EGF-TM7 receptors in rheumatoid arthritis

Kop, E.N.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Deletion of either CD55 or CD97 ameliorates arthritis in mouse models

Robert M. Hoek¹, Daphne de Launay¹, Else N. Kop¹, A. Seda Yilmaz-Elis², Feng Lin³, Kris A. Reedquist³, J. Sjef Verbeek², M. Edward Medof³, Paul P. Tak¹, and Jörg Hamann¹

¹ Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.
² Leiden University Medical Center, Leiden, The Netherlands.
³ Case Western Reserve University, Cleveland, Ohio.

The definitive version is available at www3.interscience.wiley.com
ABSTRACT

Objective: CD55 (decay-accelerating factor) is best known for its role in the negative regulation of the complement system. Indeed, lack of this molecule leads to disease aggravation in many autoimmune disease models. However, CD55 is abundantly present on fibroblast-like synoviocytes and is also a ligand of the adhesion-class heptahelical receptor CD97, which is expressed by infiltrating macrophages. Treatment with antibodies to CD97 ameliorates the collagen-induced model of rheumatoid arthritis (RA) in DBA/1 mice, but the net contribution of CD55 is unknown. This study was undertaken to investigate the role of CD55 in experimental RA.

Methods: Arthritis was induced in wild-type, CD55−/−, and CD97−/− mice using collagen-induced and K/BxN serum-transfer models. Incidence of arthritis was monitored over time, and disease activity was assessed by clinical and immunohistochemical evaluation.

Results: In contrast to observations in many inflammatory disease models, lack of CD55 resulted in decreased arthritis in experimental models of RA. Consistent with the previously reported effects of anti-CD97 antibody treatment, CD97−/− mice had reduced arthritis activity compared with wild-type controls.

Conclusion: Our findings indicate that the lack of CD55 or CD97 in 2 different models of arthritis increases resistance to the disease. These findings provide insight into a role for CD55 interaction with CD97 in the pathogenesis of RA and suggest that therapeutic strategies that disrupt CD55/CD97 may be clinically beneficial.
Deletion of either CD55 or CD97 ameliorates arthritis in mouse models

INTRODUCTION

The glycosyl phosphatidylinositol–anchored cell surface protein CD55 is well known for its role in protecting self cells against autologous complement attack.1,2 Its alternative name, decay-accelerating factor, refers to the role of CD55 in promoting the dissociation of C3 and C5 convertases that assemble on self cells. Thus, CD55 regulates complement activation at the point of convergence between the classical, lectin, and alternative pathways.3 In various disease models, among which are several autoimmune diseases, the absence of CD55 aggravates clinical symptoms.4-12 In many of these cases it has been shown that overactivation of the system’s complement is the cause of the observed disease exacerbation.

Complement activation has been connected both with human rheumatoid arthritis (RA) and with several animal models of RA. For example, it has been observed that levels of terminal complement components are elevated in the synovial fluid of RA patients.13,14 Additionally, in collagen-induced arthritis (CIA), the most widely used actively induced murine arthritis model, mice lacking either C3, C5, or factor B are more resistant to disease.15-17 These findings were reiterated in the passive K/BxN serum–transfer model.18,19 The observation that CD55 is expressed at very high levels on fibroblast-like synoviocytes (FLS)20-23 has prompted speculation on its role in RA in connection with the complement system.24,25

Interestingly, CD55 can also act as a ligand for the adhesion-class heptahelical receptor CD9726, which is expressed on all leukocytes and several other cell types.27,28 Indeed, polyvalent recombinant CD97 probes efficiently adhere to FLS in a CD55-specific manner.29 Furthermore, we previously described the amelioration of actively induced arthritis using a CD97 antibody that blocks CD97–CD55 interaction.30 The question thus arose of whether mice lacking CD55 would display aggravated clinical symptoms of arthritis in either the CIA or the K/BxN serum–transfer models (due to the protective role of CD55 in relation to its regulatory role in the complement system) or reduced disease activity (due to its role as a cellular ligand for CD97). Herein we report that, contrary to many other disease models, in both actively and passively induced models of RA, CD55−/− mice showed reduced arthritis. Moreover, our findings in CD97+/+ mice were comparable to those in CD55+/+ mice in both of these models of RA.
Chapter 8

Figure 1. Joint morphology in wild-type and CD55/- mice. A and B, Sections of knee tissue from wild-type mice (A) and CD55/- mice (B) snap-frozen in Tissue-Tek compound and stained with the CD55-specific monoclonal antibody 2C6. C–F, Hematoxylin and eosin staining (C and E) and Safranin O staining (D and F) of specimens from wild-type mice (C and D) and CD55/- mice (E and F). Lack of CD55 did not lead to changes in morphology in uninflamed joints. Bar in F 25 µm in A and B; 15 µm in C–F.

MATERIALS AND METHODS

Mice

CD55/- and CD97/- mice were generated as described previously31,32 and kept in the animal facility of the Academic Medical Center. The gene-targeted mice that were used were backcrossed to C57BL/6J mice at least 8 times. C57BL/6J wild-type mice were either purchased from Charles River (Lyon, France) or obtained from a colony maintained at the Academic Medical Center. Age- and sex-matched mice were used in all experiments. The institutional Animal Care and Use Committee of the Academic Medical Center approved all experiments.

Induction and scoring of arthritis

CIA was induced as previously described33, with minor changes. Briefly, isoflurane-anesthetized mice (10–12 weeks of age) were immunized intradermally at the base of the tail with 100 µl of inoculum containing 1 mg/ml of chicken type II collagen (Sigma-Aldrich, St. Louis, MO) in Freund’s complete adjuvant (CFA) with 2.5 mg/ml of Mycobacterium tuberculosis H37Ra (Difco, Detroit, MI) on day 0 and day 21. Over a period of ~60 days, clinical manifestations of the disease were scored on a scale
Deletion of CD97 and CD55 in arthritis models

of 1–3, where 1 = swelling of a single joint, 2 = swelling of ≥2 joints, and 3 = swelling of the entire paw. Scores were summed over all 4 paws to give a maximum possible score of 12. Furthermore, the cumulative incidence of arthritis was determined by permanently including mice in the group of sick animals from the day of the first occurrence of clinical disease. In addition, ankle swelling was assessed using dial calipers (POCO 2T 0–10-mm test gauge; Kroeplin Längenmesstechnik, Schlüchtern, Germany).

The K/BxN serum–transfer model was induced as previously described, using recipient C57BL/6J, CD55−/−, and CD97−/− mice that were 6–7 weeks of age. Briefly, K/BxN serum pools were prepared from 5–6-week-old arthritic mice generated from the cross between the KRN and Bl10g7 mice (carrying the NOD-derived g7 major histocompatibility complex allele) and stored at 20 °C. Intraperitoneal injection of 190 µl and 180 µl of serum was performed on day 0 and day 2, respectively. Mice were evaluated over a period of 21 days using the method for caliper measurements and the clinical scoring system described above.

**Histologic and immunohistochemical staining**

Hind limbs of mice were decalcified by means of a procedure described by Jonsson et al. Following this treatment, the tissue was either processed for embedding in paraffin or snap-frozen in Tissue-Tek compound. Four-micron–thick microtome sections of paraffin-embedded tissues were stained with hematoxylin and eosin (H&E) or Safranin O–fast green, using previously described procedures. These sections were evaluated for the level of inflammation and loss of proteoglycans. Inflammation was graded on a scale of 0 (no inflammation) to 3 (severely inflamed joint) based on infiltration by inflammatory cells in the synovium. Safranin O staining was scored using a semiquantitative scoring system with a scale of 0–3, where 0 represents no loss of proteoglycans and 3 indicates complete loss of staining for proteoglycans.

For immunohistochemistry, hind limbs were embedded in OCT compound and snap-frozen in the gas phase of liquid nitrogen without decalcification. Six-micron–thick cryostat sections of this material were stained with a monoclonal antibody specific for mouse CD55 (2C6; a kind gift from Dr. P. Morgan). This antibody was visualized by staining with a horseradish peroxidase–conjugated anti-rat antibody (Jackson ImmunoResearch, Newmarket, UK).

**Anticollagen antibody enzyme-linked immunosorbent assay (ELISA)**

High-binding 96-well ELISA plates (catalog no. 3590; Costar, Cambridge, MA) were coated overnight at 4 °C with 5 µg/ml of chicken collagen (5 mg) (catalog no. C9301; Sigma-Aldrich) dissolved in 2.5 ml of sterile filtered 0.1M acetic acid on a rotator and dissolved overnight at 4 °C in phosphate buffered saline (PBS; 50 µl/well) for IgG1 and for IgG2a collagen-specific antibodies. Plates were washed 3 times with 0.05% Tween 20 in PBS (100 µl/well). Plates were then blocked for 1 hour with PBS/1% bovine
serum albumin (BSA; 100 µl/well). Mouse sera were added in serial dilutions in 2% milk/PBS and incubated overnight at 4 °C, followed by washing and incubation with 1 µg/ml of biotinylated rat anti-mouse IgG1 or IgG2a (SouthernBiotech, Birmingham, AL) in 2% milk/PBS for 1 hour at room temperature. After washing, plates were incubated with streptavidin-conjugated alkaline phosphatase (Jackson ImmunoResearch) for 1 hour at room temperature, washed, and developed with p-nitrophenyl phosphate substrate (Sigma-Aldrich). The reaction was quenched with 1M H₂SO₄. The resulting optical density was measured at 450/540 nm.

**Statistical analysis**
The effects of the lack of CD55 or CD97 on paw swelling and mean clinical score were assessed by calculating the area under the curve using the trapezoidal rule, followed by the Wilcoxon rank sum test. Differences in cumulative incidence were analyzed on a per-day basis, using chi-square analysis-of-contingency tables. In some cases, data for individual days were analyzed using the Mann-Whitney U test.

**RESULTS**

**Unaffected joint morphology in mice lacking CD55**
CD55 is abundantly expressed by FLS in humans²¹,²₃,³⁷ and rats³⁸. As shown in Figures 1A and B, CD55 was also expressed by intimal lining layer cells in the synovium of wild-type mice, the staining of which was lost in CD55⁻/⁻ animals. However, this loss of CD55 on FLS did not lead to morphologic changes or changes in proteoglycan expression, as shown by H&E staining (Figures 1C and E) and Safranin O staining (Figures 1D and F).

**Mitigation of actively induced arthritis in mice with CD55 deficiency**
To analyze the contribution of CD55 to arthritis, we induced CIA in CD55⁻/⁻ and wild-type mice by intradermal immunization near the base of the tail with chicken type II collagen in CFA. To our surprise, the CD55⁻/⁻ mice showed diminished disease, as assessed by several different parameters (Figure 2). First, CD55⁻/⁻ mice lagged behind the wild-type mice in disease development until day 46, as shown by the cumulative disease incidence (Figure 2A). Second, the area under the curve of the clinical scores in CD55⁻/⁻ mice was significantly smaller compared with wild-type mice from day 25 to day 32 (mean ± SEM 8.25 ± 3.01 in wild-type mice and 0.95 ± 1.00 in CD55⁻/⁻ mice) (Figure 2B). Third, the mean clinical scores on days 30 and 32 after immunization were significantly lower in CD55⁻/⁻ mice (Figure 2B). Fourth, there were significant differences in mean paw swelling from day 28 until day 44 (Figure 2C).

Anticollagen antibody titers measured at the end of this experiment (63 days after initial immunization) showed no significant differences for the IgG1 isotype and marginally
Deletion of CD97 and CD55 in arthritis models

Mean ± SEM titers of IgG1 were 2,226 ± 592 in wild-type mice (n = 13) and 3,871 ± 1,833 in CD55−/− mice (n = 11). Mean ± SEM titers of IgG2a were 29,103 ± 5,495 in wild-type mice and 14,340 ± 3,426 in CD55−/− mice (P ≤ 0.05). Histopathologic analysis of hind paws obtained from wild-type and CD55−/− mice on day 63 showed no significant differences, although CD55−/− mice showed a trend toward less erosion and leukocyte infiltration (Figures 2D and E).

Amelioration of actively induced arthritis in CD97−/− mice
To test whether the observed alleviation of CIA was attributable to loss of CD97–CD55 interaction, we evaluated CD97−/− mice in the same experimental model. As with the
CD55−/− mice, CD97−/− mice developed signs of the disease later than their wild-type counterparts (Figure 3A), with a significant difference in cumulative incidence on days 33 and 35 after immunization. The difference in disease severity, however, did not reach statistical significance (Figure 3B).

**Diminished serum-transfer arthritis in mice lacking either CD55 or CD97**

To address the question of whether hampered complement regulation due to the lack of CD55 could have an effect on an arthritis model that is strongly affected by a dysregulated complement system, we used the K/BxN serum–transfer model.\(^{18,19}\) We adoptively transferred serum from spontaneously arthritic K/BxN mice to either C57BL/6J wildtype, CD55−/−, or CD97−/− mice. One would anticipate that lack of CD55 would lead to disease exacerbation compared with wild-type mice, while loss of CD97 would result in findings consistent with those described above. Surprisingly, the CD55−/− mice failed to display enhanced disease, but rather, had somewhat less disease activity. Although the cumulative disease incidence did not differ significantly between CD55−/− and wild-type mice, CD55−/− mice showed a trend toward lower clinical scores until 11 days after serum transfer (Figure 4A), and disease incidence was 100% in wild-type mice by day 7, whereas disease incidence did not reach 100% in CD55−/− or CD97−/− mice until day 10.

CD55−/− mice showed significantly less paw swelling over time, as measured by the area under the curve between days 2 and 6, and on average, paw swelling was below that in wild-type mice until day 10 (Figure 4B). Furthermore, the CD97−/− mice showed significantly lower disease severity as compared with wild-type mice from day 5 to day 6, as measured by the area under the curve (Figure 4A), and there was a significant difference in cumulative disease incidence between CD97−/− mice and wild-type mice on day 5 only. At this time point, 3 of 5 wild-type mice and 2 of 5 CD55−/− animals had clinical signs of disease, while none of the CD97−/− animals were affected. Interestingly,
from 2 days after the start of the passive immunization until the end of the experiment on day 14, the CD97⁻/⁻ mice showed significantly less paw swelling over time, as measured by the area under the curve (Figure 4B).

These findings were consistent with the results of a second experiment showing a marginal delay in onset of arthritis and overall lower mean clinical score in CD55⁻/⁻ compared with wild-type animals (Table 1 and data not shown). The CD97⁻/⁻ mice had significantly lower clinical scores from day 4 until the end of the experiment on day 14 (data not shown). Furthermore, a significant delay in disease onset was noted in CD97⁻/⁻ mice (mean ± SEM day of onset 3.5 ± 0.6 in wild-type mice and 7.8 ± 0.9 in CD97⁻/⁻ mice; P < 0.05) (Table 1). Because of the high disease penetrance, unaffected hind paws at the end of the experiment were rare, being observed in the first experiment only once in the CD55⁻/⁻ group and twice in the CD97⁻/⁻ group, but never in wild-type mice. In the second experiment, in which disease was more severe, all hind paws in all groups were affected. As in the actively induced arthritis model, histopathologic analysis of Safranin O–stained sections of inflamed hind paws from wild-type, CD55⁻/⁻, or CD97⁻/⁻ mice did not show major differences (results not shown).
It is well established that the primary function of CD55 is to regulate complement activation in self tissues.\(^1\),\(^2\) It blocks progression of the protease conversion cascade by accelerating the decay of the components that lead to C3 and C5 convertases that assemble on self tissues. As indicated above, this is the point at which the 3 pathways (the classical, lectin, and alternative pathways) converge and give rise to the effector molecules/complexes (C3a/C5a and C5b–9) of the complement system.\(^3\)

Indeed, CD55-/- mice show enhanced complement activation and, to our knowledge, in all disease models tested so far show augmented complement activation and clinical symptoms.\(^4\)-\(^9\) Hence, it was surprising that subjecting CD55 -/- mice to CIA, an actively induced model of RA, resulted in a significant reduction in arthritis activity, especially in terms of paw swelling.

Alternatively, CD55 can act as a ligand for CD97, an interaction that can be blocked by anti-CD97 antibody treatment.\(^26\),\(^39\),\(^40\) We previously showed that treatment of CIA with CD97 antibody ameliorates disease\(^30\), a finding that has been corroborated in an extended study.\(^41\) However, a recent study has also shown that antibody treatment can have a different outcome than the use of gene-targeted mice.\(^32\) To verify that a lack of interaction between CD55 and CD97 was the cause of the observed improvement in clinical outcome, we subjected CD97 -/- mice to CIA. Consistent with the results of the antibody treatment studies, CD97 -/- mice showed reduced disease scores in the course of developing CIA. This finding supports the notion that the effect of the antibody treatment is likely attributable to interference with ligand binding to CD97 in this disease model, and our findings in CD55 -/- mice further support this interpretation.

To further assess whether CD55–CD97 interaction rather than complement dysregulation in CD55 -/- mice impacts arthritis, we analyzed CD55 -/- and CD97 -/- mice in a K/BxN serum–transfer model. In this model, serum from spontaneously arthritic K/BxN mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Disease incidence</th>
<th>Days of onset in each animal</th>
<th>Maximum clinical score, mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>wild-type mice</td>
<td>5/5</td>
<td>5, 5, 5, 6, 7</td>
</tr>
<tr>
<td></td>
<td>CD55 -/- mice</td>
<td>5/5</td>
<td>5, 5, 6, 6, 10</td>
</tr>
<tr>
<td></td>
<td>CD97 -/- mice</td>
<td>5/5</td>
<td>6, 6, 7, 9, 10</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>wild-type mice</td>
<td>4/4</td>
<td>2, 4, 4, 4</td>
</tr>
<tr>
<td></td>
<td>CD55 -/- mice</td>
<td>4/4</td>
<td>2, 4, 5, 5</td>
</tr>
<tr>
<td></td>
<td>CD97 -/- mice</td>
<td>4/4</td>
<td>6, 7, 9, 9*</td>
</tr>
</tbody>
</table>

* P ≤ 0.05 versus wild-type mice, by Mann-Whitney U test.

### DISCUSSION

It is well established that the primary function of CD55 is to regulate complement activation in self tissues.\(^1\),\(^2\) It blocks progression of the protease conversion cascade by accelerating the decay of the components that lead to C3 and C5 convertases that assemble on self tissues. As indicated above, this is the point at which the 3 pathways (the classical, lectin, and alternative pathways) converge and give rise to the effector molecules/complexes (C3a/C5a and C5b–9) of the complement system.\(^3\)

Indeed, CD55 -/- mice show enhanced complement activation and, to our knowledge, in all disease models tested so far show augmented complement activation and clinical symptoms.\(^4\)-\(^9\) Hence, it was surprising that subjecting CD55 -/- mice to CIA, an actively induced model of RA, resulted in a significant reduction in arthritis activity, especially in terms of paw swelling.

Alternatively, CD55 can act as a ligand for CD97, an interaction that can be blocked by anti-CD97 antibody treatment.\(^26\),\(^39\),\(^40\) We previously showed that treatment of CIA with CD97 antibody ameliorates disease\(^30\), a finding that has been corroborated in an extended study.\(^41\) However, a recent study has also shown that antibody treatment can have a different outcome than the use of gene-targeted mice.\(^32\) To verify that a lack of interaction between CD55 and CD97 was the cause of the observed improvement in clinical outcome, we subjected CD97 -/- mice to CIA. Consistent with the results of the antibody treatment studies, CD97 -/- mice showed reduced disease scores in the course of developing CIA. This finding supports the notion that the effect of the antibody treatment is likely attributable to interference with ligand binding to CD97 in this disease model, and our findings in CD55 -/- mice further support this interpretation.

To further assess whether CD55–CD97 interaction rather than complement dysregulation in CD55 -/- mice impacts arthritis, we analyzed CD55 -/- and CD97 -/- mice in a K/BxN serum–transfer model. In this model, serum from spontaneously arthritic K/BxN mice
is used to induce arthritis, and the disease is strongly complement dependent.\textsuperscript{18,19} Importantly, in this experiment, the CD55\textsuperscript{-/-} mice did not show aggravated disease, but instead showed some protection against disease development, although it was not as clear as that seen in the CD97\textsuperscript{-/-} animals. Thus, although altered contributions of complement activation due to the lack of CD55 cannot be excluded, our results emphasize the importance of the alternative role of CD55 as interacting with CD97 in arthritis.

Our results do not address the question of how CD55–CD97 interaction contributes to the development of arthritis. It is noteworthy that CD55 expression in synovial tissue is known to be much higher than necessary for protection against complement attack. For example, CD55 expression on leukocytes, endothelial cells, and epithelia is considerably lower, while it still adequately protects these cells against complement lysis. Because of this, CD55 is widely used as a defining marker for FLS.\textsuperscript{21,23,42} This might reflect an alternative role of CD55 at the interface that the synovial tissue constitutes. At this location, it is likely that CD55 acts as an instigator for immune cells to pass into the joint. Interestingly, Lawrence et al. addressed this possibility with respect to neutrophil transmigration across mucosal epithelia.\textsuperscript{43} In an altered and highly proinflammatory environment, as constituted by the arthritic joint, such instigator molecules would be counterproductive and facilitate the inflammation to culminate. These insights have potential clinical relevance, since blocking the CD55–CD97 interaction in RA might have a beneficial effect on the clinical outcome of the disease.

Acknowledgements
We are grateful to Carin de Cortie for expert technical assistance, Dr. Margriet J. Vervoordeldonk for experimental help, and Dr. Sigrid R. Ruuls for critical reading of the manuscript.

Author contributions
All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Hoek had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References


