IgA antibodies against CD74 are associated with structural damage in the axial skeleton in patients with axial spondyloarthritis

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Abstract

Objective

To study the association between the presence of antibodies against CD74 and structural damage in the sacroiliac joints and spine in patients with axial spondyloarthritis (axSpA).

Methods

Antibodies against CD74 were measured in the sera of patients with axSpA from 2 cohorts: 1. An observational cohort from Damp in Northern Germany and 2. from a clinical trial (ENRADAS), in which the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) had been evaluated by two readers blinded to the time point at baseline and two years later. The presence of antibodies against CD74 was correlated with the presence and grade of radiographic sacroiliitis in the observational cohort, and with baseline mSASSS in the ENRADAS cohort.

Results

The sensitivity of IgA anti-CD74 antibodies for axSpA was 50% in the Damp cohort and 42% in ENRADAS. The presence of IgA antibodies against CD74 was associated with a higher grade of sacroiliitis (observational cohort) and a higher baseline mSASSS in the ENRADAS cohort.

Conclusion

IgA antibodies against CD74 are not only markers of AS, but are associated with structural damage development in the sacroiliac joints and in the spine.

Key words

axial spondyloarthritis, CD74 antibodies

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Competing interests: N. Baerlecken owns the patent application for Anti-CD74; M. Rudwaleit has received honoraria/ consultation fees from Abbvie, BMS, Celgene, Chugai, Janssen, Lilly, MSD, Novartis, Pfizer and UCB; the other co-authors have declared no competing interests.

Introduction

The pathogenesis of axial spondyloarthritis (axSpA) and the mechanism of disease progression leading to new bone formation and ankylosis of the spine is not entirely understood. We have recently discovered antibodies against CD74, which are associated with axial spondyloarthritis (1). CD74 is the invariant chain of the MHC class II complex and regulates the peptide loading of the molecule, but can also be expressed on the cell surface of all cells expressing MHC class II, including monocytes, dendritic cells and osteoblasts (2). The cell surface expressed CD74 is one of the receptors of macrophage migration inhibitory factor (MIF) (3). MIF has been shown to be upregulated in the sera of patients with axial spondyloarthritis (4) and to stimulate osteoblasts *in vitro* (5). Provided that antibodies against CD74 mimic MIF effects, they may also activate osteoblasts, therefore be involved in the pathogenesis of axSpA and be associated with a faster radiological progression.

We therefore measured antibodies against CD74 in the sera of patients with axSpA and investigated their association with 1. presence and grade of radiographic sacroiliitis in the observational cohort, and 2. with the baseline mSASSS in the ENRADAS cohort (6).

Patients and methods

Patients

• Observational cohort

An observational discovery cohort was established in Damp, Germany. 117 consecutive axSpA patients visiting the outpatients' clinic for routine examinations were recruited after informed consent in a study approved by the local ethics committee (number 6330). The diagnosis of axial spondyloarthritis had been made at least 10 years before the entry into our study and all the patients fulfilled the ASAS criteria (7). Information on the following parameters were obtained in the study visit:

Age, gender, disease duration since onset of inflammatory back pain, uveitis, psoriasis, inflammatory bowel disease, peripheral arthritis, current and past therapy, the Bath Ankylosing Spondylitis Disease Activity Score (BASDAI), tragus wall distance, thoracic excursion and lumbar flexion, HLA-B27 and C-reactive protein (CRP). The details, segregated by the presence or absence of IgA antibodies against CD74, are shown in Table I. The most recent x-ray of the sacroiliac joints was graded by the local radiologist according to the modified New York criteria for ankylosing spondylitis (AS) (8). According to that definition a sacroiliitis grade 0 is characterised by a normal x-ray picture, grade I by some blurring of the joint margins, grade II by minimal sclerosis with some erosion, grade III by definite sclerosis on both sides of joint and/or severe erosions with widening of joint space with or without ankyloses and grade IV by complete ankyloses. Based on that grading, 96 of the 117 axSpA patients had radiographic axSpA (AS) and 21 non-radiographic axSpA. The highest grade of the two sacroiliac joints of each patient was used for the correlation with antibodies against CD74.

• ENRADAS

A total of 118 out of 122 patients who completed the ENRADAS study of whom sera were still available were included. The sera had been stored in a -80°C freezer without previous thawing. The ENRADAS cohort has been described in detail earlier (6). In short, patients with radiographic axSpA (AS) had been recruited and were treated with NSAIDs, either in a maximum dosage or on demand. Spinal radiographs (baseline and year 2) were obtained and assessed for modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) (9) by two trained readers blinded for the time-point and for all clinical data. In the mSASSS only the anterior edges of both the lumbar and cervical spine are evaluated on a lateral x-ray view. The following definitions are used: 0 = no abnormality; 1 = erosion and/or sclerosis and/or squaring; 2 = syndesmophyte (non-bridging); 3 = total bony bridging between upper and lower vertebral edges (ankylosis). Since 24 vertebral edges are included in the lumbar and cervical scoring system, the total mSASSS can range from 0–72).

There was no outcome difference between the two treatment arms. There-

Table I. Baseline data of the observational and the ENRADAS cohort.

Parameter	Observational cohort (n=117)		ENRADAS (n=118)	
	with CD74 antibodies (n=59)	without CD74 antibodies (n=58)	with CD74 antibodies (n=50)	without CD74 antibodies (n=68)
Age, mean ± SD years	47.5 ± 12.6	44.3 ± 13.6	45.0 ± 10.3	40.8 ± 9.6
Symptom duration, mean ± SD years	25.2 ± 11.2	23.3 ± 15.1	15.6 ± 13.9	13.8 ± 10.1
Male sex (%)	81.4	77.6	74.0	67.6
HLA–B27 positive (%)	91.5	94.8	78.0**	97.1**
Peripheral arthritis ever (%)	46.6	38.6	22.0	30.9
Uveitis ever (%)	32.8	24.6	20.0	14.7
Psoriasis ever (%)	8.6	12.3	12.0	7.4
Inflammatory bowel disease ever (%)	8.6	8.8	0	0
Smoking ever (%)	50.0	50.9	48.0	47.1
CRP in mg/l (at time of antibody measurement), mean ± SD, 0–10	5.7 ± 7.9	7.0 ± 14.7	14.7 ± 15.7***	7.3 ± 7.9***
BASDAI (at time of antibody measurement), mean ± SD, 0–10	3.0 ± 2.2	2.6 ± 2.0	4.3 ± 1.4	4.1 ± 1.6
ASDAS (at time of antibody measurement), mean ± SD	Not done	Not done	3.0 ± 3.7	2.6 ± 0.9
BASFI, mean ± SD, 0–10	Not done	Not done	3.4 ± 2.0	3.3 ± 2.3
Treatment with a TNFα blocker	91.5***	65.5***	0	0
Sacroiliitis score	3.0 ± 1.2**	2.4 ± 1.1**	Not done	Not done
Modified SASSS, mean ± SD	Not done	Not done	17.6 ± 18.9**	10.8 ± 15.4**
Syndesmophytes	Not done	Not done	5.0 ± 6.0	3. 6 ± 5.7
Tragus wall distance	15.8 ± 7.9*	12.8 ± 6.9*	15.4 ± 5.1	14.2 ± 4.6
Thoracic excursion	3.1 ± 1.8	3.7 ± 1.6	3.6 ± 1.9	3.9 ± 2.1
Lumbar flexion	12.4 ± 1.3*	13.5 ± 1.7*	13.9 ± 1.7	13.8 ± 1.7

Differences between patients with and without antibodies against CD74 that significant in univariate analysis are marked with *($p<0.05$), **($p<0.01$), or ***($p<0.001$).
BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; BASFI: Bath Ankylosing Spondylitis Functional Index; TNFα: tumour necrosis factor α; SASSS: Stoke Ankylosing Spondylitis Spine Score.

fore, we did not separate the two groups in our current study. Information on HLA-B27 status, baseline CRP and smoking status of the patients enrolled was included in the analysis as well as further parameters shown in Table I.

Blood donors

Forty-one blood donors recruited in the blood bank of the Medical University Hannover served as controls for the ELISAs measuring CD74 antibodies in order to confirm the previously established cut-off. For data protection regulations, we are only informed about the age (which was 30.5 ±8.1 years) and gender (61% male) of the blood donors, but do not get any additional information. In general, potential blood donors are rejected from the blood donation in case they took any medication within the last 7 days.

ELISA

IgA antibodies against CD74 were measured using the SpADetect ELISA of Aesku.Diagnostics (Wendelsheim, Germany) according to the manufacturer's instructions. On the ELISA plate, complete human CD74 recombinantly produced in HEK293 cells was coated as an antigen. The measurements were performed blinded to the evaluations of the radiographs. The cut-off of the ELISA was 25 U/ml.

Statistical analysis

The primary endpoint of the study was to show an association of IgA antibodies against CD74 with baseline structural damage in axSpA. Therefore, the patients in both cohorts were segregated into positive or negative for IgA antibodies against CD74, dependent on whether the ELISA test results were higher or lower than the upper normal reference value. Then, the association of CD74 antibodies with the baseline sacroiliitis grade (observational cohort) and the baseline mSASSS (ENRADAS) were calculated using the Mann-Whitney t-test using GraphPad prism v. 6.01. Since structural progression of axSpA has previously been found to be associated with CRP, smoking, male gender and with the presence of HLA-B27, the association of these parameters with antibodies against CD74 (divided into positive or negative) was calculated using Fisher's exact test for dichotomous and Mann Whitney U-test for continuous variables. When the CRP values in ENRADAS (but not in the observational cohort) were found to be correlated with both antibodies against CD74 and with the baseline mSASSS, multivariate logistic regression analysis was performed to investigate whether CRP and/or anti-CD74 were independently associated with baseline mSASSS.

In addition, the distribution of the parameters in patients with and without antibodies against CD74 were compared using Fisher's exact test (for the parameters, arthritis, uveitis, psoriasis, inflammatory bowel disease, therapy with TNF inhibitors) or Mann Whitney test (for the parameters age, disease duration, BASDAI, tragus wall distance, thoracic excursion and lumbar flexion).

Finally, in the patients from the ENRADAS cohort the association of the mSASSS increase after 2 years of follow-up was compared between the patients with and without antibodies against CD74 using Mann Whitney U-test.

Results

In the observational cohort, 94/117 patients were male, 108 (92.3%) were

HLA-B27 positive. The average age was 46.3 years (range 24–80 years) and the average disease duration 24.6 (range 1–65 years) years (Table I).

The sensitivity of IgA anti-CD74 antibodies for axSpA was 59/117 (50%) in the observational cohort and 50/118 (42%) in ENRADAS, and the specificity was 95 % (2/41 in blood donors) (Fig. 1).

In the observational cohort, the mean sacroiliitis grade was significantly higher in the patients with compared to those without IgA antibodies against CD74 (p=0.01) (Fig. 2). In addition, the presence of IgA anti-CD74 antibodies significantly correlated with the presence of grade 4 sacroiliitis (52.9% (IgA positive) *vs*. 25% (IgA negative), (p=0.004, OR 3.17 (95% CI 1.36–7.47) (Fig. 2).

In ENRADAS, the presence of IgA antibodies against CD74 was associated with a higher mSASSS at baseline, both when calculated with Mann Whitney test (Table I) as well as with two-sided Spearman rank correlation (p=0.0059) (Fig. 3). Multivariate analysis including IgA antibodies against CD74 and CRP revealed, that only IgA antibodies against CD74 correlated with the mSASSS (p<0.001).

There was an association of IgA antibodies against CD74 with a higher tragus wall distance and with a lower lumbar flexion in the observational cohort, but not in ENRADAS (Table I).

Information on the current and previous treatment was available in all of the patients. In the observational cohort, patients with IgA antibodies against CD74 were significantly more frequently treated with a TNF inhibitor (54/59 = 91.5%) than patients without IgA antibodies against CD74 (38/58 = 65.5%) (p=0.0006) (Table I). Therapy with TNF inhibitors was not allowed in ENRADAS. In both cohorts, patients with and without IgA antibodies against CD74 did not differ with regard to gender, age, duration of disease and BASDAI.

In the ENRADAS cohort, significantly more of the HLA-B27negative patients (11/13) compared to HLA-B27positive patients (39/104) had IgA antibodies against CD74 (p=0.002) (Table I).

Finally, in the ENRADAS cohort IgA antibodies against CD74 were associated with a higher mSASSS progression in the subsequent 2 years (1.49±2.81 (IgAp ositive) *vs*. 0.69±1.85 (IgA negative) (p=0.046) (Fig. 4).

Discussion

The current studies reveal, that IgA antibodies against CD74 are associated with the baseline structural damage in axSpA as well as with its progression in the next two years.

CD74 is the receptor of macrophage migration inhibitory factor (MIF), which is upregulated in axial spondyloarthritis. MIF has multiple *in vivo* effects on bone metabolism. Both overexpression of MIF as well as a knockout of CD74 in mice induce high-turnover osteoporosis and enhanced osteoclastogenesis (5). On the other hand, MIF stimulates the proliferation of osteoblasts (10) and elevated MIF levels are associated with the subsequent structural progression of axSpA (11). Antibodies against CD74 may be mechanistically involved in structural progression of axSpA in two ways: (1) They may mimic the effect of MIF on osteoblasts and on immune cells by binding to the same receptor (CD74), and (2) they may bind and block soluble CD74, which has been shown to bind and neutralise MIF in the serum (12).

In the observational cohort, the percentage of patients receiving TNF inhibitors was significantly higher in the patients with compared to patients without IgA antibodies against CD74. This suggests, that in the view of the rheumatologists, the subset of patients with the autoantibodies is more severely affected from axSpA. A higher disease activity may also contribute to the faster structural progression.

In the ENRADAS cohort, lack of HLA-B27$^-$ was associated with the presence of IgA antibodies against CD74. This was not observed in the observational cohort, in which however only 8% of the participants lacked HLA-B27, and in the recent InterSpA study on patients with axial spondyloarthritis and a disease duration of less than two years (13). The data from the ENRADAS cohort suggests, that the progression of

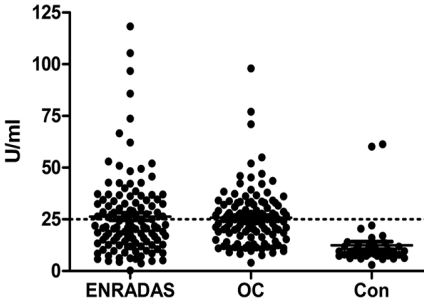

Fig. 1. Distribution of IgA antibodies against CD74 in the patients from ENRADAS, the observational cohort (OC) and from 41 blood donors.

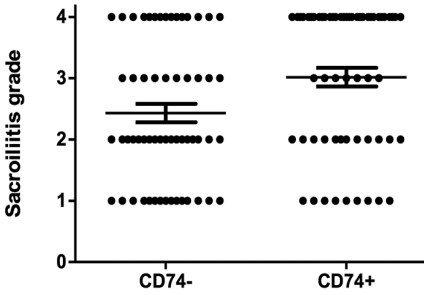

Fig. 2. Comparison of the highest sacroiliitis grade in patients with (CD74+) or without (CD74-) IgA antibodies against CD74 (observational cohort).

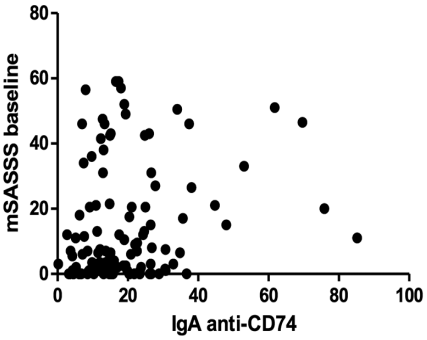

Fig. 3. Correlation of the concentration of IgA antibodies against CD74 with the mSASSS at baseline (ENRADAS cohort).

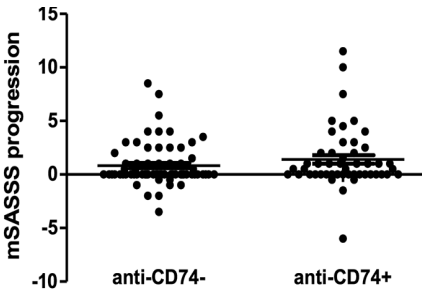

Fig. 4. Progression of the mSASSS in 2 years in patients with (IgACD74pos) or without (IgACD74neg) IgA antibodies against CD74 (ENRADAS cohort).

axial spondyloarthritis to AS is driven by the presence of either HLA-B27 or IgA antibodies against CD74.

In conclusion, the current study shows, that IgA antibodies against CD74 are an interesting biomarker to predict a faster radiographic progression of axSpA, may be used to stratify patients for therapeutic studies and possibly be relevant even for therapeutic decisions. We therefore now measure antibodies against CD74 in all the new patients in which we consider axial spondyloarthritis.

Acknowledgments

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