The cartilage protein melanoma inhibitory activity contributes to inflammatory arthritis

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Abstract

Objective. Melanoma inhibitory activity (MIA) is a small chondrocyte-specific protein with unknown function. MIA knockout mice (MIA−/−) have a normal phenotype with minor microarchitectural alterations of cartilage. Our previous study demonstrated that immunodominant epitopes of MIA are actively presented in an HLA-DR4-restricted manner in the inflamed RA joint. The objective of this study was to investigate the potential role of MIA as an autoantigen.

Methods. Collagen-induced arthritis (CIA) and anti-collagen antibody-induced arthritis (CAIA) were induced in MIA−/− mice. Anti-type II collagen (anti-CII) antibodies were measured by ELISA. T cell proliferation and cytokine production were assessed by flow cytometry.

Results. MIA−/− mice had a markedly reduced incidence and severity of CIA and CAIA compared with wild-type (WT) mice. Attenuation of disease was not related to defective binding of anti-CII antibodies to cartilage in the absence of MIA. However, MIA−/− mice had significantly reduced anti-CII IgG1 and IgG2a antibody levels accompanied by an increase in FoxP3-expressing CD25+CD4+ regulatory T cells. This was paralleled by a significant reduction in CII-specific IFN-γ production by T cells in MIA−/− but not WT animals, suggesting a qualitative impact of MIA on the collagen-induced Th1 response. Furthermore, Ag-specific proliferation of T cells after restimulation with MIA in WT but not MIA−/− mice indicated the existence of MIA-specific T cells in the context of CIA.

Conclusion. These data support a role for MIA as an autoantigen during arthritis development. Whether MIA can influence the balance of pathogenic vs regulatory responses in human RA remains to be investigated.

Key words: autoantigen, rheumatoid arthritis, collagen-induced arthritis, MIA, melanoma-inhibiting activity.

Introduction

Melanoma-inhibitory activity (MIA) is an 11 kDa soluble protein originally cloned from a human melanoma cell line in 1994 [1]. A bovine homologue was subsequently cloned from chondrocytes as cartilage-derived retinoic acid-sensitive protein [2]. Additional work revealed that this small protein was expressed and secreted by malignant melanomas, chondrosarcomas and, to a lesser extent, different types of adenocarcinomas [3, 4]. Multiple studies have now demonstrated that MIA is not only a useful serum biomarker for these malignancies, but also plays an important functional role in tumour progression and metastasis through multiple mechanisms [5–8].

In non-malignant tissue, the expression of MIA is specifically restricted to cartilage [4]. In vivo and in vitro studies have shown that MIA expression is closely associated with the degree of chondrocyte differentiation, as it is correlated with expression of Sox9, collagen type II (CII), and aggrecan during the differentiation and de-differentiation of chondrocytes [9–12]. Accordingly, MIA production by chondrocytes is not constitutive, but
is regulated by the same anabolic (e.g. IGF-1) and catabolic (e.g. IL-1β) and TNF signals that control the production of extracellular cartilage matrix [11, 13, 14]. The specific expression of MIA by chondrocytes in anabolic conditions and the diffusion of this secreted small protein to serum make MIA a good candidate biomarker for cartilage homeostasis [11, 15–17]. As in malignant conditions, there is increasing evidence that MIA is not only a biomarker for cartilage metabolism, but also plays a functional role in chondrocyte differentiation. MIA can act as a chemotactic factor for mesenchymal stem cells and, in conjunction with bone morphogenetic protein-2 and transforming growth factor β3, promote chondrogenic differentiation and extracellular matrix deposition, while at the same time inhibiting bone morphogenetic protein-2 and transforming growth for mesenchymal stem cells and, in conjunction with cyte differentiation. MIA can act as a chemotactic factor metabolism, but also plays a functional role in chondro-
dence that MIA is not only a biomarker for cartilage
small protein to serum make MIA a good candidate
anabolic conditions and the diffusion of this secreted
bone morphogenetic protein-2 and transforming growth
factor β3, promote chondrogenic differentiation and extra-
cellular matrix deposition, while at the same time inhibiting
osteogenic differentiation [18, 19]. In vivo, mice lacking
functional MIA protein under physiological conditions
display no major abnormalities but do exhibit subtle ultra-
structural cartilage changes, including defects in collagen
fibres, a disordered arrangement of chondrocytes and an
enhanced calcified cartilage layer of articular cartilage [19,
20]. Under pathological conditions such as OA and fracture
healing, however, MIA deficiency enhances cartilage
repair by increasing the proliferation of mesenchymal
stem cells and delaying differentiation towards chondro-
cytes through an upstream effect on Sox9 [21]. Taken
together, these data indicate that MIA regulates chondro-
cyte differentiation and that delay of this differentiation
improves the regenerative capacity of cartilage.
MIA may modulate joint pathology not only by its direct
functional effect, but also by its immunological properties.
A broad screening analysis of HLA-DR4-restricted cartil-
age epitopes identified molecules such as human cartil-
age glycoprotein-39 (HC gp-39) and MIA as potential
candidate antigens in human RA [22]. The presence of
the antigen and of MHC class II/HC gp-39 complexes in
the inflamed target tissue and the presence of HC gp-39
peptide-specific T cell responses was observed in human
RA [23–26]. The role of HC gp-39 as an autoantigen has
been firmly established in experimental models, where
immunization with HC gp-39 induced a modest arthritis
and intranasal administration of HC gp-39 was able to
induce cross-tolerance in collagen-induced arthritis (CIA)
[27]. A potential role for MIA as an autoantigen is
supported by our demonstration of endogenous HLA-
DR-restricted presentation of MIA by RA synovial fluid
cells [28]. However, it remains unknown whether MIA-
reactive T cells contribute to inflammatory arthritis.
Therefore this study aimed to investigate whether MIA
contributes to inflammatory arthritis in experimental
models and whether its potential effect relates to immuno-
logical or biochemical properties.

Material and methods

Collagen-induced arthritis

MIA-deficient mice, generated as previously described
[20], were backcrossed for eight generations on a
C57BL/6J background and maintained on a C57BL/6J
background. Wild-type (WT) C57BL/6 mice (6–8 weeks
old) were purchased from Charles River Laboratories
(CRL stock #027). Animals were housed up to six per
Cage under specific pathogen-free conditions in accord-
ance with institutional and national guidelines. All experi-
ments were blinded and the mice of different experimental
groups were mixed in cages to avoid cage-dependent
effects. CIA was induced in MIA−/− and WT mice as pre-
inviously described [29]. Briefly, chicken CII (cCII) (Sigma
Chemical, St Louis, MO, USA) was dissolved in 0.1 M
acetic acid to a concentration of 2.0 mg/ml by overnight
rotation at 4 C and mixed with an equal volume of com-
plete Freund’s adjuvant (5 mg/ml of Mycobacterium tuber-
culosiss; Chondrex, Redmond, WA, USA). The mice were
immunized intradermally at the base of the tail with 100 μl
of emulsion (containing 100 μg of collagen and 250 μg
of M. tuberculosis) on day 0; this procedure was repeated
as a booster 21 days later. The severity of arthritis was
assessed using an established semi-quantitative scoring
system of 0–4 per paw as described previously [29].
The cumulative score for all four paws of each mouse
(maximum possible score 16) was used to represent over-
all disease severity and progression: 0 = normal joint,
1 = swelling of one joint (toe/wrist/ankle/footpath),
2 = swelling of more than one joint, 3 = swelling of all joints
and 4 = bursting of the skin/dysfunction or distortion of
the joint. For the evaluation of incidence, mice were
considered to have arthritis if their clinical arthritis
scores increased by at least one point for 2 consecutive
days. All animal experiments were approved by the animal
ethics committee of the Academic Medical Center/
University of Amsterdam, The Netherlands.

Collagen-antibody induced arthritis

Arthritogenic antibody cocktail was obtained from
Chondrex (Redmond, WA, USA) and arthritis was induced
according to the manufacturer’s instructions. Briefly,
MIA−/− and WT mice were injected intraperitoneally
on day 0 with a mixture of four anti-CII mAbs (3 mg
each) and on day 4 with 50 μg of lipopolysaccharide
(Chondrex). The severity of arthritis was assessed by a
single-blinded observer using the same semi-quantitative
scoring system as for CIA.

Measurement of collagen-specific IgG titres

On day 58, after the first immunization with CII, sera were
collected from immunized mice. A total of 5 μg/ml of CII
in PBS was coated onto immunoplates (Nunc) at 4°C
overnight. After blocking with 1% BSA in PBS, serially
diluted (1:2500–1:15000) serum samples were added to
CII-coated wells and incubated for 1 h at room tempera-
ture (RT). After a washing step, plates were incubated with
rat anti-mouse IgG1-HRP and rat anti-mouse IgG2a-HRP
(both from BD Pharmingen) for 1 h at RT. Following a
series of washes with PBS, that plates were developed
with tetramethylbenzidine (TMB) and reactions were
stopped by the addition of stop solution. Absorbance
values were measured using an ELISA reader (Bio-Rad)
at 450/540 nm.
Cell staining and flow cytometry

For flow cytometric analysis, spleens were collected on day 10 after primary immunization. Single-cell suspensions were obtained by crushing spleens through a 40 µm cell strainer (Becton Dickinson). Erythrocytes were lysed with ice-cold isotonic NH4Cl solution (155 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA, pH 7.4). The remaining cells were washed twice with complete Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich) supplemented with 10% FCS, 1% L-glutamate, 1% HEPES [ฟ-(2-hydroxyethyl)-1-piperazineethanesulphonic acid], 0.05% gentamicin and 1% penicillin-streptomycin. Cells were seeded in 96-well round-bottom culture plates at a cell density of 1 x 10^6 cells/well. Cells were stained with fluorochrome-conjugated primary antibodies for 30 min at 4°C in PBS and 0.5% BSA in the presence of anti-CD16-CD32 (clone 2.4G2). The following fluorochrome-labelled mAbs were obtained from eBioscience: anti-B220 (RA3-6B2), anti-CD3 (17A2), anti-CD4 (GK1.5), anti-CD8 (53-6.7), anti-CD62L (MEL-14), anti-CD44 (IM7), anti-CD25 (PC61.5) and anti-FoxP3 (FJK-16s). Intracellular staining for Foxp3 was performed using Foxp3 fixation/permeabilization concentrate and diluent (eBioscience) according to the manufacturer’s protocol. Data were collected on an FACS Canto (BD Biosciences) and were analysed with FlowJo (Tree Star) and GraphPad software (GraphPad Software, La Jolla, CA, USA).

Splenocyte stimulation, cytokine production and T cell proliferation assay

Splenocytes were plated at a concentration of 5 x 10^6 cells/ml in a 96-well round-bottom plate and stimulated with plate-bound anti-CD3 (10 µg/ml) and soluble anti-CD28 (2 µg/ml) (both provided by Dr L. Boon, Bioceros BV, Utrecht, The Netherlands), cCII (100 µg/ml) or recombinant MIA (100 µg/ml) for 3 days in complete RPMI medium. Recombinant MIA was obtained as described previously [28]. Briefly, full-length complementary DNA sequences were cloned onto the expression vector pNGV-1. The resulting constructs were subsequently stably transfected into Chinese hamster ovary (CHO) K1 cells, from which transfectants that produced MIA at levels of 50 µg/l under serum-free culture conditions were selected and further purified to near homogeneity by capture on a cation-exchange SP Sepharose column. For the detection of intracellular cytokines, Brefeldin A (5 µg/ml; Sigma-Aldrich) was added for the last 4 h of stimulation and cells were harvested and stained with CD4 and CD8 antibodies. Cells were then fixed and permeabilized using Cytofix/Cytoperm (BD Biosciences) and labelled with IL-17A (eBioTC11-18H10.1) and INF-γ (XMG1.2) (eBioscience) antibodies, thoroughly washed, and analysed by flow cytometry. To access proliferative capacity, cells were labelled with carboxyfluorescein succinimidyl ester (CFSE, 1 µg/ml; Molecular Probes, Eugene, OR, USA) and subsequently stimulated for 72 h in the absence or presence of anti-CD3/CD28 antibodies, cCII or MIA proteins. Cells were then analysed for CFSE dilution and precursor frequencies were determined by flow cytometry.

Histology

Hind paws were fixed in 10% buffered formalin for 24 h, decalcified in 15% EDTA and then embedded in paraffin. Serial sagittal 5 µm sections of whole hind paws were cut and stained with haematoxylin and eosin (H&E). Three independent observers (N.Y., D.B., M.V.) assessed the tissue for the degree of synovitis and cartilage degradation by microscopic evaluation under blinded conditions, as described previously [30, 31]. Synovitis and cartilage degradation in the knee joints were graded on a scale of 0 (no inflammation) to 3 (severely inflamed joint) based on the extent of infiltration by inflammatory cells into the synovium.

Binding of anti-cCII antibodies to cartilage

Cartilage of WT and MIA⁻/⁻ mice [hip joint; three mice (six pieces of cartilage) each] was isolated and each piece was incubated with a mixture of four anti-cCII mAbs, as used for the collagen antibody induced arthritis (CAIA) model, in PBS buffer in one well of a 96-well plate for 4 h at RT. After washing three times with PBS, binding of anti-CII antibodies to cartilage was quantified using a secondary peroxidase from horseradish-labelled antibody followed by incubation with TMB substrate and stopping with 2 M H2SO4. Binding of the antibody was evaluated by measurement of absorbance at 450 nm.

Statistical analysis

Statistical significance was determined using Mann–Whitney U test. P-values <0.05 were considered significant (GraphPad Prism version 4.03; GraphPad Software). The difference in clinical scores between groups was calculated as the area under the curve (AUC) (SPSS version 12.0.2; SPSS, Chicago, IL, USA). Incidence was compared using Kaplan–Meier survival analysis (GraphPad Prism).

Results

Reduced incidence and severity of CIA in MIA-deficient mice

To investigate the potential role of MIA in chronic inflammatory arthritis, we induced CIA in MIA⁻/⁻ (n = 24) and WT mice (n = 33) in three independent experiments. The clinical arthritis score was significantly reduced in MIA⁻/⁻ mice compared with WT controls from day 24 onwards until the end of the experiment at day 58 (P < 0.05 at all time points) (Fig. 1A). Accordingly, analysis of the AUC, which integrates disease activity during the whole experiment, indicated a significant protection of MIA⁻/⁻ mice vs WT controls (P = 0.0005) (Fig. 1B). More detailed analysis revealed that this difference was mainly due to a reduced incidence of arthritis in the MIA⁻/⁻ mice (reaching 50.0%) compared with the controls (reaching 84.8%) (P = 0.003) (Fig. 1C). A subanalysis of the animals that did develop arthritis in both groups (n = 11 in MIA⁻/⁻ and n = 28 in WT)
showed that clinical disease severity was modestly reduced, reaching significance from day 47 onwards (Fig. 1D). In addition, analysis of synovial inflammation was assessed by H&E staining of knee joints (Fig. 2A and B). Histological scoring showed comparable inflammation and cartilage degradation in arthritic MIA$^{-/-}$ mice and WT (Fig. 2C and D). Taken together, these data indicate that MIA deficiency reduced the incidence and, to a lesser degree, severity of CIA.

Reduced anti-collagen antibody responses in MIA-deficient mice

As anti-collagen antibodies play a pathogenic role in CIA, we first assessed whether the reduced disease incidence and severity observed in MIA-deficient mice were related to an alteration of the humoral autoimmune response. We determined production of collagen-specific IgG2a, which is typically produced during a Th1 response, and collagen-specific IgG1, which is typically produced during a Th2 response. Analysis of serum obtained 58 days after induction of CIA showed that both Th1-related IgG2a [27.55 μg/ml (s.d. 22.31) vs 57.87 (s.d. 29.18), $P = 0.0003$] and Th2-related IgG1 [2.43 μg/ml (s.d. 2.75) vs 7.87 (s.d. 5.55), $P = 0.0005$] (Fig. 3A) were significantly decreased in MIA$^{-/-}$ mice compared with WT animals. However, the relative ratio of Th1- vs Th2-type antibodies (IgG1/IgG2a) was not significantly different between these experimental groups (data not shown). To confirm this finding, we tested MIA$^{-/-}$ ($n = 14$) and WT ($n = 12$) animals in the CAIA model. In this model, transient arthritis is induced by the transfer of pathogenic anti-CII antibodies, thereby bypassing the need to mount an active humoral autoimmune response. As previously described, mice developed the first signs of disease 4 days after initial administration of anti-CII antibodies and arthritis reached its maximum at about days 7–8 (Fig. 3B and C). The clinical arthritis score was significantly lower in MIA$^{-/-}$ mice compared with WT at days 8 and 10 ($P < 0.05$) (Fig. 3B and D) due to a reduced incidence of arthritis in the MIA$^{-/-}$ mice (14.3%) compared with the controls (66.7%). The clinical arthritis score value among only arthritic mice was similar between groups (Fig. 3C). This partial protection of MIA$^{-/-}$ mice from CAIA indicates that the difference in clinical disease cannot be explained solely by quantitative changes in the anti-collagen antibody
response. Since MIA deficiency affects the cartilage microstructure, we also investigated whether the binding of the anti-CII antibodies to cartilage was affected in MIA\textsuperscript{−/−} animals. The cartilage binding assay revealed no differences in binding of the anti-CII antibodies comparing WT [0.59 (S.D. 0.08), \(n=3\)] with MIA\textsuperscript{−/−} [0.52 (S.D. 0.05), \(n=3\)] mice. Taken together, these data suggest that MIA might have a qualitative impact on the immune cells, such as CII-reactive T cells, in enhancing arthritis development at the effector level of arthritis pathogenesis.

MIA-induced T cell proliferation in CIA

Next we investigated whether MIA affects T cell responses, as the presentation of MIA epitopes in the context of HLA-DR4 in human RA suggests the existence of autoreactive CD4\textsuperscript{+} T cells directed against this cartilage antigen. To assess MIA-specific T cell responses, we recovered spleens at day 10 after primary immunization with CII and restimulated them in vitro using anti-CD3/anti-CD28 antibodies as a positive control. Assessment of proliferation by CFSE dilution showed that T cells from MIA\textsuperscript{−/−} and WT mice proliferated equally upon stimulation with anti-CD3/anti-CD28, suggesting that the global proliferative capacity of T cells is not affected by MIA deficiency (Fig. 4A). Restimulation with CII showed no difference in splenic T cell proliferation between WT and MIA\textsuperscript{−/−} animals (Fig. 4A). In contrast, in vitro restimulation with MIA induced a clear proliferative response in CD4\textsuperscript{+} T cells from WT but not from MIA\textsuperscript{−/−} mice immunized with collagen (\(P=0.007\)) (Fig. 4A). Qualitative analysis of the T cell cytokine production showed that anti-CD3/anti-CD28 stimulation induced a clear IFN-\(\gamma\) and IL-17 response in both WT and MIA\textsuperscript{−/−} animals (Fig. 4B and C). In contrast, antigen-specific stimulation with MIA induced an IFN-\(\gamma\) response (Fig. 4B) but no IL-17 response (Fig. 4C). Taken together, these data indicate the existence of IFN-\(\gamma\)-producing T cells reacting to autologous MIA in CIA.

Decreased anti-CII Th1 response in MIA\textsuperscript{−/−} mice

To understand how this T cell autoreactivity towards MIA may influence CIA, we next assessed the anti-CII T cell responses during CIA in WT vs MIA\textsuperscript{−/−} mice. Analysis of cytokine production showed that CII restimulation induced IFN-\(\gamma\) production in WT animals but not in MIA\textsuperscript{−/−} mice (Fig. 4B). No differences were observed in IL-17 production (Fig. 4C). These data demonstrate that primed in vivo splenocytes of MIA\textsuperscript{−/−} mice did not react...
to stimulation with CII by production of Th1-associated cytokine IFN-γ, indicating a significant impact of MIA deficiency on the collagen-induced Th1 response.

Increase of FoxP3+CD25+CD4+ T cells in MIA−/− mice

Since both T cell proliferation to MIA and CII-induced IFN-γ production were suppressed in MIA−/− mice, we investigated whether this was associated with an expansion of the Treg population in these animals. Under homeostatic conditions we observed no difference in the percentages of total CD4+ T cells [57.17% (S.D. 4.54) in MIA−/− vs 51.13% (S.D. 5.77) in WT] and FoxP3+CD25+CD4+ regulatory T cells [4.50% (S.D. 0.67) in MIA−/− vs 4.47% (S.D. 0.32) in WT] in spleens of MIA−/− vs WT mice. In sharp contrast, analysis of the lymphocyte populations 10 days after primary immunization with CII revealed a small but significant increase in total CD4+ T cells in MIA−/− mice vs WT controls (P=0.004) (Fig. 5A). More strikingly, however, was an almost 50% increase in FoxP3+CD25+CD4+ T cells in MIA−/− mice vs WT controls (P=0.004) (Fig. 5B). These data suggest that protection from arthritis in MIA−/− mice is associated with an expansion of the Treg pool upon arthritis induction.

Discussion

Several lines of experimental and clinical evidence indicate that RA is an autoimmune disease driven by T and/or B lymphocyte reactivity to self-antigens. However, one of the major challenges to define the precise role of autoreactive T cells in the disease pathogenesis remains the identification of relevant autoantigens. Key criteria for the identification of putative autoantigens are a demonstration of T cell reactivity to the specific antigen in human RA and experimental evidence that T cells directed towards this antigen contribute to pathology in arthritis models. The latter can be achieved by using T cell receptor (TCR) transgenic animals and/or MHC-antigen polymers to trace autoreactive T cells in vivo [32, 33]. In the absence of such tools for a specific candidate autoantigen, an alternative strategy is to assess whether overexpression or deletion of the antigen modulates experimental arthritis. In the present study we used this strategy to assess whether...
T cell reactivity against MIA has a functional impact on experimental arthritis. The reason for choosing MIA as a potential antigen was based on several important findings. First of all, MIA is primarily expressed by chondrocytes in the human joint [4]. Moreover, it has been shown that levels of MIA are elevated in the synovial tissue and fluid in patients with rheumatic diseases [15]. Most importantly, MIA is the only cartilage antigen besides HC gp-39 for which it has been formally demonstrated that its immunodominant epitopes are actively presented in an HLA-DR-restricted fashion in the inflamed RA joint [28].

In the present study we investigated CIA, an animal model of RA, and demonstrated that the incidence and severity of disease is decreased in MIA-deficient mice compared with WT controls, thus indicating that endogenous MIA contributes to the pathogenesis of CIA. MIA deficiency could have a dual role in this model, since MIA may not only function as an autoantigen, but can also directly impact cartilage homeostasis [10, 21]. Three lines of evidence support a direct role for T cell reactivity to MIA in our model. First, ex vivo proliferation of T cells towards MIA in WT but not MIA-deficient mice after induction of CIA indicated that MIA-specific T cells are indeed present during arthritis development. Second, proliferation to CII was similar in WT and MIA-deficient mice, but the production of IFN-γ was clearly impaired in the latter. Whereas it remains unknown how MIA deficiency modulates the cytokine profile of CII-specific T cells, it is well established that this Th1 response contributes to CIA. The impact of MIA on the Th1 response is also strongly supported by significantly lower levels in MIA-deficient mice of anti-CII IgG2a associated with these responses. It is also interesting to note that another HLA-DR4-restricted cartilage autoantigen, HC gp-39, was previously shown to cross-regulate anti-CII responses and thereby modulate CIA [27]. Third, we found that MIA-specific T cell proliferation and CII-induced INF-γ production in WT vs MIA-deficient mice were associated with a relative decrease in the splenic CD4+CD25+ Treg population in WT mice. As the role of Treg cells in the suppression of CIA is well established [34, 35], this could contribute to the observed clinical differences. Whether this alteration in the Treg frequency directly impacts upon the Th1 response to CII remains to be determined.

We attempted to substantiate the role of these MIA-specific T cells in arthritis by active immunization of mice with MIA but did not detect any signs of arthritis in these experiments (data not shown). This may indicate that although MIA contributes to arthritis, exposure to this putative autoantigen is not sufficient to trigger clinical disease, or that repeated immunization, as reported in proteoglycan-induced arthritis [36], rather than two immunizations (day 0 and day 21) would be required. Additionally, we were not able to study mechanistically how MIA exactly regulates anti-CII T cells and Tregs, as our analyses are limited by the absence of specific tools (including MHC-peptide tetramers) to track and sort the antigen-specific cell populations in vivo.
Despite these limitations, a role for MIA as a T cell autoantigen was further supported by the fact that we did not find evidence for a direct biochemical effect of MIA deficiency in this model. Indeed, cartilage appeared histologically similar in both groups and binding of anti-CII antibodies to the cartilage was not affected.

Conclusion

Taken together, the data of the present study support the concept that several cartilage proteins can act as T cell autoantigens during arthritis. These proteins include CII, proteoglycans, HC gp-39 and MIA. Even if not all these autoantigens are potent enough to individually induce arthritis, they can be useful tools to modulate T cell responses and thereby dampen arthritis in experimental models [37]. Whether these autoantigens can also be used to regulate the balance of pathogenic vs regulatory responses in human RA remains to be established.

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Clinical vignette

Clinical images: PET-CT and contrast-enhanced ultrasound in Takayasu’s arteritis

A 31-year-old woman was diagnosed with Takayasu’s arteritis (TA) in 2007 and treated sequentially with different immunosuppressant agents including mycophenolate, infliximab, adalimumab and tocilizumab for refractory disease. When we saw her, she had systemic symptoms and increased inflammatory markers. At that time, no immunosuppressant therapy was ongoing and physical examination showed bruits in the supraaortic vessels. Vascular imaging, contrast enhanced ultrasound (CE-US) and PET-CT scan were performed to assess disease activity (Fig. 1). The right panel (D) shows increased 18F-fluorodeoxyglucose vascular uptake on PET-CT, especially in the carotid district bilaterally. The ultrasound grey-scale image on the left side shows marked intima media complex thickness (*) of the left common carotid artery (A). After contrast injection, a progressive enhancement represented by diffuse bright spots can be observed within the vessel wall (*B: basal image; *C: enhancement of the artery wall). This observation was consistent with the PET-CT findings and with the clinical features. The degree of enhancement of the parietal wall reflects vasa vasorum contrast uptake, a surrogate marker of vascular inflammation. CE-US may represent a useful tool in the diagnosis and follow-up of TA, providing real-time information about ongoing vascular inflammation.

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Fig. 1 PET-CT and CE-US in TA.

(A) Left common carotid artery; (*) intima media complex thickness; (*B) basal image; (*C) enhancement of artery wall; (D) 18F-fluorodeoxyglucose vascular uptake on PET-CT.