c–CD18, on monocytes. Full-length recombinant CD23 incorporated into fluorescent liposomes was shown to bind to COS cells transfected with cDNA encoding either CD11b–CD18 or CD11c–CD18 but not with CD11a–CD18. The interaction was specifically inhibited by anti-CD11b or anti-CD11c, respectively, and by anti-CD23 MAbs. The functional significance of this ligand pairing was demonstrated by triggering CD11b and CD11c on monocytes with either recombinant CD23 or anti-CD11b and anti-CD11c MAbs to cause a marked increase in nitrite-oxidative products and pro-inflammatory cytokines (IL-1β, IL-6, and TNFα). These CD23-mediated activities were decreased by Fab fragments of MAbs to CD11b, CD11c, and CD23. These results demonstrate that CD11b and CD11c are receptors for CD23 and that this novel ligand pairing regulates important activities of monocytes.

Introduction

CD23, a low affinity receptor for immunoglobulin E (IgE) (Yukawa et al., 1987; Bonnefoy et al., 1987), belongs to the C-type lectin family (Kikutani et al., 1986), some members of which have been identified as adhesion molecules. CD23 is expressed on a wide variety of haemopoeitic cell types including B and T lymphocytes, follicular dendritic cells, monocytes, platelets, Langerhans cells, eosinophils, and natural killer (NK) cells (Delespesse et al., 1992). By means of its interaction with CD21 (Aubry et al., 1992), CD23 is implicated in a variety of biological activities, including cell–cell adhesion, regulation of IgE synthesis, germinal center B cell survival, and histamine release from basophils (Bonnefoy et al., 1993). CD23 is also involved in B cell growth, prothymocyte maturation, myeloid precursor proliferation, inhibition of macrophage migration, and antigen presentation (Gordon, 1991); not all of these activities can be attributed to the interaction of CD23 with IgE or CD21. Increased levels of CD23 have been reported in various chronic inflammatory diseases including rheumatoid arthritis (Hellen et al., 1991), systemic lupus erythematosous (Bansal et al., 1992), inflammatory bowel disease (Kaiserlian et al., 1993), Sjögren's syndrome, and glomerulonephritis (Yano et al., 1992).

These findings led us to investigate whether CD23 interacts with a new ligand. We report that CD23 also interacts specifically with the cell surface proteins CD11b and CD11c, the α chains of the β2 integrin adhesion molecule complexes CD11b–CD18 and CD11c–CD18, on human monocytes. This new ligand pairing causes a marked increase in nitrite (NO_2^-), oxidative products (H_2O_2), and pro-inflammatory cytokines (interleukin-1β [IL-1β], IL-6, and TNFα) production by monocytes.

Results and Discussion

CD23 Binds to Human Blood Monocytes

To search for the existence of another receptor for CD23, total blood mononuclear cells were incubated with recombinant full-length CD23 incorporated into fluorescent liposomes and analyzed by flow cytometry (Pochon et al., 1992). A fraction (10%–20% depending on the donor) bound CD23 liposomes (Figure 1a), which was then shown by double staining to consist mainly of CD14-positive cells (i.e., monocytes). A weak binding of CD23 liposomes on resting blood B cells was observed through an interaction with CD21 (data not shown). To confirm that monocytes were able to bind CD23 liposomes, blood mononuclear cells were FACS sorted into CD14-positive and CD14-negative populations. CD23 liposomes were shown to bind mainly to the CD14-positive population (Figure 1a).

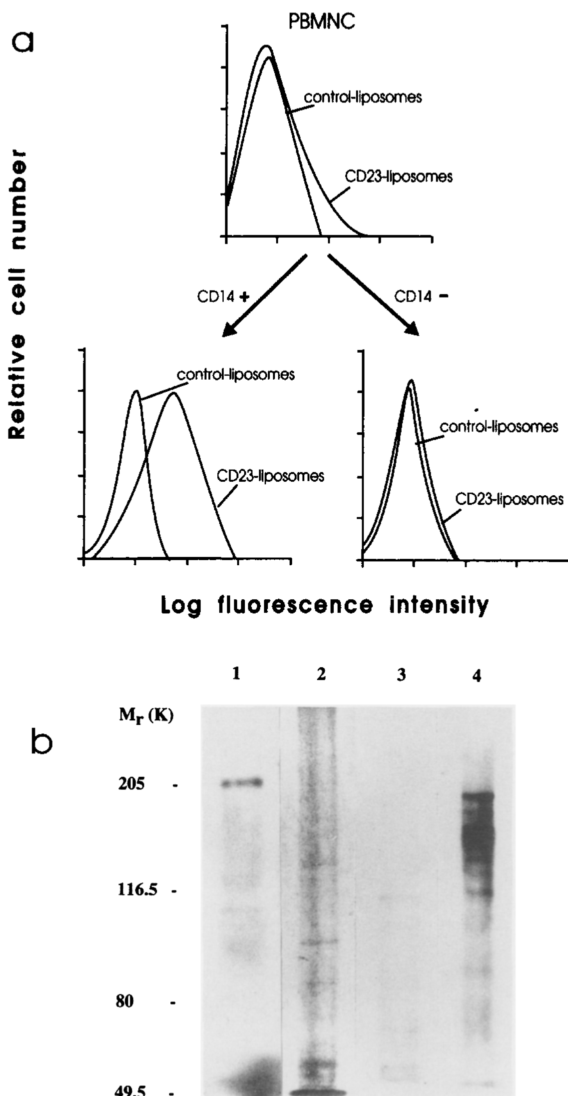

Figure 1. CD23 Binding to Blood Monocytes

(a) CD23 liposomes bind to CD14-positive blood mononuclear cells. In the experiment presented here, 12% of peripheral blood mononuclear cells were stained with CD23 liposomes. Blood mononuclear cells were stained with anti-CD14 MAb followed by sheep FITC-conjugated $(Fab')_2$ antibodies to mouse IgG and IgM prior to FACS sorting into CD14-positive and CD14-negative cell populations. Separated cells were then stained with CD23 liposomes or control (glycophorin A) liposomes and analyzed by FACS.

(b) Apparent molecular weight of CD23 affinity-purified blood monocyte proteins and immunoreactivity with an anti-CD11c MAb. Lysates of blood monocytes were affinity-purified on a BSA (lane 1) or CD23 column (lane 2), eluted proteins separated on SDS–PAGE gels, and silver stained (lanes 1 and 2). CD23 affinity-purified proteins were transferred onto nitrocellulose and filters were incubated with either an isotype-matched antibody (lane 3) or with an anti-CD11c MAb (lane 4), then with horseradish peroxidase–conjugated goat anti-mouse antibody. M_r markers are shown on the left.

CD23 Binds to CD11b and CD11c on Human Monocytes

Since monocytes were found to express neither membrane IgE nor CD21 (data not shown), the known ligands for CD23, we investigated whether monocytes express a different receptor for CD23. Monocytes were lysed and cell extracts purified over an affinity column coupled with recombinant soluble CD23 or bovine serum albumin (BSA) (as control). SDS–PAGE and silver staining analysis of the eluted material from the CD23 column revealed bands of around 80 and 160 kDa molecular mass (Figure 1b, lane 2), bands that were absent in the BSA-purified material (Figure 1b, lane 1). Antibodies identifying antigens within this range of molecular mass and reported to be expressed on monocytes were tested by FACS for their capacity to inhibit CD23 liposome binding to monocytes (Figure 2). Anti-CD11b and anti-CD11c monoclonal antibodies (MAbs) both inhibited CD23 liposome binding to monocytes, with varying degrees of potency (Figure 2). Anti-CD13, anti-CD49d, anti-CD21 (not expressed on monocytes), and anti-CD11a (the third member of the β2 integrin family of adhesion molecules) had no significant effect (Figure 2). Antibodies against MHC class I, class II, CD14, and CD45, all of which highly expressed on monocytes, were also tested for their effect on CD23 liposome binding. None, however, had any effect (data not shown). Anti-CD18 MAbs gave a partial inhibition of CD23 binding. This could be due either to steric hindrance or to the induction of a conformational change in the CD11b and CD11c molecules upon anti-CD18 Mab binding. The monocyte-derived proteins eluted from the CD23 affinity column were immunoreactive with anti-CD11c (see Figure 1b, lane 4) and anti-CD11b (data not shown) antibodies.

CD23 Binds to CD11b-CD18 and CD11c-CD18 Transfectants

To confirm that the α chains of CD11b–CD18 and CD11c–CD18 were receptors for CD23, full-length cDNAs encoding CD11b and CD11c were transiently cotransfected with CD18 cDNA into COS cells. Transfectants expressing CD11b–CD18 and CD11c–CD18 were both shown to bind CD23 liposomes, in contrast with transfectants expressing CD11a–CD18 (Figure 3). This might be explained by the higher degree of homology between CD11b and CD11c when compared with their homology to CD11a (Corbi et al., 1987, 1988; Larson et al., 1989). The specificity of the interaction was demonstrated by inhibiting CD23 liposome binding using anti-CD11b, anti-CD11c, and anti-CD23 MAbs. The same results were obtained using BHK cells expressing CD11b–CD18 and CD11c–CD18 (data not shown). As further proof of the specificity of the CD23 interaction, activated blood monocytes from a leukocyte adhesion-deficiency patient, lacking β2 integrin expression due to a mutation in the gene encoding the β subunit (Fischer et al., 1988), were unable to bind CD23 liposomes (data not shown). Together, these data demonstrate that CD23 interacts with CD11b and CD11c on normal human monocytes and on transfectants.

CD23 Binding to CD11b and CD11c Is Inhibited by Factor X and Is Cation Dependent

CD11b and CD11c are adhesion molecules that participate in many cell–cell and cell–matrix interactions (Springer, 1990). The initial results of this study show that CD11b–CD18 and CD11c–CD18 may exhibit an additional

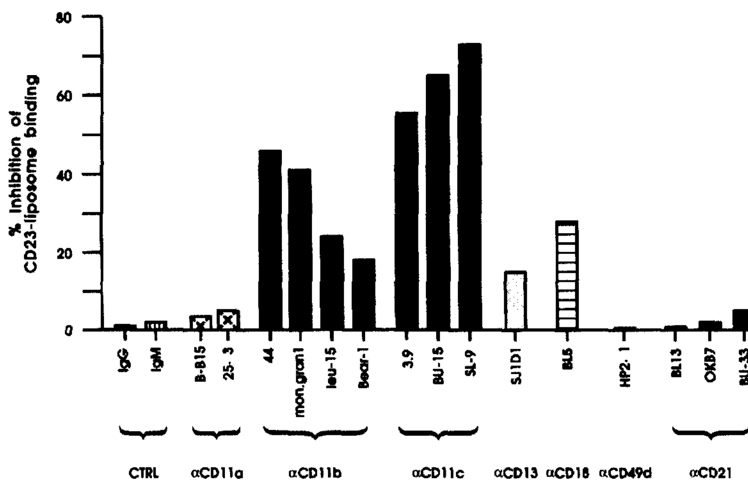

Figure 2. Effect of a Panel of MAbs on CD23 Binding to Blood Monocytes

Anti-CD11b and anti-CD11c MAbs decrease CD23 liposome binding to activated blood monocytes. Activated monocytes were incubated with CD23 liposomes in the presence of different MAbs (αCD) or isotype-matched controls (CTRL) (all used at 10 μg/ml). Cells were analyzed by FACS and mean fluorescence intensity (MFI) measured. Data of a representative experiment are presented. MFI of cells stained with control liposomes was 6.5 and with CD23 liposomes was 84.5. Percentage inhibition using arithmetic linear MFI values is calculated according to the following formula:

$$\text{Percent of inhibition} = \text{MFI}\frac{([\text{CD23–lipo}]) - ([\text{CD23–lipo}] + \text{MAb})}{(\text{CD23–lipo})} \times 100$$

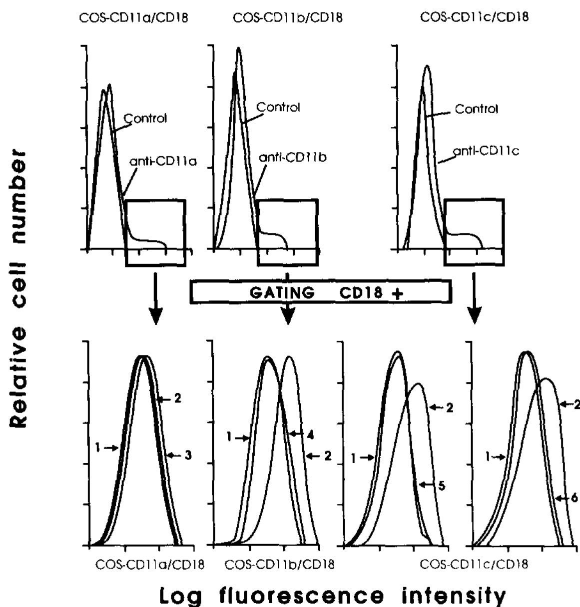

Figure 3. CD23 Binding to CD11b–CD18 and CD11c–CD18 COS Transfectants

CD23 liposomes bind to α chains of CD11b–CD18 and CD11c–CD18 on recombinant transfectants. cDNAs coding for CD11a, CD11b, and CD18 were transfected together with CD18 in COS-7 cells to get expression of the β2 integrins at the cell surface. Controls were done with single chain transfections. COS cells were stained 48 hr after transfection with anti-CD11a, anti-CD11b, and anti-CD11c MAbs or isotype-matched MAbs (control), followed by FITC-labeled sheep anti-mouse antibody. Between 10%–15% of the cells were shown to express CD11a, CD11b, CD11c, or CD18 by staining with the respective MAbs. Prior to staining with CD23 liposomes, CD18-positive transfected COS cells were then FACS-sorted to increase the percentage of cells expressing β2 integrins. CD11a–CD18, CD11b–CD18, and CD11c–CD18 transfectants were then incubated with CD23 liposomes (histogram 2) or control (glycophorin A) liposomes (histogram 1). The specificity of CD23 interaction with CD11b and CD11c was demonstrated by inhibition of CD23 liposome binding to CD11b–CD18 and CD11c–CD18 transfectants using anti-CD11b (histogram 4), anti-CD23 (histogram 5), and anti-CD11c (histogram 6) MAbs, respectively. No binding of CD23 liposomes was observed on CD11a–CD18 transfectants and no effect of anti-CD11a MAb was found (histogram 3).

adhesive function by virtue of their ability to bind CD23. CD11b–CD18 and CD11c–CD18 have been reported to bind several ligands, including CD54, fibrinogen, factor X, lipopolysaccharide (LPS), concanavalin A, and zymosan (Springer, 1990). CD23 seems to identify an epitope close or identical to factor X as observed by the capacity of factor X to inhibit in a dose-dependent manner CD23 liposome binding without affecting surface expression of CD11b or CD11c on monocytes (Figure 4). None of the other ligands tested had any effect. CD23 may be acting as a C-type lectin in its interaction with CD11b and CD11c. EDTA decreases CD23 binding to monocytes (Figure 4) by chelation of Ca^{2+}, which is necessary to CD23 binding, or by chelation of the divalent cations, which are necessary for the binding of ligands to CD11b and CD11c (Altieri, 1991). In this context, addition of Ca^{2+} or Mn^{2+} resulted in a dose-dependent increase of CD23 binding to monocytes (Figure 4), like fibrinogen or factor X (Altieri, 1991). CD23–CD11b/CD11c interaction seems to involve sugars, but not sialic acid, as observed by the capacity of tunicamycin, but not neuraminidase, to decrease CD23 binding to monocytes. Quantification of the number of binding sites for CD23 on activated monocytes was determined by FACS: 20,000 CD23 molecules, 50,000 anti-CD11b and 20,000 anti-CD11c antibodies bound per activated blood monocyte. Those results indicate that CD23 does not bind to all CD11b and CD11c molecules. This could be due to heterogeneity in CD11b and CD11c glycosylation, and sugars are critical for CD23 binding, as indicated by the tunicamycin experiment (Figure 4). Moreover, the affinity of CD23 for CD11b and CD11c is too low to be determined accurately, inasmuch as 100 × more CD23 than antibodies to CD11b and CD11c is required to achieve saturation.

CD23 bears extracellularly a triplet of amino acids (Asp, Gly, Arg) (Kikutani et al., 1986), which in the reverse orientation is a common recognition site for the integrin receptors. Therefore, the effect of a polyclonal antibody directed against this tripeptide was tested for its capacity to inhibit CD23 binding to monocytes. No inhibition was observed, confirming the absence of inhibition obtained with fibrino-

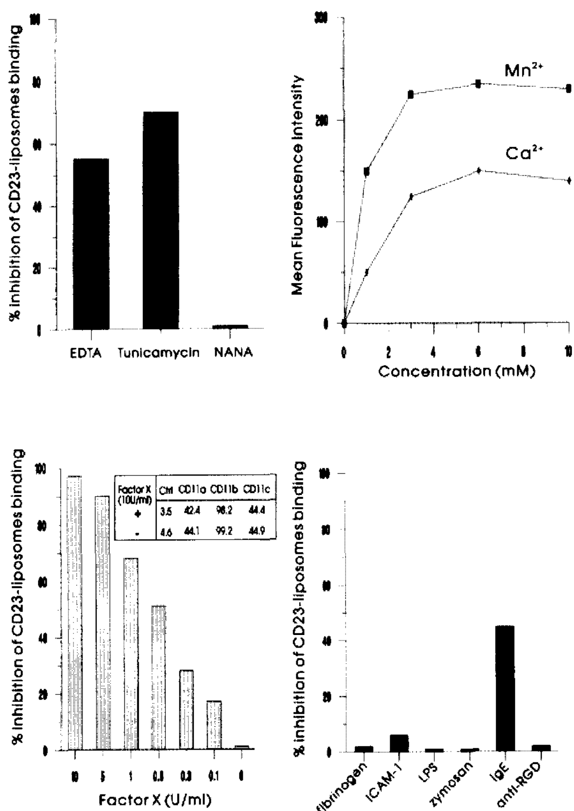

Figure 4. Structural Characterization of CD23–CD11b and CD23–CD11c Interaction

(a) Involvement of sugars and divalent cations. Purified activated blood monocytes were treated or not with tunicamycin or with neuraminidase. Cells were then incubated with CD23 liposomes or control liposomes in absence or presence of EDTA (top left), Ca^{2+} or Mn^{2+} (top right).

(b) Factor X does inhibit CD23 binding to monocytes. Purified activated blood monocytes were incubated with CD23 liposomes in absence or presence of factor X (bottom left). Inset, factor X-treated (plus) or untreated (minus) cells were stained with isotype-matched control antibody (ctrl), anti-CD11a, anti-CD11b, and anti-CD11c (all at 10 µg/ml) followed by FITC-conjugated sheep anti-mouse antibody. Purified activated blood monocytes were incubated with CD23 liposomes in presence of fibrinogen, purified recombinant ICAM-1, LPS, human serum opsonized-zymosan, IgE, or polyclonal antibody to RGD peptide (bottom right). Cells were analyzed by FACS and MFI measured. Percentage inhibition was calculated as in Figure 2.

gen (Figure 4). IgE ,which is binding in the lectin domain of CD23 (Kikutani et al., 1986), partially inhibits CD23 binding to monocytes (Figure 4). Those results indicate that CD23 would seem to be acting as a C-type lectin recognizing partly sugar and protein structures, reminiscent of what was observed for CD23 interaction with CD21 (Aubry et al., 1994).

Binding of CD23 to CD11b and CD11c on Monocytes Increases Nitric Oxide, H_2O_2, IL-1β, IL-6, and TNFα Production

To evaluate the functional significance of the interaction of CD23 with CD11b or CD11c, we investigated whether CD23–CD11b/CD11c interaction could trigger monocytes

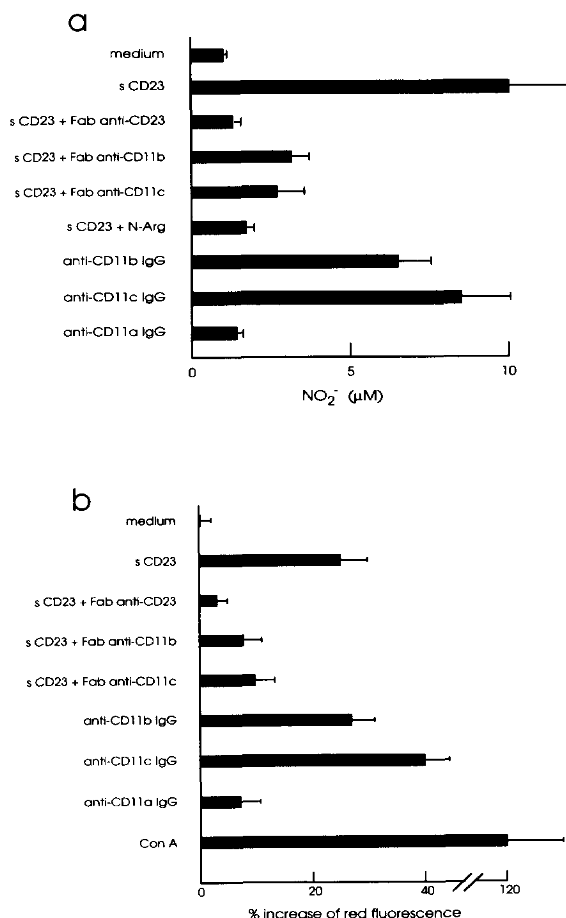

Figure 5. CD23 Binding to CD11b and CD11c Increases Nitrite Production and Oxidative Burst by Blood Monocytes

Recombinant CD23 by binding to CD11b and CD11c specifically increases (a) the nitrite production and (b) the oxidative burst by monocytes. Monocytes were incubated in the absence or presence of recombinant soluble CD23, anti-CD11a, anti-CD11b, anti-CD11c MAbs.

(a) To assess the amount of NO produced, the culture supernatants were assayed for the stable end products of NO, NO_2^-, and NO_3^-. The specificity of CD23-mediated increase of NO_2^- production was demonstrated by inhibition of NO_2^- production by Fab fragments of anti-CD23, anti-CD11b, anti-CD11c MAbs and by inhibition with nitroarginine (N-Arg).

(b) Activated monocytes were incubated with hydroethidine and analyzed by FACS. Percentage increase in red fluorescence of stimulated monocytes is shown in comparison to untreated monocytes. MFI values of monocytes alone were 159 ± 10. Mean ± SD values of six experiments are presented. Concanavalin A, which is known to induce a respiratory burst in monocytes, was used as a positive control. The specificity of the CD23 interaction with CD11b and CD11c was demonstrated by inhibition of CD23-mediated increase of H_2O_2 production by Fab fragments of anti-CD11b, anti-CD11c, and anti-CD23 MAbs.

to release proinflammatory mediators such as nitric oxide (NO), H_2O_2, and cytokines. Triggering of adherence-activated normal monocytes using recombinant soluble CD23, anti-CD11b or anti-CD11c antibodies increased the generation of NO_2^-, indicating the activation of the NO pathway (Moncada et al., 1991). The effect of CD23 on nitrite production was inhibited by Fab fragments of anti-CD23, anti-CD11b, anti-CD11c MAbs and by nitroarginin, a specific inhibitor of the NO synthase pathway (Figure

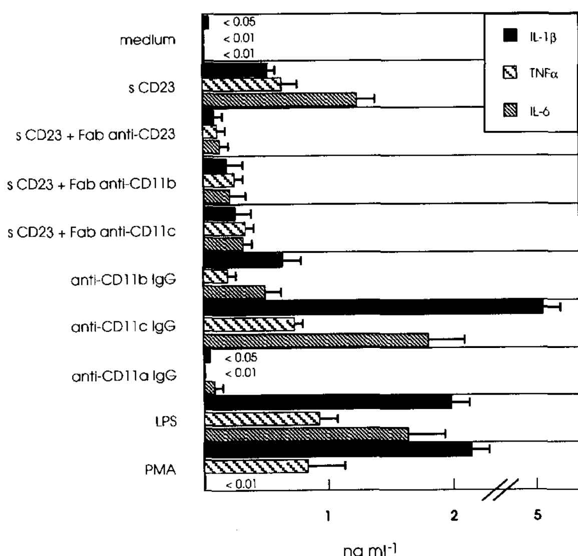

Figure 6. CD23 Binding to CD11b and CD11c Increases Proinflammatory Cytokine Production by Blood Monocytes

Monocytes were incubated overnight in the absence or presence of recombinant soluble CD23, anti-CD11a, anti-CD11b, anti-CD11c, anti-CD23 MAbs, concanavalin A, LPS, or PMA. Cytokines were measured in the culture supernatant by specific ELISA. The specificity of CD23 interaction with CD11b and CD11c was demonstrated by inhibition of CD23-mediated increase of cytokine production by Fab fragments of anti-CD11b, anti-CD11c, and anti-CD23 MAbs. Mean ± SD values of four experiments are presented.

5a). The oxidative burst was also shown to be regulated through CD11b and CD11c, since recombinant soluble CD23, anti-CD11b, and anti-CD11c MAbs all caused oxidation of hydroethidine to ethidium bromide in monocytes. The specificity of the CD23 effect on the oxidative burst was demonstrated by using Fab fragments of anti-CD23, anti-CD11b, and anti-CD11c MAbs (Figure 5b). This confirms and extends the finding that anti-CD11b MAbs induce an oxidative burst in monocytes (Trezzini et al., 1991). CD23 binding to CD11b and CD11c was associated with an early specific Ca^{2+} flux in blood monocytes (data not shown).

Since activated macrophages are an important source of proinflammatory cytokines, we evaluated the effect of recombinant soluble CD23 and of anti-CD11b and anti-CD11c MAbs on the production of such cytokines by monocytes. Recombinant soluble CD23, anti-CD11b, and anti-CD11c MAbs were potent stimulators of IL-1β, IL-6, and TNFα. Again, the specificity of this induction was demonstrated by using Fab fragments of anti-CD11b, anti-CD11c, and anti-CD23 MAbs (Figure 6). Interestingly, IL-1 and TNFα were potent inducers of CD23 liposome binding to monocytes (data not shown), suggesting a potential cytokine autocrine loop through CD11b and CD11c stimulation and regulation. Moreover, engagement of surface CD23 expressed on monocytes has been shown previously to result in an increase of NO, free radicals, and cytokine production (Paul-Eugene et al., 1992). Therefore, the CD23–CD11b and CD23–CD11c pairing could exacerbate the inflammatory reaction by autocrine and paracrine stimulatory pathways.

In view of the increased levels of CD23 reported in various inflammatory diseases including rheumatoid arthritis (Hellen et al., 1991), we have also studied the effect of a neutralizing antibody to CD23 in a murine collagen type II–induced arthritis model. Administration of IgG anti-CD23 resulted in marked amelioration of established arthritis (Plater-Zyberk and Bonnefoy, 1995). One potential mechanism of action of anti-CD23 antibody therapy could be by blocking the interaction between CD23 and CD11b, CD11c, therefore decreasing the release of proinflammatory cytokines that are known to participate in the inflammatory process. The recent generation of CD23-deficient mice (Fujiwara et al., 1994; Stief et al., 1994; Yu et al., 1994) will also permit to investigate the role of this molecule in inflammatory processes in vivo.

We have shown that CD23 is a functional ligand for CD11b and CD11c and that the interaction between these two types of adhesion receptors is likely to have important roles in controlling a number of monocytic activities, cells that play a key role in the immune response.

Experimental Procedures

Cells

Blood mononuclear cells were separated into T cells, B cells, and monocytes. T and B cells were separated by rosetting with sheep red blood cells. Monocytes were enriched by Ficoll and overnight adherence to plastic at 37°C.

For tunicamycin treatment, cells were cultured for 48 hr in complete medium (RPMI 1640; Seromed, Berlin, Germany) supplemented with 2 mM glutamine and 10% heat inactivated fetal calf serum (Flow Laboratories, Irvine, Scotland) containing 10 μg/ml of tunicamycin (Boehringer Mannheim, Mannheim, Germany).

For neuraminidase treatment, cells were treated for 45 min at 37°C with neuraminidase 0.1 U/ml (Boehringer Mannheim).

The binding of CD23 liposomes was compared on treated and nontreated cells by flow cytometry (FACStar Plus, Becton Dickinson, Erembodegem, Belgium).

Mabs and Reagents

Anti-CD11a Mabs 25.3 and B-B15 were obtained from Immunotech (Luminy, France) and Serotec (Oxford, England). Anti-CD11b Mab 44 was from Serotec, mon.gran 1 was from Janssen (Beerse, Belgium), Leu-15 was from Becton Dickinson, and (Bear-1) was from Sera-Lab, Limited (Sussex, England). Anti-CD11c Mabs 3.9 was from Serotec, SL9 was from Sera-Lab, and BU-15 was from The Binding Site (Birmingham, England). Anti-CD13 Mab SJ1D1, anti-CD18 Mab BL5, anti-CD23 Mab (MAb 25), and anti-CD49d Mab (HP2.1) were from Immunotech. Anti-CD21 Mab BL13 was from Immunotech, OKB7 was from Ortho, and BU-33 was obtained from Professor I. C. M. MacLennan; HB-5 was from American Type Culture Collection (ATCC), OKB7 was from Ortho Diagnostics System, Incorporated (Raritan, New Jersey). Anti-CD14, anti-CD3, anti-CD16, and anti-CD20 Mabs were from Becton Dickinson. Sheep fluorescein isothiocyante (FITC)-conjugated $(Fab')_2$ antibodies to mouse IgG and IgM were obtained from Bioart (Meudon, France). The isotype Mab controls were purchased from Becton Dickinson.

Recombinant cytokines IL-1, IL-6, TNFα and, soluble CD23 were produced by Glaxo Institute for Molecular Biology and were devoided of LPS contamination (Pyrotell-kit-LAL; SKAN, Basel, Switzerland). IL-4 was purchased from Amersham International (Buckinghamshire, England). Phorbol myrisate acetate (PMA) and ionomycin were obtained from Calbiochem (La Jolla, California). LPS was from Sigma (St Louis, Missouri).

Affinity Purification

Activated monocytes (5 × 10⁸) were washed once with phosphate-buffered saline (PBS) and homogenized (Bellco) in 50 ml of extraction buffer: 50 mM Tris–HCl, 1% w/v Triton X-100, 100 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM TLCK, 1 mM PMSF, 1 mM benzamidine, and 10 mM iodoacetamide (pH 8.0). The cell lysate was further incubated 60 min on ice to allow proper extraction and was centrifuged 1 hr at 100 000 × g. The supernatant was first applied with constant recycling at 25 ml/hr to a BSA–affigel–15 affinity resin (Bio-Rad Laboratories, Glattbrugg, Switzerland) coupled with 2 mg BSA/ml resin to remove nonspecific binding. The flow-through of this column was then applied under the same conditions to a CD23–affigel–15 affinity resin (5 ml) coupled with 3 mg of recombinant sCD23 (25 kDa)/ml resin. The CD23 affinity resin was washed first with 100 ml of extraction buffer then with 100 ml of extraction buffer containing only 0.1% Triton X-100. The material was finally eluted from the CD23 column with 50 mM Tris–HCl, 3 M NH_4SCN, 0.1 % w/v Triton X-100, 2 mM $CaCl_2$, and $MgCl_2$ (pH 8.0) and immediately dialysed extensively against the same buffer, omitting the NH_4SCN. The eluate was concentrated about 20-fold using a centrifugal concentrator Macrosep having a 10^4 cut off membrane (Filtron). All procedures were carried out at 4°C.

Western Blotting Analysis

The concentrated material was then analyzed on a 7.5% nonreducing SDS–PAGE and transfered onto a 0.45 μm nitrocellulose membrane (Bio-Rad Laboratories, Glattbrugg, Switzerland) according to the instructions of the manufacturer. The membrane was first incubated either with an anti-CD11c or with an anti-CD11b (Serotec) MAb at 10 μg/ml in PBS and 0.05% Tween-20 and stained using a goat anti-mouse peroxidase conjugate (Kirkegaard and Perry, Gaithersburg, Maryland). The immunoreactive bands were finally detected using the enhanced chemiluminescence Western blotting technology on Hyperfilm enhanced chemiluminescence (Amersham Rahn, Zurich, Switzerland).

Flow Cytometric Analysis

Liposome Binding Assays

Cells were resuspended in liposome suspension diluted in 0.5% BSA, 0.1 sodium azide, 2 mM $CaCl_2$, 140 mM NaCl, 20 mM HEPES (pH 7) and incubated for 2 hr at 4°C (Pochon et al., 1992).

Inhibition of Liposome Binding

Cells were incubated with CD23 liposomes or control liposomes in absence or presence of EDTA (5 mM), Ca^{2+} or Mn^{2+} (1–10 mM). Purified activated blood monocytes were incubated with CD23 liposomes in absence or presence of factor X (0.1–10 U/ml; Sigma), fibrinogen (50 μg/ml; Sigma), purified recombinant ICAM-1 (produced in our laboratory), LPS (1 μg/ml; Sigma), human serum opsonized zymosan (1 mg/ml; Sigma), IgE (50 μg/ml; The Binding Site, Birmingham) or polyclonal antibody to RGD peptide (1/500, ATCC).

CD Staining

Cells were incubated with diluted Mabs and sheep FITC-conjugated anti-mouse antibodies diluted in PBS, 0.5% BSA, and 0.05% sodium azide. Cells (5,000 events/condition) were analyzed by FACS (Bonnefoy et al., 1987).

Quantification of Binding Sites per Cell by FACS

Cells were incubated with saturating concentrations of purified Z-Z-CD23 fusion protein (P. G. et al., unpublished data), anti-CD11b, or anti-CD11c followed by sheep FITC-conjugated anti-mouse antibodies. The QIFI-beads (Dako, Glostrup, Denmark) have been used. A calibration curve was built and the mean fluorescence intensity (MFI) arbitrary units are transformed in binding sites/cell (Poncelet and Carayon, 1985).

Transfections

cDNAs coding for CD11a (Larson et al., 1989) were recloned in pCDNA1 (Invitrogen, San Diego, California). cDNAs for CD11b (Corbi et al., 1987) and CD18 (Kishimoto et al., 1987) were recloned in pCDM8 (Seed, 1987). Aliquots (20 μg) of DNA were transfected in COS-7 cells (ATCC) by electroporation (260 V, 960 mFD) using a Gene Pulser device (Bio-Rad, Richmond, California) and 0.4 cm cuvettes in 20 mM HEPES (pH 7.4), 150 mM NaCl. Cotransfections of CD11a, CD11b, or CD11c with CD18 were performed to get expression of the β2 integrins at the cell surface. Controls were done with single chain transfections. Cells were harvested 48 hr after transfection.

Measurement of Nitrite and Oxidative Burst Production

Monocytes were incubated for 4 days at 37°C (nitrite) or overnight (oxidative burst) in the absence or presence of recombinant soluble CD23 (Graber et al., 1992) (50 ng/ml), anti-CD11a (clone 25.3), anti-CD11b (clone 44), anti-CD11c (clone BU-15) MAbs (all at 10 μg/ml). To assess the amount of NO produced, the culture supernatants were assayed for the stable end products of NO, NO_2^-, and NO_3^- according to Green et al. (1982). Inhibition was obtained with nitroarginine (N-Arg at 1 mM) (Sigma). For oxidative burst, activated monocytes were incubated with hydroethidine (Molecular probes, Eugene, Oregon) (0.3 μg/ml) for 30 min at 37°C (Rothe and Valet, 1990) and analyzed by FACS. Monocytes that had undergone an oxidative burst show an increase of red fluorescence signals compared with untreated monocytes reflecting oxidation of hydroethidine to ethidium bromide (Lacal et al., 1990).

Measurement of Cytokine Production

Blood monocytes were incubated overnight at 37°C in the absence or presence of recombinant soluble CD23 (50 ng/ml), anti-CD11a (clone 25.3), anti-CD11b (clone 44), anti-CD11c (clone BU-15), anti-CD23 (MAb 25) MAbs, concanavalin A (Sigma) (all at 10 μg/ml), LPS (1 ng/ml) (Sigma) or PMA (5 ng/ml) (Calbiochem, La Jolla, California). Cytokines were measured in the culture supernatant by specific enzyme-linked immunosorbent assay (ELISA). The ELISAs limit of sensitivity is 0.05 ng/ml for IL-1β (Ferrua et al., 1988), 0.01 ng/ml for TNFα (Medgenix, Biotechnie, Rungis, France), and <0.01 ng/ml for IL-6 (Manie et al., 1993).

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