Nuclear Factor-κB–inducing Kinase Is Expressed in Synovial Endothelial Cells in Patients with Early Arthritis and Correlates with Markers of Inflammation: A Prospective Cohort Study

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ABSTRACT. ***Objective.*** The nuclear factor-κB (NF-κB) family of transcription factors is strongly involved in synovial inflammation. We have previously shown that NF-κB–inducing kinase (NIK) is a key regulator of inflammation-induced angiogenesis in rheumatoid arthritis (RA) synovial tissue (ST). Here, we investigated synovial NIK expression in patients with early arthritis and in autoantibody-positive individuals at risk of developing RA.

Methods. ST biopsies were obtained by arthroscopy from 154 patients with early arthritis (duration < 1 yr) with various diagnoses and 54 IgM rheumatoid factor–positive and/or anticitrullinated protein antibodies–positive individuals without evidence of arthritis. ST was stained for NIK and endothelial cell (EC) markers. Additionally, measures of disease activity were collected and contrast-enhanced magnetic resonance imaging (MRI) was performed in a subset of these patients.

Results. In patients with early arthritis, NIK was predominantly expressed in EC of small blood vessels. Further, NIK expression correlated with erythrocyte sedimentation rate (r 0.184, p = 0.024), C-reactive protein (r 0.194, p = 0.017), joint swelling (r 0.297, p < 0.001), synovial immune cell markers (lining r 0.585, p < 0.001; sublining macrophages r 0.728, p < 0.001; T cells r 0.733, p < 0.001; and B cells r 0.264, p = 0.040), MRI effusion (r 0.665, p < 0.001), MRI synovitis (r 0.632, p < 0.001), and MRI total score (r 0.569, p < 0.001). In 18.5% of autoantibody-positive individuals, ST NIK^+EC were present, but this was not predictive of the development of arthritis.

Conclusion. NIK^+EC are present in the earliest phase of synovial inflammation and may be indicative of high angiogenic activity in the inflamed ST. Therefore, NIK^+EC may play an important role in the persistence of synovitis. Collectively, our data underscore the importance of angiogenesis in synovial inflammation and identify NIK as a potential therapeutic target in arthritis. (J Rheumatol First Release July 15 2015; doi:10.3899/jrheum.150245)

Key Indexing Terms:
NUCLEAR FACTOR-κB NF-κB–INDUCING KINASE RHEUMATOID ARTHRITIS
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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial inflammation that may lead to joint destruction. In RA synovial tissue (ST), angiogenesis can already be observed in the earliest phase of disease. Angiogenesis is defined as the formation of new blood vessels from the preexisting vasculature[1]. The number of blood vessels is already significantly increased in patients with early disease, and the vasculature is clearly activated as shown by an increased expression of adhesion molecules[2,3]. Also, dynamic contrast-enhanced magnetic resonance imaging (MRI) in patients with early arthritis demonstrates increased vascularity and suggests that angiogenesis plays a key role in the pathogenesis of RA[4]. Interestingly, anti-tumor necrosis factor (TNF) therapy and other antirheumatic therapies result in the deactivation of vascular endothelium, providing indirect experimental evidence that targeting vascular endothelium may lead to decreased cell trafficking toward the synovial compartment[5,6]. This notion is supported by experimental studies in animal models: angiogenesis is involved in the switch from acute to chronic synovial inflammation and specific targeting of neovasculature results in reduced synovial inflammation[7,8]. Angiogenesis is considered an important factor in the pathogenesis of RA and may be a good target[1,9].

The nuclear factor-κB (NF-κB) family of transcription factors is crucially important in the development and perpetuation of (synovial) inflammation[10,11]. NF-κB can be activated by 2 signal transduction pathways that have distinct roles[12]. The canonical NF-κB pathway is activated in response to proinflammatory stimuli. In this pathway, inhibitor of κB kinase (IKK) β is essential for NF-κB activation, whereas IKKα is dispensable. In contrast, the noncanonical pathway is strictly dependent on NF-κB–inducing kinase (NIK) and IKKα homodimers, and can be activated through the triggering of TNF-receptor superfamily members, such as the lymphotoxin β receptor (LTβR), B cell activating factor belonging to the TNF family receptor, and CD40. Activation of the noncanonical pathway results in the stabilization of NIK, the most important activating kinase of this pathway that results in the activation of IKKα, followed by nuclear translocation of mainly p52-RelB dimers that target specific genes. In RA ST, the noncanonical NF-κB stimuli LTβ and LIGHT (homologous to Lymphotoxins, exhibits Inducible expression, and competes with HSV Glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes), both ligands of the LTβR and CD40L, are widely expressed, mainly by B cells and T cells[13,14,15]. We have recently demonstrated that noncanonical NF-κB signaling in endothelial cells (EC) regulates pathological angiogenesis in (pre)clinical models of arthritis, independent of vascular endothelial growth factor (VEGF)[16].

Currently, little is known about the contribution of noncanonical NF-κB signaling to the onset and perpetuation of RA. The function of NIK and downstream noncanonical NF-κB signaling is to a large extent cell-type–specific: in synovial fibroblasts, osteoclasts, EC, and B cells, noncanonical NF-κB signaling contributes to the inflammatory process, whereas in macrophages, dendritic cells, and T cells, this pathway probably has a regulatory role[17]. Detailed knowledge of the role of noncanonical NF-κB signaling in angiogenesis and its contribution to chronic inflammation may provide more insight into the pathogenesis of RA and could lead to the development of novel-targeted therapeutic interventions. Therefore, we investigated synovial NIK$^+$EC in the earliest phases of RA compared with other forms of arthritis in a prospective cohort of disease-modifying antirheumatic drug (DMARD)–naive patients with early arthritis, as well as in a cohort of autoantibody-positive individuals at risk of developing RA.

MATERIALS AND METHODS

Study subjects. First, 154 patients with early arthritis who were included in the prospective early arthritis cohort "Synoviomics" at the Academic Medical Center (AMC)/University of Amsterdam, the Netherlands, were enrolled[18]. At inclusion, all patients had < 1 year of disease duration, as measured from the first clinical evidence of joint swelling. All patients had an active arthritis of at least a wrist, ankle, or knee joint and were DMARD-naive. These patients are collectively referred to as "patients with early arthritis." The second group consisted of 54 individuals from the prospective observational cohort "PreSynoviomics" at the AMC. This cohort included individuals with either arthralgia and/or a positive family history for RA, but without (a history of) arthritis (as determined by an experienced rheumatologist), who were positive for IgM rheumatoid factor (IgM-RF) and/or anticitrullinated protein antibodies (ACPA). These individuals can be classified as Phase C (systemic autoimmunity associated with RA) with/without Phase A (genetic risk factors for RA) and/or Phase D (symptoms without clinical arthritis) according to the European League Against Rheumatism (EULAR) Study Group for Risk Factors for Rheumatoid Arthritis[19], and are collectively referred to as "autoantibody-positive individuals". Both studies were approved by the Medical Ethics Committee of the AMC and performed according to the Declaration of Helsinki. All patients gave written informed consent.

Study design. At baseline, demographics were collected and the following clinical and laboratory variables were obtained: patient's visual analog scale (VAS; range 0–100 mm) for pain in the biopsied joint, Disease Activity Score at 28 joints (DAS28), the severity of swelling of the biopsied joint as assessed by the investigator (range of 0 = no swelling to 3 = severe swelling)[4], IgM-RF levels using IgM-RF ELISA [upper limit of normal (ULN) 12.5 IU/ml; Sanquin] until December 2009 and thereafter using IgM-RF ELISA (ULN 49 IU/ml; Hycor Biomedical), ACPA using anticyclic citrullinated peptide antibodies 2 ELISA CCPlus (ULN 25 kAU/l; Eurodiagnostica), erythrocyte sedimentation rate (ESR), and serum levels of C-reactive protein (CRP). Radiographs were taken of hands and feet.

For the patients with early arthritis, a diagnosis was made at baseline, and after followup, patients were reclassified based on the 2-year clinical diagnosis (cumulative) founded on fulfillment of standard classification criteria for established rheumatic diseases or the absence of these criteria [unclassified arthritis (UA), Phase E according to the EULAR Study Group for Risk Factors for RA][19]. Further, patients with UA at baseline were categorized as "UA-RA" if they converted to RA during this followup, or as "UA-UA" if their arthritis remained unclassified. All patients diagnosed with RA at baseline were categorized as "RA-RA". Finally, after 2 years of followup, patients with early arthritis fulfilling the 2010 American College of Rheumatology (ACR)/EULAR criteria for RA were classified for arthritis

outcome: self-limiting disease, defined as no arthritis on examination and no use of DMARD or steroids in the preceding 3 months; persistent nonerosive disease, defined as presence of arthritis in at least 1 joint and/or requirement of DMARD or steroids in the preceding 3 months; or erosive disease, defined as presence of joint erosions on radiographs of the hands and/or feet[20].

In the autoantibody-positive individuals, yearly study visits were performed, and for individuals who developed arthritis, an additional visit was performed at which the presence of arthritis was independently assessed by 2 investigators.

Synovial tissue biopsy sampling, stainings for immunohistochemistry and immunofluorescence, and quantification. At baseline, all study subjects underwent arthroscopic ST biopsy sampling as described[21,22,23]. In patients with early arthritis, ST biopsy sampling was performed in inflamed wrist, ankle, knee, or other joints (metacarpophalangeal or metatarsophalangeal). Autoantibody-positive individuals underwent ST biopsy sampling from a knee joint[23]. ST of all patients was stained using an anti-NIK monoclonal mouse antibody (sc-8417, Santa Cruz Biotechnology) and/or a monoclonal anti-von Willebrand factor antibody (vWF; F8/86; Dako) as described[20].

In a random subset of the patients with early arthritis (based on availability), sections were stained for CD68 to detect macrophages (n = 51), CD3 to detect T cells (n = 51), and CD22 to detect B cells (n = 61), and analyzed by semiquantitative analysis, as described[24]. In 10 randomly selected patients with early arthritis from this subset, we performed double-immunofluorescence stainings on NIK (sc-8417; Santa Cruz Biotechnology) with vWF (0082; Dako). See Supplementary Materials and Methods (available online at jrheum.org) for staining protocols and quantification.

MRI. At baseline, prior to the arthroscopy, a contrast-enhanced MRI (CE-MRI) of the knee was performed in a subset of both patients with early arthritis (n = 36) and autoantibody-positive individuals (n = 40)[4]. Subsets were selected based on presentation with a knee arthritis (for patients with early arthritis only), the absence of contraindications for CE-MRI, such as claustrophobia, metal implants, or elevated serum creatinine, and was only performed during a specific timespan in these studies. Images were acquired on a 1.5-T MRI scanner (GE Signa Horizon Echospeed, LX9.0, General Electric Medical Systems) as described[4]. The MRI was scored for effusion in 4 compartments (lateral, medial, central, and suprapatellar), a Baker's cyst was scored as an extra compartment. Synovitis was scored in 4 compartments. For effusion and synovitis, a score of 0 (normal) to 3 (large volume) for each compartment was given. Edema, cartilage defects, and erosions were scored as being present (1) or absent (0) in 6 joint compartments (lateral/medial femoral surface, lateral/medial tibial plateau, patellar surface, and trochlea)[25]. A total MRI score was calculated (0–45). Scoring was done by 2 musculoskeletal radiologists (CvdL and MM), who were blinded to the patients' diagnoses.

Statistical analysis. Categorical data were depicted as n (%) and differences between study groups were analyzed using the chi-square test. Variables not normally distributed were depicted as median [interquartile range (IQR)]. To compare baseline characteristics and NIK expression between the different classification groups, the Kruskal-Wallis test was used when more than 2 groups were compared; subsequently, the Mann-Whitney U test was used to compare differences between 2 subgroups. Bivariate correlations of not normally distributed variables were analyzed using the Spearman rank correlation test. All statistical analyses were performed using SPSS v19.0 software (IBM Corp.). A p value of < 0.05 was considered statistically significant.

RESULTS

Patients with early arthritis. Baseline characteristics of the patients with early arthritis are shown in Table 1.

Of the 154 included patients with early arthritis, 64 were classified as having RA at baseline, 61 as UA, 11 as crystal arthropathy (CA), 4 as inflammatory osteoarthritis (OA), and 14 as spondyloarthritis (SpA). Overall median (IQR) disease duration at baseline was 3 months (1–8). Of the 61 patients who were initially classified as UA, 18 fulfilled the 2010 ACR/EULAR criteria after 2 years of followup (UA-RA), 31 remained unclassified after followup (UA-UA), and 12 were lost to followup and therefore excluded from the diagnostic outcome analysis. Of the 82 patients fulfilling the RA criteria after 2 years of followup, 12 had self-limiting disease, 38 had persistent nonerosive disease, and 11 had erosive disease. For 21 patients the arthritis outcome data were not available and those patients were excluded from these analyses.

Table 1. Baseline characteristics of patients with early arthritis. Values are median (IQR) unless otherwise specified.

Characteristics	Patients with Early Arthritis, n = 154
Female, n (%)	90 (58)
Age, yrs, mean (SD)	49 (14)
IgM-RF–positive, n (%)*	39 (25)
ACPA-positive, n (%)**	35 (23)
IgM-RF- and ACPA-positive, n (%)	28 (18)
ESR, mm/h	25 (11–43)
CRP, mg/l	9.3 (3.0–28.3)
VAS pain biopsied joint, mm	59 (27–81)
Swelling biopsied joint, score 0–3	2 (1–2)
DAS28	4.4 (3.2–5.4)
Disease duration, mos	3 (1–8)

* Missing for 2 patients. ** Missing for 3 patients. IQR: interquartile range; IgM-RF: immunoglobulin M rheumatoid factor; ACPA: anticitrullinated protein antibodies; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; VAS: visual analog scale (range 0–100 mm); DAS28: Disease Activity Score at 28 joints.

NIK is expressed in synovial blood vessel EC in patients with early arthritis. In this exploratory study, we found that NIK was almost exclusively expressed in EC of synovial blood vessels of patients with early arthritis (Figure 1A, Figure 1B). This is substantiated by the significant positive correlation between NIK and vWF in these patients (r 0.628, $p < 0.001$; Figure 1C). This is in line with previous results showing that about 70% of EC in established RA ST were NIK-positive[16]. NIK was expressed in all different diagnostic groups (RA, UA, CA, OA, and SpA) and no significant difference was observed in the number of NIK^+EC between these different diagnoses at baseline (Figure 2A). When patients were reclassified based on the 2-year clinical diagnosis, the number of NIK^+EC at baseline was significantly higher in patients with UA compared with patients with RA ($p = 0.003$; Figure 2B). In patients with UA who were reclassified as RA after 2 years of followup, the number of NIK^+EC at baseline was in the same range as patients with RA who fulfilled the 2010 ACR/EULAR criteria for RA already at baseline (Figure 2C).

Interestingly, NIK was significantly differentially expressed in the various biopsied joints ($p < 0.001$). NIK expression [median (IQR)] was highest in the knee joint [154.9 (24.6–444.9), n = 100], lowest in the wrist joint [0.0

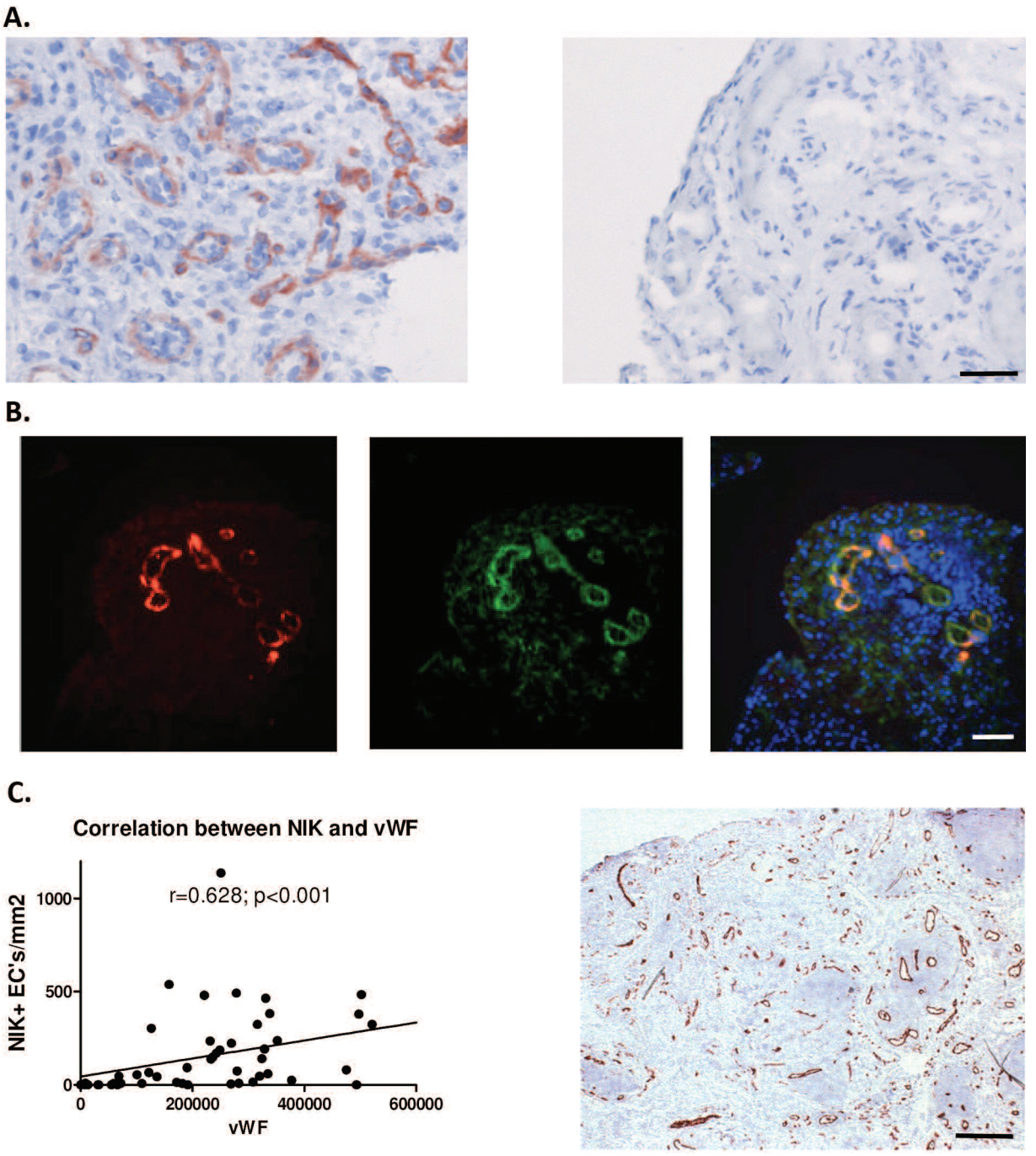

Figure 1. Baseline synovial NIK expression in patients with early arthritis. A. Representative immunohistochemical staining of a NIK-positive patient with early arthritis (left) and of a NIK-negative patient with early arthritis (right). Magnification 400×, scale bar 50 μm. B. Immunofluorescence staining of NIK (red) with vWF (green) and nuclei (blue) in a NIK-positive patient with early arthritis. Magnification 250×, scale bar 100 μm. C. The correlation between NIK expression and vWF (n = 52; left) as analyzed using Spearman rank correlation test and a representative immunohistochemical staining of a vWF-positive patient with early arthritis (right), scale bar 200 μm. NIK: nuclear factor-κB-inducing kinase; EC: endothelial cell; vWF: anti-von Willebrand factor antibody.

(0.0–18.7), n = 19], and intermediate in the ankle joint [4.7 (0.0–56.0), n = 33] and in the other joints [32.4 (30.2–34.5), n = 2; Supplementary Table 1 available online at jrheum.org].

Baseline expression of NIK is not related to arthritis outcome.

We next investigated synovial NIK expression in relation to arthritis outcome (self-limiting, persistent nonerosive, or erosive disease) in patients fulfilling the 2010 ACR/EULAR criteria for RA after 2 years of followup. However, NIK

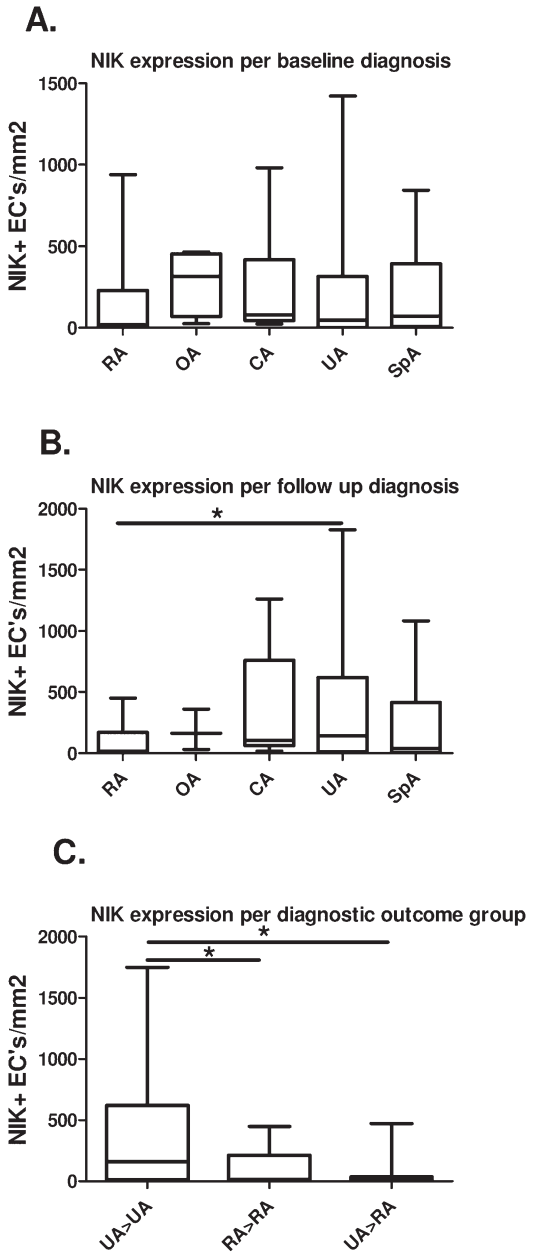

Figure 2. ST NIK expression in relation to different classification groups. A. NIK ST expression of patients with early arthritis diagnosed at baseline with RA (n = 64), inflammatory OA (n = 4), CA (n = 11), UA (n = 61), and SpA (n = 14). B. NIK ST expression after reclassifying patients based on the 2-year clinical diagnosis with RA (n = 82), OA (n = 4), CA (n = 11), UA (n = 31), and SpA (n = 14). C. Patients with UA at baseline were categorized as "UA-RA" (n = 18) if they converted to RA during followup, or as "UA-UA" (n = 31) if their arthritis remained unclassified. Patients with RA at baseline were categorized as "RA-RA" (n = 64). The Kruskal-Wallis test was used and data are presented as box plots (25th to 75th percentiles), lines within the box mark the median value, lines outside the boxes denote the 10th and 90th percentiles. Lines connecting datasets indicate statistically significant differences between groups. Twelve patients with UA were lost to followup after 2 years. * $p < 0.01$. NIK: nuclear factor-κB-inducing kinase; EC: endothelial cell; ST: synovial tissue; RA: rheumatoid arthritis; OA: osteoarthritis; CA: crystal arthropathy; UA: unclassified arthritis; SpA: spondyloarthritis.

expression [median (IQR)] did not differ among the different arthritis outcome groups [self-limiting: 14.7 (1.0–69.9), persistent nonerosive disease: 11.6 (0.0–203.9), and erosive disease: 11.9 (0.0–59.3), p = 0.850].

NIK expression correlates with markers of disease activity in patients with early arthritis. NIK expression correlated positively with systemic markers of disease activity, such as ESR (r 0.184, p = 0.024) and CRP (r 0.194, p = 0.017), and with local assessment of swelling of the biopsied joint (r 0.297, $p < 0.001$; Figure 3A), but not with the VAS pain for the biopsied joint and DAS28 (data not shown).

NIK expression also correlated positively with cellular markers of inflammation in the ST; NIK correlated highly significantly with CD68+ lining and sublining macrophages (r 0.585, $p < 0.001$ and r 0.728, $p < 0.001$, respectively), CD3+ T cells (r 0.733, $p < 0.001$), and B cells (r 0.264, p = 0.040; Supplementary Table 1 available online at jrheum.org).

NIK expression also correlated significantly with MRI effusion (r 0.665, $p < 0.001$), MRI synovitis (r 0.632, $p < 0.001$), and MRI total score (r 0.569, $p < 0.001$) in a subset of the patients with early arthritis (n = 36; Figure 3B–D). NIK expression did not significantly correlate with MRI edema (r –0.012, p = 0.946), MRI cartilage damage (r –0.032, p = 0.855), or MRI erosion scores (r –0.026, p = 0.882).

Subjects at risk of developing RA. Baseline characteristics of the 54 autoantibody-positive individuals are depicted in Table 2.

Twenty individuals were solely IgM-RF–positive, 22 were solely ACPA-positive, and 12 were positive for both autoantibodies. Thirteen of the 54 individuals (24%) developed arthritis during followup after a median duration of 18 months (IQR 6–40). Of the 13 patients who developed arthritis, 9 fulfilled the 2010 ACR/EULAR criteria for RA[26] at arthritis onset and 3 were initially diagnosed as having UA but fulfilled the RA classification criteria later on. One patient fulfilled the ACR classification criteria for OA of the hand, but not for RA[27]. The median followup time of the 41 individuals (76%) who did not develop an arthritis was 42 months (IQR 18–59). In the individuals who developed arthritis after followup, significantly more individuals were both IgM-RF– and ACPA-positive.

NIK is expressed in synovial EC in certain autoantibody-positive individuals, but this is not predictive of the development of arthritis. In autoantibody-positive individuals, NIK expression was very low in general and NIK$^+$EC were only present in 10 out of the 54 autoantibody-positive individuals (Figure 4; Supplementary Table 2 available online at jrheum.org).

Synovial NIK expression in the 13 patients who developed arthritis was in the same range as in the autoantibody-positive individuals who did not develop arthritis during followup (Supplementary Table 2 available online at jrheum.org). Therefore, the presence of NIK$^+$EC was not predictive of the development of arthritis in autoantibody-positive individuals.

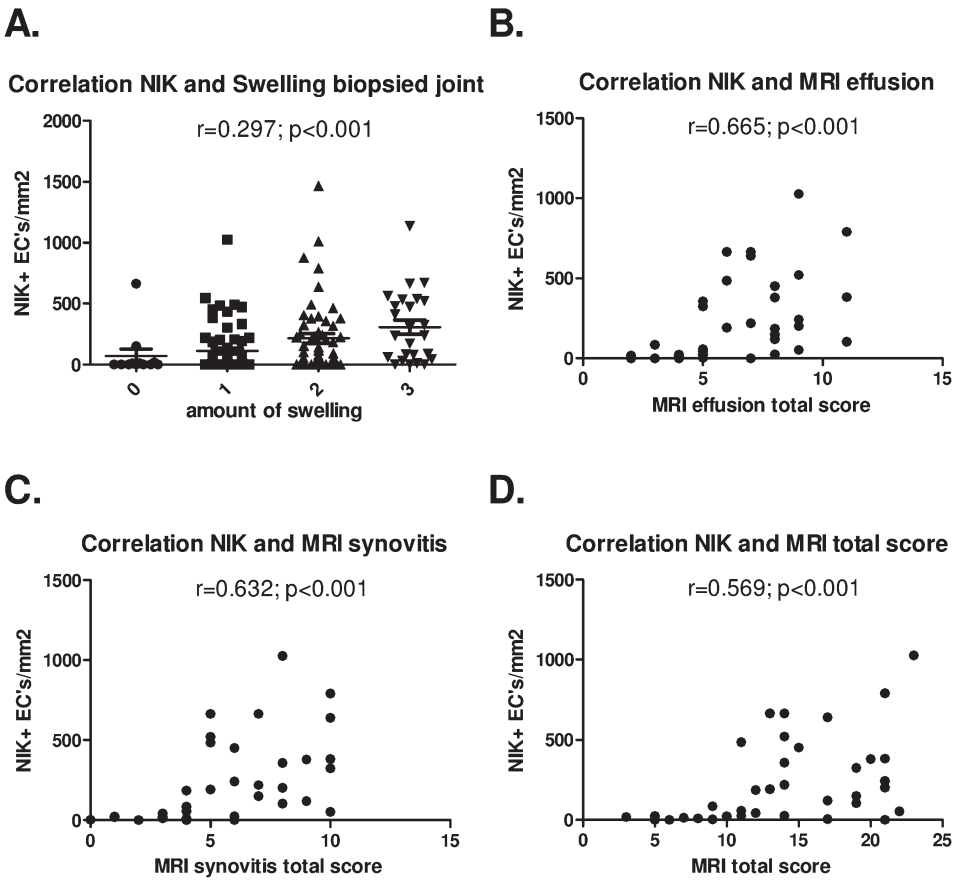

Figure 3. Correlations between baseline synovial NIK expression and local markers for disease activity in patients with early arthritis. A. Comparison of NIK expression and the amount of swelling of the biopsied joint as assessed by the doctor (score of 0 = no swelling to 3 = severe swelling, n = 145). The MRI (n = 36) was scored for effusion in 5 compartments, for synovitis in 4 compartments, and for edema, cartilage degeneration, and erosions in 6 compartments. A score of 0–3 for each compartment was given. A total MRI score was calculated (0–45). B. The correlation between NIK expression and MRI effusion. C. The correlation between NIK expression and MRI synovitis. D. The correlation between NIK expression and total MRI score. Spearman rank correlation test was used to analyze all correlations. NIK: nuclear factor-κB-inducing kinase; MRI: magnetic resonance imaging.

NIK expression is not associated with markers of inflammation in autoantibody-positive individuals. The autoantibody-positive individuals had normal inflammatory variables [median (IQR)], such as ESR [8 (2–15)] and CRP [2.1 (1.0–7.5)]. Synovial NIK expression was not associated with the systemic markers of inflammation ESR (p = 0.544) and CRP (p = 0.227), or with the VAS pain of the biopsied joint (p = 0.526). Previously, we demonstrated that the ST in these autoantibody-positive individuals contains very low numbers of immune cells, comparable with healthy controls[28]. In line with this, we did not observe a correlation between NIK$^+$EC and CD68+ macrophages, CD3+ T cells, or B cells in these tissues (data not shown). Further, the presence of NIK$^+$EC also did not correlate with the MRI data (MRI effusion, p = 0.881; MRI synovitis, p = 0.475; MRI cartilage damage, p = 0.293; MRI edema, p = 0.415; and MRI erosion scores, p = 0.804) in autoantibody-positive individuals (data not shown).

DISCUSSION

We demonstrated that NIK is highly expressed in synovial blood vessels of patients with various forms of early arthritis. Additionally, we showed that synovial NIK expression is associated with systemic and local markers of disease activity in patients with early arthritis. Interestingly, NIK$^+$EC could also be found in some individuals at risk of developing RA. We demonstrate that NIK is expressed in synovial blood vessels already in the earliest phases of inflammatory joint disease.

NIK was not differentially expressed between the different diagnoses at baseline. Nevertheless, reclassification of patients based on the 2-year clinical diagnosis showed that ST from patients with UA contained significantly more NIK$^+$EC at baseline compared with the other types of arthritis. Although this could be an intrinsic feature of UA, another perhaps more likely explanation for this is that the

Table 2. Baseline characteristics of autoantibody-positive individuals. Values are median (IQR) unless otherwise specified.

Characteristics	Autoantibody-positive Individuals		p
	No Arthritis Developed, n = 41	Arthritis Developed, n = 13	
Female, n (%)	29 (71)	8 (62)	0.538
Arthralgia, n (%)	30 (91)*	12 (92)	0.880
Age, yrs	48 (35–53)	48 (43–55)	0.341
IgM-RF–positive, n (%)	23 (56)	9 (69)	0.401
ACPA-positive, n (%)	24 (59)	10 (77)	0.232
IgM-RF- and ACPA-positive, n (%)	6 (15)	6 (46)	0.017
ESR, mm/h	9 (2–17)	7 (5–12)	0.660
CRP, mg/l	2.0 (1.0–5.9)	2.8 (1.4–9.4)	0.474
VAS pain biopsied joint, mm	9 (0–39)**	4 (0–28)	0.507
Followup time, mos	42 (18–59)	18 (6–40)	0.030

* Missing for 8 individuals. ** Missing for 4 individuals. IQR: interquartile range; IgM-RF: immunoglobulin M rheumatoid factor; ACPA: anticitrullinated protein antibodies; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; VAS: visual analog scale (range 0–100 mm).

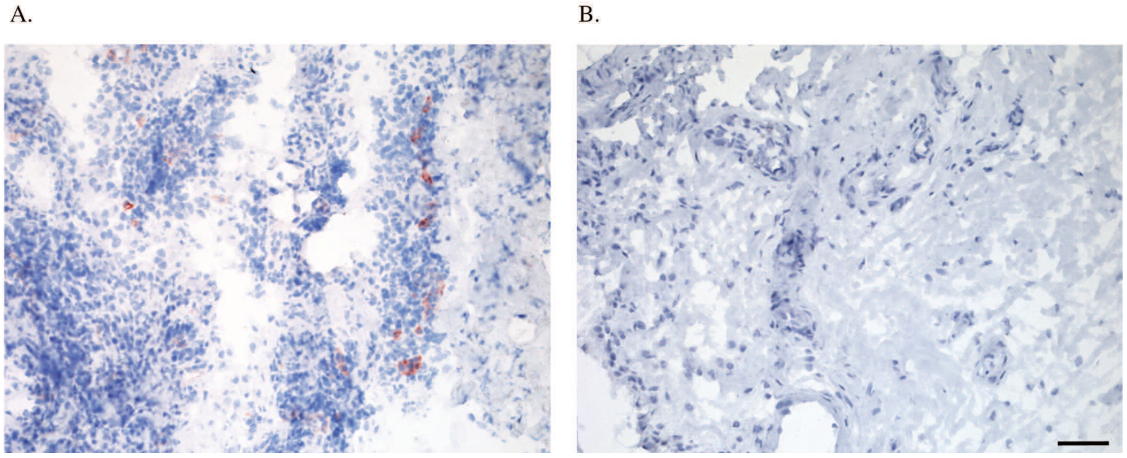

Figure 4. Baseline synovial NIK expression in autoantibody-positive individuals. A. Representative immunohistochemical staining of a NIK-positive individual and (B) of a NIK-negative individual. Magnification 400×, scale bar 50 μm. See Supplementary Table 1 (available online at jrheum.org) for quantification of these data. NIK: nuclear factor-κB-inducing kinase.

mean swelling of the biopsied joint was also higher in this group (data not shown). Also, the variation in NIK expression was high, which makes it unsuitable in clinical practice as a prognostic marker for individual patients with UA. Therefore, we do not advocate the routine use of synovial biopsy to establish the final diagnosis in patients with UA, except in specific cases to rule out infectious causes, crystal-induced arthritis, or neoplasms, or in early drug-development studies[29].

We also observed differential NIK expression in the various joint types. Although this could be a specific characteristic of the individual joints, a more likely explanation is again the increased mean swelling and cellular inflammation scores in the joints that contained the highest NIK expression irrespective of the joint type (Supplementary Table 1 available online at jrheum.org). Nevertheless, we cannot completely rule out that joint-specific features, such as positional gene expression patterns, can affect synovial NIK expression or predispose joints to the development of arthritis[30].

The presence of NIK$^+$EC may be indicative of high angiogenic activity in the inflamed ST, which is in line with previous work from our group and others demonstrating that NIK is a key regulator of pathological angiogenesis and is requisite for pathology in animal models of arthritis[16,31]. In light of the importance of angiogenesis in the progression from acute to chronic inflammation, and the fact that NIK also regulates endothelial expression of CXCL12, an important chemokine in the attraction of immune cells,

NIK+EC may contribute to the persistence of synovial inflammation[7,32].

Of interest, we also showed that NIK+EC are present in ST in 18.5% of autoantibody-positive individuals. However, NIK expression was very low compared with the expression in patients with early arthritis and did not correlate with cellular or other local and systemic markers for (subclinical) disease activity. Also, the presence of NIK+EC at baseline did not predict development of arthritis in this relatively small cohort. Previously, we have demonstrated that features of the synovium are similar between autoantibody-positive individuals and healthy controls, all showing very low scores for phenotypic cellular markers, adhesion molecules, and vascularity[28]. This may explain the lack of an association between synovial NIK+EC and (subclinical) inflammation or the development of arthritis. Nonetheless, NIK+EC are sometimes already present before the onset of clinical arthritis, and therefore targeting these cells in the earliest phases of the disease may be beneficial.

With respect to arthritis outcome (self-limiting, persistent nonerosive, or erosive disease), we did not find a correlation with synovial NIK expression. This may seem surprising given the important role of NIK and the downstream NF-κB subunit RelB in osteoclast biology and the bone-destructive components of inflammatory arthritis[31,33]. In a human study, genetic association analysis showed that single nucleotide polymorphisms in *MAP3K14*, the gene encoding for NIK, affect bone mineral density and bone turnover[34]. However, we did not study the synovial tissue-bone interphase (pannus), but regular ST biopsies in which NIK was predominantly expressed in EC. Therefore, we cannot exclude the possibility that any potential expression of NIK in osteoclasts in pannus tissue may be predictive of the development of erosive disease, but this was outside the scope of our present study.

Our study had some limitations. There was a limited number of patients with UA who progressed to RA (n = 18), and multiple subsets were used for various analysis that was largely based on the availability of ST sections. Nevertheless, we provide clear evidence that NIK+EC correlate positively with markers of local inflammation, such as ST B and T cells and MRI scores. Given the important role of angiogenesis in the perpetuation of arthritis, targeting this process has been proven to be beneficial in (pre)clinical disease models[35,36]. Importantly, targeting angiogenesis will probably not result in the severe immune suppression that is induced by treatment with biologics that target cytokines or specific immune cells, and may cause infectious complications. We have recently established that NIK is only involved in pathological angiogenesis in a preclinical model of arthritis and not in normal developmental angiogenesis[16]. Therefore, selective targeting of NIK in EC may have several advantages over targeting other well-known proangiogenic pathways, such as VEGF, which also blocks physiological angiogenesis and can have adverse effects such as hypertension and thromboembolic events[37].

Our combined data identify NIK as a potential therapeutic target in arthritis[16]. To target NIK selectively in EC, a multimodular recombinant protein that specifically binds to cytokine-activated endothelium under inflammatory conditions, including arthritis, could be used[38]. Because we demonstrate that NIK+EC can already be observed in the earliest phase of the disease, targeting NIK in patients with early arthritis may block pathological angiogenesis and prevent the switch from acute to chronic inflammation. This could be done with a local intraarticular treatment (e.g., gene therapy) or by using small molecule inhibitors[39]. The crystal structure of the catalytic domain of NIK has been identified[40,41], which may facilitate the development of new potent NIK inhibitors. This may lead to new treatment strategies that could be beneficial in RA, and in other types of arthritis and other inflammatory diseases.

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ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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