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Targeting the chemotactic function of CD147 reduces collagen-induced arthritis

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Summary

CD147 is a type I transmembrane glycoprotein expressed on a wide variety of cell types, including all leucocytes. While CD147 is best known as a potent inducer of matrix metalloproteinases, it can also function as a regulator of leucocyte migration through its cell surface interaction with chemotactic extracellular cyclophilins. A potential role for CD147–cyclophilin interactions during inflammatory diseases, including rheumatoid arthritis (RA), is suggested from several studies. For example, CD147 expression is increased on reactive leucocytes in the synovial fluid and tissues of patients with arthritis. In addition, the synovial fluid of patients with RA contains high levels of extracellular cyclophilin A. In the current studies we investigated the contribution of the chemotactic function of CD147–cyclophilin interactions to joint inflammation using the mouse model of collagen-induced arthritis. Our data demonstrate that proinflammatory leucocytes, specifically neutrophils, monocytes and activated $CD4^+$ T cells, lose their ability to migrate in response to cyclophilin A *in vitro* when treated with anti-CD147 monoclonal antibody. Furthermore, *in vivo* treatment with anti-CD147 monoclonal antibody can reduce the development of collagen-induced arthritis in mice by > 75%. Such findings suggest that CD147–cyclophilin interactions might contribute to the pathogenesis of RA by promoting the recruitment of leucocytes into joint tissues.

Keywords: autoimmunity; chemokines; collagen-induced arthritis; inflammation; rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by the infiltration of proinflammatory leucocytes such as neutrophils, monocytes and activated $CD4^+$ T cells into the joint space and tissue. These invading leucocytes secrete many cytokines, including tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), which activate resident fibroblast-like synoviocytes,[1] resulting in fibroblast hyperproliferation and the production of tissue-degrading matrix metalloproteinases (MMPs).[2] Chemokines are known to be critical factors for the recruitment and infiltration of inflammatory leucocytes from the circulation into joint tissues.[3] Indeed, many different chemokines including CXCL8, CCL2, CCL3 and CCL5 have been detected in the synovial fluid of patients with RA.[4]

CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN), is a type I transmembrane glycoprotein expressed on all cell types, including haematopoietic, epithelial, endothelial and tumour cells. CD147 was first described as a contributing factor to tumour cell invasion because of its ability to enhance MMP production.[5] More recently, the capacity of CD147 to stimulate MMP secretion has also been examined in the context of different inflammatory diseases, including RA.[6,7] For

Abbreviations: CIA, collagen-induced arthritis; CypA, cyclophilin A; ELISA, enzyme-linked immunosorbent assay; EMMPRIN, extracellular matrix metalloproteinase inducer; IgG2a, immunoglobulin G2a; IL, interleukin; mAb, monoclonal antibody; MMP, matrix metalloproteinase; PBS, phosphate-buffered saline; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

example, *in vitro* studies have demonstrated that cell-surface CD147 on proinflammatory leucocytes, such as macrophages, can interact with CD147 expressed on synovial fibroblasts and induce the production of MMPs that contribute to the pathology of RA.[8,9] However, other than its EMMPRIN activity, CD147 exhibits additional functions that may also contribute to RA. One of these is a capacity to interact with extracellular proteins, most notably extracellular cyclophilins.[10]

Cyclophilins are a family of ubiquitously expressed intracellular proteins functioning as peptidyl-prolyl *cis*–-*trans* isomerases. Cyclophilin A (CypA) is the best characterized and most abundant of the cyclophilins, accounting for 0·1–0·4% of total cellular protein.[11] Additionally, CypA has been identified as the intracellular binding partner for the immunosuppressive drug, cyclosporine A.[12] Importantly, cyclophilins can be actively secreted,[13] making these proteins able to function in an extracellular manner. Extracellular cyclophilins possess a potent chemotactic capacity for several human and mouse leucocyte subsets, including neutrophils, T cells and monocytes.[8,14,15] Cyclophilins therefore represent a novel family of extracellular proteins with the ability to function as chemokines.

Previous studies have demonstrated that CD147 is the principal cell surface signalling receptor for the chemotactic activity of extracellular cyclophilins.[16] Moreover, monoclonal antibodies (mAbs) specific for CD147 can inhibit the *in vitro* migration of leucocytes mediated by extracellular cyclophilins, demonstrating the dependence on CD147 interaction for this chemotactic activity.[14,15,17,18] As a result of the relevance of CD147 and extracellular cyclophilins to leucocyte migration, we have previously proposed that CD147–cyclophilin interactions may play a role in the development of inflammation, by promoting infiltration of leucocytes into tissues during ongoing inflammatory responses.[10,18] Indeed, various lines of indirect evidence suggest a role for CD147 and/or extracellular cyclophilins in several inflammatory diseases, including RA. For example, up-regulated expression of CD147 has been reported in patients with atherosclerosis[19] and systemic lupus erythematosus.[20] Several studies have reported up-regulated CD147 expression in patients with RA, including on synovial monocytes/macrophages, granulocytes and fibroblast-like cells.[6–9] On the side of cyclophilins, elevated levels of extracellular cyclophilins have been detected in the serum of patients with severe sepsis,[21] and in the synovial fluid of patients with active RA.[22] In fact, levels of extracellular CypA within the synovial fluid of patients with RA were found to directly correlate with the number of neutrophils present in the same fluid.[22] In recent experimental studies, we investigated the direct contribution of CD147–cyclophilin interactions to inflammatory responses. Using two different mouse models of acute lung inflammation, we demonstrated that inhibiting the capacity of CD147 to interact with extracellular cyclophilins reduced the influx of neutrophils into lung tissues during acute lung injury[14] and eosinophils and T helper type 2 $CD4^+$ T cells during acute allergic asthma.[17] These findings led us to conclude that CD147–cyclophilin interactions probably contribute directly to the development of acute inflammatory responses, by recruiting leucocytes to inflamed tissues.

In the current study, we examined whether CD147–cyclophilin interactions might also contribute to chronic types of inflammatory responses, specifically RA. For these studies, we made use of the collagen-induced arthritis (CIA) mouse model that shares many of the clinical and pathological features of human RA, including an infiltration of inflammatory leucocytes into the synovium.[23] Our findings demonstrate that treating CIA mice with anti-CD147 at the onset of CIA disease reduces joint inflammation by > 75%. These findings provide a potentially novel target (CD147–cyclophilin interactions) to consider for reducing tissue inflammation in RA, as well as other types of chronic inflammatory disease.

Materials and methods

Animals

In vivo studies were conducted using male DBA/1J mice (age 9–10 weeks) purchased from Jackson Laboratories (Bar Harbor, ME). *In vitro* studies were conducted using female C57BL/6 mice aged 6 weeks or older purchased from the National Cancer Institute (Bethesda, MD). All studies were approved by the Institutional Animal Care and Use Committee at The George Washington University Medical Center.

Antibodies and reagents

Immunization grade bovine collagen II (CII) and complete Freunds' adjuvant were purchased from Chondrex (Redmond, WA). Rat anti-mouse CD147 mAb was purified from the RL73.2 hybridoma originally donated to us by H. R. MacDonald (Ludwig Institute for Cancer Research, Switzerland). The rat immunoglobulin G2a (IgG2a) hybridoma (HB-189) obtained from the American Type Culture Collection (Manassas, VA) was used as a source of isotype control antibody. Both mAbs were purified by the National Cell Culture Center (Minneapolis, MN). The UM-8D6 clone and its corresponding IgG1 isotype control were purchased from Ancell (Bayport, MN). MC57 fibroblasts were maintained in RPMI-1640 + L-glutamine + 10% fetal bovine serum. Fluorescein-conjugated $F(ab')_2$ anti-rat IgG (secondary) antibody was purchased from Jackson ImmunoResearch Laboratories (West Gove, PA). Cy-Chrome-conjugated anti-mouse CD4 mAb was purchased from BD Biosciences (Franklin Lakes, NJ). Rabbit

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anti-cyclophilin A antibody was obtained from U.S. Biological (Swampscott, MA). Horseradish peroxidase-conjugated anti-rabbit secondary antibody was purchased from Amersham Biosciences (Piscataway, NJ). 3.3′,5.5′-tetramethylbenzidine (TMB) substrate was purchased from Dako (Carpinteria, CA). Human recombinant CypA was purchased from Calbiochem (San Diego, CA). Bovine serum albumin fraction V and concanavalin A were purchased from Sigma (St Louis, MO).

Regimen for induction of CIA

Male DBA/1J mice were immunized with 100 μg CII emulsified in complete Freunds' adjuvant on day 0 via a tail-base injection. On day 21, immunized mice were boosted via an intraperitoneal injection with 100 μg CII in phosphate-buffered saline (PBS). Untreated mice were used in some experiments as a negative control. To evaluate the severity of the disease, a macroscopic clinical scoring method was used. Scoring took place every 3–4 days with the following scale: 0 = normal joint; 1 = mild swelling and/or redness; 2 = pronounced edema or redness of the paw or several digits; 3 = severe swelling of entire paw. A clinical score was generated for each mouse by combining the scores of all four paws (maximum score of 12).

Anti-CD147 intervention regimens

For intervention studies, doses of 5 μg anti-CD147, or isotype control mAb, were administered per injection by intraperitoneal delivery in 100 μl PBS. This dose was determined to be optimal based on preliminary titration studies. Antibody was given daily for 10 days starting on the day of CII challenge. Animals were killed at various times during the course of disease for tissue analysis (see Figure legends).

Analysis of joint proteins

At the indicated time-points during disease, animals were killed and joint tissues were isolated, cleaned and then frozen at −80° until analysis. Frozen joint tissues were pulverized on dry ice and protein was extracted using RIPA buffer (50 mmol/l Tris–HCl, pH 7·5, 150 mmol/l NaCl, 1% Nonidet P-40, 0·5% sodium deoxycholate, 0·1% sodium dodecyl sulphate). For Western blot analysis, equal volumes of protein samples were fractionated by 4–20% sodium dodecyl sulphate–polyacrylamide gel electrophoresis, blotted onto nitrocellulose membrane, and then probed with anti-CypA antibody. Densitometric analysis of developed autoradiograph bands was conducted using a Molecular Dynamics Personal Densitometer SI. To measure levels of myeloperoxidase, 50 μl joint protein extract was combined with 50 μl TMB substrate[24] and the resulting colorimetric reaction was quantified using an enzyme-linked immunosorbent assay (ELISA) plate reader. Levels of TNF-α were measured by ELISA using a kit purchased from R&D Systems (Minneapolis, MN).

Leucocyte isolation and generation

Neutrophils were isolated from the peritoneal cavity of mice 3 hr after intraperitoneal injection of 1 ml 3% thioglycollate broth. Isolated peritoneal cells were washed three times with cold PBS and then overlaid onto a sequential gradient of Histopaque-1007 and -1119 (Sigma). Enriched neutrophils (> 80%) were collected from the interface of the two Histopaque layers. Monocytes were isolated from blood collected via cardiac puncture. Peripheral blood mononuclear cells were enriched by centrifugation over Lymphocyte Separating Medium (Mediatech Inc., Herndon, VA) and the monocytes were then enriched to 90% by magnetic antibody cell sorting (MACS) separation using a positive selection kit specific for $CD11b^+$ cells (Miltenyi Biotec, Auburn, CA). Activated $CD4^+$ T cells were generated by overnight stimulation of total splenocytes (3×10^6 cells/well) with concanavalin A (1 μg/ml). The $CD4^+$ T cells were then purified from these populations using a MACS negative selection kit. Naïve $CD4^+$ T cells were purified from populations of unstimulated spleen cells.

Chemotaxis assays

Chemotaxis assays were conducted using 48-well modified Boyden chambers (Neuroprobe, Gaithersburg, MD) with the two compartments separated by a 5-μm polycarbonate membrane (Neuroprobe). The chemotaxis of purified neutrophils, monocytes and $CD4^+$ T cells was assessed by adding 10^4 cells in medium (RPMI-1640 + 1% bovine serum albumin) to the upper compartment and medium containing 100 ng/ml CypA for neutrophils, 200 ng/ml CypA for monocytes, and 400 ng/ml CypA for $CD4^+$ T cells to the wells of the lower compartment. (These doses were established to be optimal for each cell subset by preliminary titration studies.) For blocking experiments, 25 μg/ml anti-CD147 or isotype control was added to each compartment for the neutrophil experiments and 10 μg/ml anti-CD147 or isotype for the monocyte and $CD4^+$ T-cell experiments. Lower compartment wells containing medium alone were utilized as a negative control. The loaded chemotaxis chambers were incubated at 37° in 5% CO_2 for 50 min. Following incubation, the membrane was removed, non-migrated cells were scraped off, and the membrane was stained with Wright–Giemsa (CAMCO, Fort Lauderdale, FL). A chemotactic index was generated for each well by dividing the number of cells migrating within each test well by the average number of cells migrating to medium alone.

Analysis of CD147 expression on leucocytes

The expression of CD147 on neutrophils and monocytes was analysed on peripheral blood leucocytes obtained from blood collected via cardiac puncture. Red blood cells were removed by treating blood with red blood cell lysis buffer (1·6 g ammonium chloride, 0·2 g potassium bicarbonate, and 0·03 g ethylenediamine tetraacetic acid in 100 ml diH_2O). Activated $CD4^+$ T cells were generated as described above. All cell populations were stained with anti-mouse CD147, or isotype control mAb, followed by fluorescein isothiocyanate-conjugated anti-rat IgG (secondary). Flow cytometric analysis was performed using forward scatter/side scatter plots to distinguish individual populations.

MMP assays

Macrophages were purified from mouse spleens using MACS $CD11b^+$ positive selection. Purified macrophages (2×10^4 per well) were cocultured with MC57 fibroblasts (10^4 per well) in RPMI + 10% fetal bovine serum in a 48-well plate with a total volume of 500 μl/well. Following a 72-hr incubation at 37°, levels of pro-MMP-9 in supernatants were measured with an ELISA kit (R&D Systems). For blocking experiments, MC57 cells were incubated with 10 μg/ml anti-CD147 (either RL73.2 or UM-8D6), or their respective isotype control mAbs, for 15 min at room temperature before adding purified macrophages.

Results

CD147 expression and CypA in CIA

In preparation for our *in vivo* studies investigating how CD147–extracellular cyclophilin interactions contribute to CIA, we first established that the two components of the interaction, CD147 and cyclophilins, were present in mouse CIA. Figure 1 shows the expression profile of CD147 on three subsets of proinflammatory leucocytes known to contribute to tissue pathology during CIA and RA, specifically neutrophils, monocytes and activated $CD4^+$ T cells. Flow cytometric analysis confirmed that CD147 was readily detectable on all three subsets. We next confirmed that elevated levels of cyclophilins were present in the inflamed joints of mice with CIA, as observed in the joints of human patients with RA.[22] Figure 2(a) shows the time–course of joint inflammation induced during CIA, based on a macroscopic scoring system. Since inflammation in mouse CIA is restricted to wrist and ankle joints where the synovial space cannot be sampled directly, changes in cytokines and chemokines are typically examined in homogenized joint tissue.[25] Proteins were extracted from the joints of CIA mice killed at various clinical scores and Western blot analysis was performed to establish the presence of CypA. As shown in Fig. 2(b), levels of CypA were elevated in the joints of mice with CIA relative to the joints of naive control mice, and the levels increased with increasing clinical scores. It was not possible for us to conclude that the observed increases in CypA were the result of changes in extracellular CypA alone because the proteins analysed were a mixture of intracellular, as well as extracellular, CypA. However, using reverse transcription–polymerase chain reaction for CypA messenger RNA, we established that the differences in levels of CypA proteins induced during CIA were not the result of a per cell increase in messenger RNA transcription (data not shown). Therefore, the increase in synovial CypA protein observed during CIA was probably the result of an increase in numbers of cells expressing or secreting CypA.

Leucocyte migration mediated by CypA is blocked by RL73.2 anti-CD147

Since our main focus for the current studies was to establish the contribution of CD147–cyclophilin inter-

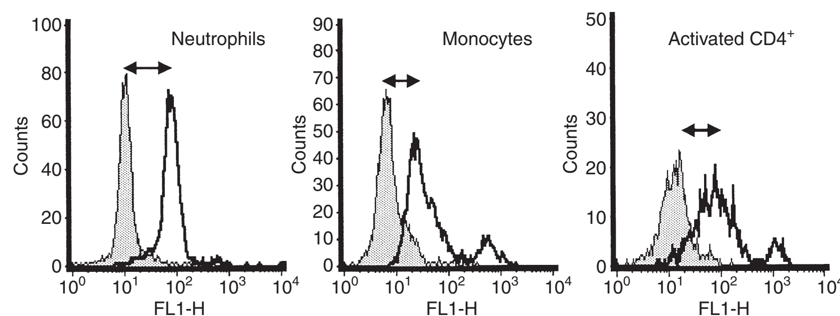

Figure 1. Mouse CD147 expression. Neutrophils, monocytes and activated $CD4^+$ T cells were stained with anti-CD147, or isotype control antibody, followed by fluorescein isothiocyanate-conjugated anti-rat immunoglobulin G. Neutrophils and monocytes were distinguished based on forward scatter/side scatter characteristics and $CD4^+$ T cells were identified by costaining with Cy-anti-CD4. Histograms show CD147 staining (open) versus isotype staining (filled).

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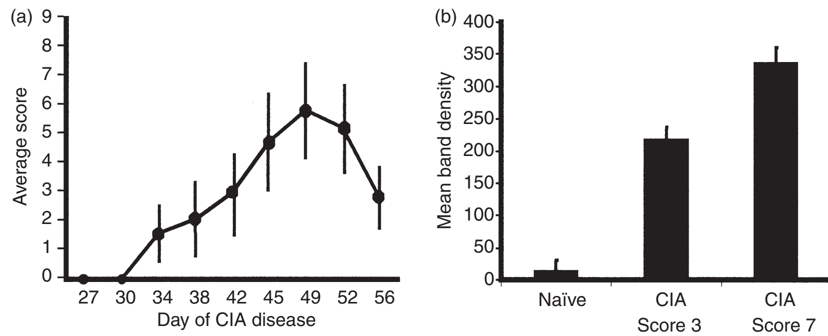

Figure 2. Presence of cyclophilin A (CypA) in collagen-induced arthritis (CIA). (a) DBA/1J mice were immunized with collagen in complete Freunds' adjuvant and then challenged 3 weeks later with collagen in phosphate-buffered saline. Starting 7 days after challenge, inflammation was scored for individual joints. Data show mean (± SE) clinical scores with $n = 6$ mice per time-point. (b) Groups of mice were killed when they reached specific CIA scores and proteins extracted from joints were analysed by Western blot analysis using anti-CypA antibody. Data show mean band densitometry of lysates from naïve mice versus mice with intermediate or high CIA clinical scores.

actions in the context of leucocyte recruitment, we next confirmed that treatment with anti-CD147 mAb could directly inhibit the migration induced by CypA *in vitro* of CIA-relevant leucocytes. For these studies, we made use of the RL73.2 clone of anti-mouse CD147.[26] We have used this clone successfully in previous studies to reduce the migration of leucocytes *in vitro*, as well as the influx of leucocytes during acute lung inflammation *in vivo*.[14,17] As shown in Fig. 3, neutrophils, monocytes and activated $CD4^+$ T cells all migrated well *in vitro* to CypA. Interestingly, naïve $CD4^+$ T cells, a subset of leucocytes known to be poorly recruited into tissues, displayed minimal migration. We have previously shown that both human and mouse naïve $CD4^+$ T cells express significantly lower levels of cell surface CD147 than activated $CD4^+$ T cells,[15,17] providing a likely explanation for their poor capacity to interact with, and respond to, CypA. In the case of neutrophils, monocytes and activated $CD4^+$ T cells, the observed migration induced by CypA was inhibited by > 90% by the RL73.2 mAb (Fig. 3). Taken together, these findings demonstrate the capacity of the RL73.2 anti-CD147 clone to directly interfere with the CypA-mediated recruitment of CIA-relevant proinflammatory leucocytes.

RL73.2 antibody does not impact on the EMMPRIN function of CD147

One of the best-established functions of CD147 is its role as an inducer of MMPs.[5] Given the importance of MMPs in tissue destruction and remodelling during RA,[6,7] we wanted to establish whether the RL73.2 antibody that we were using to inhibit the chemotactic function of CD147 might also impact on its EMMPRIN function. For example, peptide inhibitors of CD147 have been shown to block the MMP-9 production induced in cocultures of macrophages and fibroblasts isolated from patients with RA.[8,9] In other studies, several different anti-human CD147 antibodies were shown to inhibit MMP production, as well as MMP-mediated tissue invasion, by a breast cancer cell line.[27] To date, the RL73.2 anti-mouse CD147 clone has not been tested for its ability to inhibit the EMMPRIN activity of mouse CD147. As shown in Fig. 4, the coculture of mouse MC57 fibroblasts with primary mouse macrophages induced an increase in the secretion of pro-MMP-9, the precursor for active MMP-9. However, the presence of the anti-CD147 mAb RL73.2 during culture had no impact on the augmented secretion of pro-MMP-9 (Fig. 4), regardless of the dose tested (data not

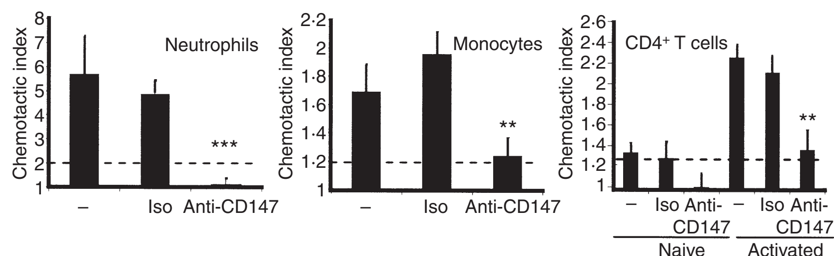

Figure 3. Leucocyte migration mediated by cyclophilin A (CypA) is blocked by RL73.2 anti-CD147. Purified neutrophils, monocytes and $CD4^+$ T cells were set up in Boyden chemotaxis chambers in the presence of extracellular CypA with or without anti-CD147 or isotype control monoclonal antibody. Bar graphs show mean (± SE) chemotactic index for each group, with $n = 4$ to $n = 6$ wells per group. Horizontal dashed lines denote the cutoff chemotactic index for significant migration. Statistical differences between anti-CD147 and isotype groups were established using a Student's *t*-test: $***P < 0{\cdot}001$ and $**P < 0{\cdot}01$.

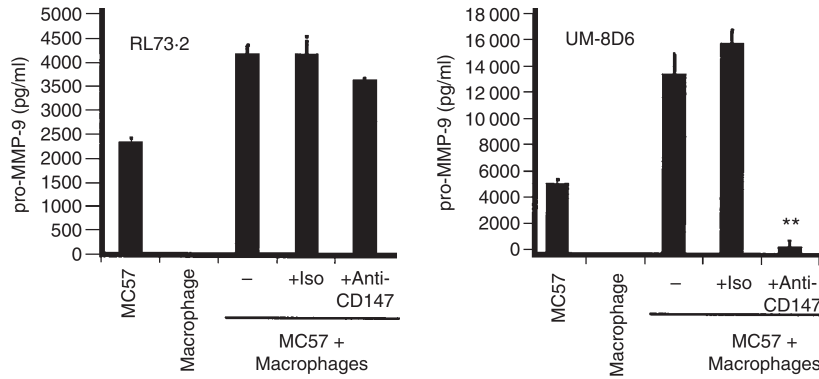

Figure 4. Pro-matrix metalloproteinase 9 (pro-MMP-9) secretion is not blocked by RL73.2 anti-CD147. MC57 mouse fibroblasts and purified mouse macrophages were cocultured in the presence of RL73.2 anti-CD147, or UM-8D6 anti-CD147, or relevant isotype control monoclonal antibodies. After 72 hr, culture supernatants were tested for pro-MMP-9 by enzyme-linked immunosorbent assay. Bar graphs show mean (± SE) concentrations of pro-MMP-9 detected in cultures. Statistical differences between anti-CD147 and isotype groups were established using a Student's *t*-test: $^{**}P < 0{\cdot}01$.

shown). This was in marked contrast to a different anti-CD147 clone, UM-8D6, which reduced pro-MMP-9 secretion by > 90%. These findings suggest that the two clones interact with different functional domains of CD147. The extracellular portion of CD147 consists of two domains (domains 1 and 2) that are thought to mediate different functions.[27] The two different clones are probably binding to, and interfering with, different domains of CD147. From the current findings, we conclude that RL73.2 anti-CD147 mAb has the capacity to inhibit the chemotactic function of CD147, but not its EMMPRIN function.

Treatment with RL73.2 anti-CD147 significantly reduces CIA severity

We next examined the impact of targeting the chemotactic function of CD147 during CIA. For these studies CIA mice were given RL73.2 mAb for 10 days, starting from the time of challenge, and then treatment was stopped. As shown in Fig. 5(a), these anti-CD147 treated mice demonstrated delayed CIA-mediated joint inflammation, as well as a highly significant reduction (> 75%) in clinical scores, compared to mice treated with an isotype control antibody. In a repeat of this intervention regimen, some mice were killed at the peak differential between anti-CD147 and isotype clinical scores (in this experiment: day 42, showing 80% reduction in inflammation) and joints were collected for protein extraction. These were then assayed for the presence of myeloperoxidase and TNF-α, products that are secreted by infiltrating neutrophils and/or macrophages. Figure 5(b) shows that both products were markedly reduced in the joints of mice receiving RL73.2 intervention.

Discussion

Chemokines have been extensively documented as playing a role in the recruitment of proinflammatory leuco-

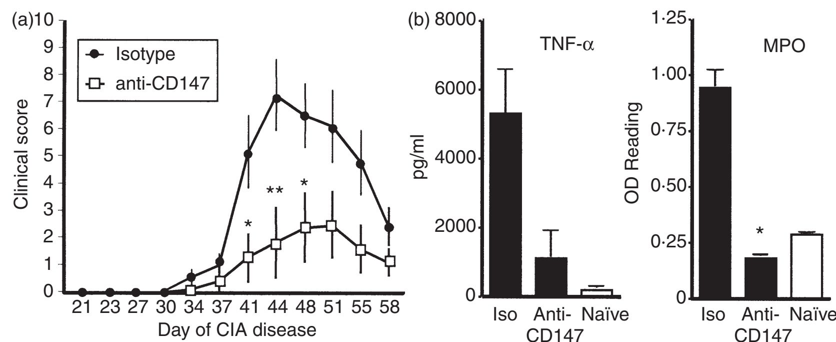

Figure 5. Anti-CD147 intervention reduces the severity of collagen-induced arthritis (CIA). DBA1/J mice were immunized and challenged with collagen II to induce CIA. (a) Half the mice were treated on days 21–30 with RL73.2 anti-CD147 monoclonal antibody (mAb) while the other half were treated with an isotype control mAb. Graph shows the mean (± SE) score for each group ($n = 9$ per group). (b) Mice were killed at the peak differential between anti-CD147 and isotype treatment and joint proteins were extracted. Myeloperoxidase levels were established using TMB substrate and tumour necrosis factor-α (TNF-α) levels were measured by enzyme-linked immunosorbent assay. A Student's *t*-test was used to establish statistical significance at individual time-points or between groups: $^{**}P < 0{\cdot}01$; $^{*}P < 0{\cdot}05$.

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cytes into joint tissues from the periphery. Recently, subsets of human leucocytes were shown to exhibit chemotactic potential in response to extracellular cyclophilins, a novel class of chemokine-like proteins. This chemotactic capacity is dependent upon interaction with CD147. Since elevated CD147 expression and high levels of extracellular cyclophilins have been reported in patients with RA, we proposed that extracellular cyclophilins attract proinflammatory leucocytes, via CD147, into joints thereby contributing to the inflammatory process. This study is the first to provide *in vivo* evidence of a likely contribution of such an interaction to joint inflammation.

Our studies made use of the RL73.2 clone of anti-CD147 mAb. Importantly, we demonstrated that RL73.2 can specifically inhibit the chemotactic function of CD147 as it blocked the CypA-mediated migration of three RA-relevant proinflammatory leucocytes. However, RL73.2 was not able to impact on the EMMPRIN function of CD147. Furthermore, using the CIA mouse model of RA we showed that targeting the chemotactic function of CD147 with RL73.2 anti-CD147 mAb intervention in CIA mice resulted in a delay in the development of arthritic symptoms, as well as a > 75% reduction in the severity of joint inflammation and a reduction in the presence of inflammatory mediators secreted by the infiltrating leucocytes. While our current studies have focused on the chemotactic function of CD147, we acknowledge that the well-studied EMMPRIN function of CD147 may be another important contributor to RA, by facilitating the secretion of cartilage-degrading MMPs. Indeed, *in vitro* studies reported that coculturing fibroblast-like synoviocytes with macrophages derived from patients with RA enhanced the secretion of MMPs in a CD147-dependent manner.[9] Targeting the EMMPRIN function of CD147 could therefore provide an alternative approach for reducing joint inflammation in RA/CIA.

The pathology of RA is characterized by two distinct phases. During the initiation phase, antigen-specific T and B cells are activated and expand within the synovium.[28] The subsequent effector phase is defined by the recruitment of neutrophils, monocytes and lymphocytes into the joint where, in conjunction with synovial fibroblasts, they contribute to the destructive events of the disease through the production of proteases, cytokines and other inflammatory mediators.[1] Our findings of a reduction in factors associated with the presence of activated neutrophils suggest that anti-CD147 intervention is more likely to be impacting on the effector phase of disease. These observations fit well with the fact that significant increases in CypA within joints are only observed once the inflammatory response is underway, arguing that any cyclophilin-mediated recruitment of leucocytes would be induced during the effector phase, rather than during the initiation phase, of the response.

Our studies have provided evidence that targeting the chemotactic function of CD147 can have a significant impact on the development and severity of joint inflammation in CIA. Although other types of intervention have also proven successful in reducing tissue inflammation in CIA/RA,[29] many of these target immune components that under normal circumstances have a beneficial immunological role to play. For example, while anti-TNF-α may be highly effective at reducing joint inflammation in RA,[30] the lack of this cytokine during infection can be detrimental to the host. Indeed, the delivery of anti-TNF-α mAbs to humans has been reported to increase the risk of serious infection and the development of malignancies.[31] We propose that targeting CD147 and its interaction with cyclophilins may provide a less detrimental therapeutic approach because elevated levels of extracellular cyclophilins are only present during situations of prolonged/persistent tissue inflammation. Blocking the capacity of CD147 to interact with extracellular cyclophilins will impact only on the leucocyte recruitment that is mediated by long-lived inflammation, such as during chronic inflammatory diseases.

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