Importance of CD23 for Collagen-Induced Arthritis: Delayed Onset and Reduced Severity in CD23-Deficient Mice

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Importance of CD23 for Collagen-Induced Arthritis: Delayed Onset and Reduced Severity in CD23-Deficient Mice

Sandra Kleinau, Pernilla Martinsson, Susanne Gustavsson, and Birgitta Heyman

Increased expression of the low affinity receptor for IgE, FcεRII/CD23 has been observed in rheumatoid arthritis. In view of this, we have investigated the expression and influence of CD23 in collagen-induced arthritis (CIA), an animal model for rheumatoid arthritis. CD23+ cells were analyzed in lymph nodes of DBA/1 mice immunized with bovine collagen type II (BCII) in CFA or with CFA only. The percentage of CD23+ lymph node cells was increased in both BCII/CFA- and CFA-immunized mice at 1, 3, and 7 wk after immunization compared with unimmunized mice, indicating a role for the adjuvant to trigger general inflammation and CD23 expression. To investigate the functional role of CD23 in CIA, CD23-deficient mice on the DBA/1 genetic background were studied. After immunization with BCII/CFA, these mice developed CIA with delayed onset and reduced severity compared with wild-type mice. These findings suggest that an increased number of CD23+ cells is part of an inflammatory response and that CD23 expression is of pathogenic importance in the arthritis process.

C CIA is influenced by MHC genes as well as non-MHC genes (2) as collagen type II (CII) emulsified in CFA (1). The development of arthritis is elicited in susceptible mice by intradermal injection of inflammation and erosions (6–9).

CD23 expression is of pathogenic importance in the arthritic process. The Journal of Immunology, 1999, 162: 4266–4270.

CD23 Ag, is a type II membrane-bound glycoprotein expressed in the mouse predominantly on mature IgM+ IgD+ B cells and follicular dendritic cells, but also on a small proportion of T cells (10–12). The membrane CD23 can be cleaved into biologically active soluble fragments (sCD23) of various molecular sizes. Both CD23 and its soluble products have been demonstrated to be involved in IgE regulation, but many activities ascribed to CD23 are also IgE independent. These include regulation of lymphocyte growth and differentiation, prothymocyte maturation, myeloid precursor proliferation, and inhibition of macrophage migration (reviewed in Ref. 13). The involvement of CD23 in RA has been suggested by reports of increased numbers of CD23+ B cells in RA patients (14, 15) as well as by detection of increased levels of sCD23 in serum (15–17) and synoval fluid (18). Furthermore, the sCD23 levels in RA patients appear to vary with disease activity and treatment with steroids (19). However, the increase in CD23 is not disease specific since high levels of sCD23 have also been reported in patients with other autoimmune diseases like Sjögren’s syndrome, systemic lupus erythematosus, autoimmune thyroiditis, and myasthenia gravis (16, 20), implying that CD23 may be involved in general chronic inflammation.

In CIA, a beneficial effect on disease severity has been observed after treatment with CD23-specific Abs (21). In view of these findings, we decided to further investigate the role of CD23 in arthritis, by using two approaches. First, we studied the kinetics of CD23 expression in DBA/1 mice after immunization with CII in CFA or with CFA only. Second, we examined the functional role of CD23 in the CIA model, by using CD23-deficient mice, backcrossed onto the DBA/1 background. We find an increased proportion of CD23-positive B cells in immunized and arthritic DBA/1 mice as well as delayed onset and reduced severity of CIA in CD23-deficient mice.

Materials and Methods

Mice

For the CD23 expression experiments, DBA/1 mice of both sexes were used. The animals were originally obtained from Bomholtgaard (Ry, Denmark) and were kept and bred at the animal unit of the Biomedical Centre, Uppsala.

CD23-deficient DBA/1 mice were obtained by backcrossing CD23-deficient C57BL/6 mice (22) with the arthritis-susceptible DBA/1 mice for a minimum of 5 generations. The backcrossed CD23+/− mice were then intercrossed to generate mice homozygous for the CD23 mutation (CD23−/−) and mice homozygous for the wild-type CD23 allele (CD23+/+). CD23−/− and CD23+/+ animals were mated to mice of the same genotype or used directly in experiments. The CD23 genotype was determined by PCR, using DNA prepared from tail biopsies. PCR was performed using one sense primer and either of two antisense primers to distinguish between homozygous and heterozygous CD23 knockout and wild-type mice: P1, 5′-AAC GCA CGG GTG TTG GTG CTG TTG-3′; P2, 5′-GCT TCC ACA CAT GCA GTC CTC-3′; and P3, 5′-TGA GAC ATT CTG CTC CCA TTC-3′. Using the first set of primers (P1/P2), only DNA from mice with at least one copy of the mutated CD23 gene produced an amplified fragment of 862 bp, while using the second set of primers (P2/P3) the wild-type CD23 gene produced a 1007-bp fragment.

The CIA experiments were performed in male mice, and, in each experiment, mutant and wild-type mice were age matched. The mice were...
backcrossed, bred, and maintained in the animal unit at the Department of Pathology, Uppsala, Sweden. All animals in the experiments were fed rodent chow and water ad libitum. Routine serological tests from animals in the different colonies were consistently negative for common pathogens.

**Antigen**

Bovine type II collagen (BCII) was prepared from nasal cartilage by pepsin digestion and a subsequent purification, as described by Miller (23). BCII was solubilized to 2 mg/ml overnight in 0.01 M acetic acid (HAc) at 4°C with constant mixing.

**Induction of CIA**

The BCII was emulsified with an equal volume (1:1) of CFA (Difco, Detroit, MI), and 50 μl of this emulsion was injected intradermally, under light ether anaesthesia, at the base of the tail of DBA/1 and CD23-deficient DBA/1 mice.

For CD23 expression experiments, a control group of DBA/1 mice immunized with CFA were used. These mice received an intradermal injection at the base of the tail with 50 μl of CFA emulsified with an equal volume of 0.01 M HAc. This immunization does not lead to arthritis development (over a 6-mo observation period, data not shown).

**Evaluation of CIA**

Arthritis development was checked by inspection three times a week during 70 days. Clinical severity of arthritis was quantified according to a graded scale from 0 to 3: 0, normal; 1, detectable swelling in one joint; 2, swelling in more than one but not in all joints; 3, severe swelling of the entire paw and/or ankylosis. Each paw was graded and, each mouse could achieve a maximum score of 12. A mean arthritic score value among only arthritic mice was calculated, starting when a minimum of three mice in a group developed arthritis.

**Flow cytometric analysis**

Cell surface expression of CD23 was analyzed on lymph node cells (LNC) from unimmunized, BCII/CFA- or CFA-immunized DBA/1 mice. The animals were killed at 1, 3, 5, and 7 wk after immunization; inguinal, popliteal, and axillary lymph nodes were dissected out; and individual single cell suspensions were prepared. CD23 expression was assessed using the mAb B3B4 (10) conjugated with phycoerythrin (PE), and the following FITC-conjugated mAbs were used for phenotyping: anti-B220 (CD45R) (B6.2H3, Bio-Rad, Hercules, CA), anti-CD4 (T helper cells) (all mAbs from Pharmingen, San Diego, CA). Double staining was performed on LNC resuspended in PBS containing 1% FCS and stained with mAbs 0.2–1 μg/5 × 10^6 cells at 4°C for 30 min. Stained cells were washed with PBS/FCS and analyzed using a FACSort (Becton Dickinson, Mountain View, CA).

**Measurement of anti-ClI Abs**

CD23<sup>+/+</sup> and CD23<sup>−/+</sup> mice were bled from the tail veins 3, 5 and 8 wk after immunization, and individual sera were analyzed for CH-specific IgG Abs by ELISA. Microtiter plates (Immunolon 2; Dynex Technologies, Chantilly, VA) were coated overnight at 4°C with 50 μl of native BCII in PBS at 200 μg/ml. After washings with PBS containing 0.05% Tween 20 (PBS/Tween), serum samples were added in serial dilution with PBS/Tween and incubated for 2 h at room temperature. The plates were then washed and incubated for 2 h at room temperature with 50 μl of sheep anti-mouse IgG conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:1000 in PBS/Tween. After additional washings, 50 μl of p-nitrophenyl phosphate substrate (Sigma, St. Louis, MO) diethylaminoethyl buffer at 1 mg/ml was applied. Absorbancces were read after 20 min at 405 nm. A polyclonal anti-BCII standard with known concentration was included on every microtiter plate to allow calculation of the Ab content by using Softmax software (Molecular Devices, Menlo Park, CA). The standard was purified by affinity chromatography from pooled sera obtained from BCII-hyperimmunized mice.

**Proliferation assays**

CD23<sup>−/+</sup> and CD23<sup>+/+</sup> mice were killed 13 days after BCII/CFA immunization, and inguinal, popliteal, and axillary lymph nodes were removed. Individual single cell suspensions were made in DMEM supplemented with 2-ME (50 μM), HEPES (10 mM), glutamine (20 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml) and adjusted to 10<sup>5</sup> cells/ml. LNC from mice immunized with CII alone were cultured in triplicate with BCII at 50, 100, and 250 μg/ml. A small proportion of the CD23<sup>−/+</sup> LNC were also subjected to the BCII/CFA protocol. The LNC were cultured in 96-well round-bottom microtiter plates and stimulated in triplicate with BCII at 50 and 250 μg/ml. BCII was dissolved in 0.01 M HAc as previously described. LNC from each type of mouse incubated in DMEM, with addition of 0.01 M HAc served as background control. The cells were incubated at 37°C in 5% CO<sub>2</sub> for 4 days, and 1 μCi/well of [3H]Tdr was added to the culture for the last 18–20 h. [3H]Tdr uptake was measured using a β-scintillation counter.

**Measurement of TNF-α**

The ELISPOT (enzyme-linked immunospot) technique was used for analyzing spontaneous TNF-α production in CD23<sup>−/+</sup> and CD23<sup>+/+</sup> mice. Mice were killed 7 days after BCII/CFA immunization, and peritoneal cells (macrophages and B cells) were harvested by rinsing the peritoneal cavity with 10 IU/ml heparin in HBSS. Individual single cell suspensions were made and adjusted to 10<sup>6</sup> cells/ml in complete DMEM with 1% FCS. One hundred microliters of the cell suspension was added in sextuplicate to each well of conventional ELISA plates, precoated over night at 4°C with 50 μl of 5 μg/ml rat anti-mouse TNF-α (MP6-XT3; Serotec, Oxford, U.K.). After 6 h of incubation at 37°C in 5% CO<sub>2</sub>, the cell suspension was flicked off and the plate washed in PBS. Thereafter 50 μl of 10 μg/ml rabbit anti-mouse TNF-α (Genzyme, Cambridge, MA) was added to each well, and the plate was left overnight at 4°C. After washings in PBS, 50 μl of alkaline phosphatase goat anti-rabbit was added and left for 1 h at room temperature. The plate was then washed in PBS, 50 μl of BCIP phosphatase substrate solution (Sigma) was added, and the plates were developed at room temperature for 2 h. The plates were thereafter washed in deionized water and left to dry. Spot forming cells were counted using an inverted microscope. Control wells with no primary Ab or cell suspension did not yield any spots.

**Statistics**

Differences in CD23 expression between unimmunized, BCII/CFA-immunized, and CFA-immunized DBA/1 mice were compared with the Student t test. The severity of arthritis in CD23-deficient vs CD23 wild-type mice was analyzed with the Mann-Whitney U test and the frequency of arthritis by Fisher’s exact test. The mean clinical onset of arthritis and Ab titers were analyzed with the Student t test.

**Results**

**CD23 expression in BCII/CFA-immunized DBA/1 mice**

CD23 expression. A kinetic study of the CD23 expression was performed in BCII/CFA-immunized mice and in control mice immunized with CFA only. The results, pooled from three repeated experiments, are shown in Fig. 1. Injection of BCII/CFA gave rise to a rapid increase of LNC-expressing CD23 1 and 3 wk after immunization, compared with baseline level in unimmunized mice (Fig. 1A). A similar increase was seen in mice immunized with CFA only. The increase of CD23<sup>+</sup> LNC declined during the following weeks, but at 7 wk the proportion of CD23<sup>+</sup> cells increased again in both groups. All BCII/CFA-immunized mice that developed clinical arthritides during the experimental period exhibited a significant increase in the CD23 population, although no correlation between frequency of CD23<sup>+</sup> cells and severity of clinical arthritis was found (data not shown). Characterization of the phenotype of the CD23<sup>+</sup> populations demonstrated that the majority of the cells were B cells. Out of 26.9% CD23<sup>+</sup> LNC in unimmunized animals 25.9% were also B220<sup>+</sup>, and out of 44.9% CD23<sup>+</sup> LNC in arthritic mice 41.6% were also B220<sup>+</sup> (not shown). A small proportion of the CD23<sup>+</sup> LNC were CD4<sup>+</sup> (1–2%), which confirms earlier observations that T cells express very little CD23 (12).

As to the intensity of the CD23 expression, the mean fluorescence intensity (MFI) was reduced in both BCII/CFA- and CFA-injected mice 1 wk after immunization compared with unimmunized mice (Fig. 1B). The MFI in BCII/CFA-immunized mice remained low although advancing to normal levels during the following weeks, whereas the MFI in CFA-immunized mice advanced to normal levels more rapidly.

**Clinical course of collagen-induced arthritis in CD23-deficient DBA/1 mice**

Two independent experiments were conducted comparing the development of arthritis in CD23-deficient mice (CD23<sup>−/−</sup>) and in
wild-type littermates (CD23\(^{+/+}\)) immunized with BCII/CFA. In Fig. 2 the cumulative incidence of disease in experiment 1 (A) and experiment 2 (B) is shown. A rapid development of arthritis beginning about 3 wk after immunization was observed in the CD23\(^{+-}\) mice in contrast to the CD23\(^{++}\) mice, where symptoms of clinical arthritis started about 10 days later. The mean day of onset was in CD23\(^{++}\) 39 (experiment 1) and 40 days (experiment 2) compared with 29 days in the wild-type mice (Table I). Four weeks after immunization, between 0 and 17% of the mutant mice had developed disease compared with 50 – 64% of the wild-type mice. In both experiments, there was a significantly lower arthritis incidence in the mutant mice during the first weeks of the study whereas at the end of the study the incidence was not statistically different between CD23\(^{+-}\) and CD23\(^{++}\) mice (Fig. 2, Table I).

In Fig. 3, the average arthritic score (i.e., calculated only from arthritic mice) in the two experiments is shown. In both experiments, the CD23\(^{+-}\) mice had less severe arthritis than CD23\(^{++}\) mice, with significant differences primarily in the mid phase of the observation period (days 42–58).

**Immune response against CII in CD23-deficient DBA/1 mice**

Anti-CII IgG Abs were measured in sera from arthritic and non-arthritic wild-type and CD23-deficient mice at 3, 5, and 8 wk after BCII/CFA immunization (Table I). Although CD23\(^{++}\) mice had higher CII-specific IgG levels, they were not statistically different from levels in CD23\(^{+-}\) mice.

We also compared the specific proliferative response against CII in BCII/CFA-immunized wild-type and CD23-deficient mice (Fig. 4). Results presented as cpm values of thymidine incorporation show that LNC from both types of mice responded to BCII. While there was a trend toward greater stimulation at 250 \(\mu\)g/ml BCII in CD23\(^{+-}\) LNC compared with LNC from CD23\(^{++}\) mice, the differences were not statistically significant (Student’s \(t\) test).

**TNF-\(\alpha\) production in CD23-deficient DBA/1 mice**

The important role of the proinflammatory mediator TNF-\(\alpha\) in the development of CIA has previously been shown (8, 24), and in

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**TABLE I. The day of onset, incidence, and Ab titers in CD23-deficient and wild-type mice immunized with BCII/CFA**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Mean Day of Onset</th>
<th>Incidence (%)</th>
<th>Anti-CII IgG(^{a}) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 21</td>
</tr>
<tr>
<td>Expt 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD23(^{+-})</td>
<td>29</td>
<td>9/11 (81.8)</td>
<td>994 ± 553</td>
</tr>
<tr>
<td>CD23(^{++})</td>
<td>39*</td>
<td>11/12 (91.7)</td>
<td>576 ± 469</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD23(^{+-})</td>
<td>29</td>
<td>8/10 (80)</td>
<td>874 ± 459</td>
</tr>
<tr>
<td>CD23(^{++})</td>
<td>40*</td>
<td>4/8 (50)</td>
<td>510 ± 307</td>
</tr>
</tbody>
</table>

\(^a\)The table shows the results from two repeated experiments in which CD23\(^{+-}\) and CD23\(^{++}\) DBA/1 mice were immunized with a single injection of BCII in CFA as described in Materials and Methods. The mice were checked three times a week for clinical signs of arthritis for 70 days.

\(^*\)Mean ± SD anti-CII IgG levels in sera of all animals.

\(^{p}<0.05\).
vitro studies have shown that CD23 can trigger TNF-α production via binding to CD11b on macrophages (25). To investigate whether the reduced CIA in CD23-deficient mice may be a result of low TNF-α levels, we analyzed the spontaneous TNF-α production in peritoneal cells from CD231/1 and CD232/2 mice after BCII/CFA immunization. As depicted in Fig. 5, we found in two repeated experiments that the TNF-α production 7 days after immunization did not differ between CD23+/+ and CD23−/− mice. Similar results were also obtained when analyzing TNF-α production at 2 and 17 days after immunization (data not shown).

Discussion

We have observed that BCII/CFA- and CFA-immunized DBA/1 mice have similar kinetics of CD23 expression, with a significant increase of CD231 cells starting 1 wk after immunization. This suggests that the expanded CD23 population is a result of general inflammation caused by immunization with adjuvant. As a characteristic feature of a chronic inflammatory response, an increase of CD231 LNC could be observed in all mice that had developed CIA after BCII/CFA immunization. The increase of CD231 cells was almost exclusively the result of an expansion of B cells, which agrees well with what has been reported for RA (14, 15).

Another interesting finding was that, while the number of CD23+ B cells was increased in BCII/CFA- and CFA-immunized mice, the cells exhibited a marked reduction in CD23 expression during the first weeks. An explanation for the decreased expression of CD23 in BCII/CFA- and CFA-immunized mice can be that an increased cleavage of membrane CD23 into sCD23 occurs in response to inflammatory stimuli. In human monocytes, TNF-α has been shown to decrease membrane CD23 and increase sCD23, apparently by increasing CD23 cleavage (26).

The functional involvement of CD23 in murine CIA was investigated by Plater-Zyberk et al. (21), who showed that prophylactic and therapeutic administration of Abs against CD23 reduced the severity of CIA. We were interested to further analyze the role of CD23 for the development of CIA, in particular to study the disease over a longer period of time. We used a genetic approach, which has the advantage of a defined and complete absence of CD23 throughout the experiment and also avoids undefined effects of Ab treatment. CD23-deficient mice exhibited a pronounced reduction of CIA, particularly in the induction phase, compared with wild-type mice, suggesting a significant contribution of CD23 in the disease process.

CD23 is expressed on B cells, follicular dendritic cells, and a fraction of T cells (10–12), indicating that CD23 could be involved in the development of CIA through several pathways. In vivo, IgE/Ag complexes (via CD23) generate a several hundred-fold higher specific Ab response than immunization with Ag alone (22, 26).
27). A possible mechanism behind this phenomenon is that endocytosis of the IgE/Ag complexes by CD23+ B cells leads to efficient presentation of antigenic peptides to T cells, as shown to take place in vitro (28–30). We asked the question whether IgE/CII complexes, generated after immunization with CII, could be of importance for the induction of arthritis through a similar mechanism. However, the CD23+/- mice did not develop a stronger specific Ab or T cell response than CD23-/- mice.

An alternative role for CD23 in arthritis is involvement in the inflammatory cascade. That CD23 is involved in inflammation is suggested by in vitro experiments, showing that it interacts with the adhesion molecule \( \beta_2 \) integrin CD11b on monocytes/macrophages, thereby stimulating production of proinflammatory cytokines such as TNF-\( \alpha \) (25, 31). Considering this, the generation of TNF-\( \alpha \) could be expected to be lower in CD23-deficient mice in response to BCII/CFA. We chose to study the TNF-\( \alpha \) production pre-onset of disease, because it has previously been shown that TNF-\( \alpha \) plays a critical role at an early phase after immunization with collagen (32). However, the spontaneous TNF-\( \alpha \) production was similar in CD23+/- and CD23-/- mice, suggesting that other contributing factors are responsible for the reduced CIA in CD23-deficient mice.

In conclusion, our data provide strong support for a role of CD23 in inflammation and in the development of arthritis and imply that a pharmacologic approach to chronic reduction of CD23 may be of therapeutic value in human inflammatory joint disease.

Acknowledgments

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References