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**Author:** Willemze, Annemiek  
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Anti-citrullinated protein antibody response associated with synovial immune deposits in a patient with suspected early rheumatoid arthritis

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Annemiek Willemze
Andreea Ioan-Facsinay
Hani S. El-Gabalawy
Anti-citrullinated protein antibodies (ACPA) are highly specific for rheumatoid arthritis (RA) and precede the onset of clinical symptoms by several years, with increasing titers as patients approach disease onset.\(^1\)\(^2\) The synovium is the primary site of pathology in RA, and ACPA are readily detectable in the synovial fluid and tissue of patients with RA.\(^3\) The transition from asymptomatic autoimmunity to clinically detectable synovitis is not well understood. We describe the serological and synovial features of a young woman from a multi-case RA family who initially had asymptomatic autoimmunity, then subsequently developed clinical features suggestive of early RA.

An 18-year-old Native North American (Cree) woman from Central Canada was recruited to a study that aimed to identify high-risk family members of RA probands and follow them longitudinally for disease onset. This study was approved by the Research Ethics Board of the University of Manitoba, and by the Band Councils of the First Nations communities. At baseline she was asymptomatic and had an entirely normal joint examination. Within 6 months of enrolment she developed pain and stiffness in her left knee and left wrist. Examination revealed joint-line tenderness in these 2 joints, along with several small joints of the hands, although no effusion was detectable. She was suspected of having early RA and started taking hydroxychloroquine and naproxen. After informed consent, at that time she also underwent a Parker-Pearson synovial biopsy of the knee, per an established study protocol. With treatment, her symptoms rapidly improved, and within 3 months she spontaneously discontinued the medications, as she had become pregnant for the first time. She continued to be free of synovitis throughout the pregnancy. After delivering a healthy baby she once again developed tenderness and swelling in the left wrist and left knee, and was again given hydroxychloroquine and naproxen, with improvement in the symptoms over the ensuing 2 months. To date, her symptoms continue to be well controlled with this regimen.

The investigations at baseline had revealed that she was negative for IgM and IgA rheumatoid factor (RF), but was positive for anti-CCP2 (Eurodiagnostica) and anti-CCP3 (Inova) antibodies, at a titer of 52 and 38 units respectively. C-reactive protein and erythrocyte sedimentation rate were in the normal range. HLA testing showed her to have the HLA-DRB1*0901 and *1402 alleles. Her serum samples were tested for ACPA isotypes (IgM, IgA, IgG1-4) using ELISA as described.\(^4\) Anti-Sa was tested by ELISA as described.\(^5\) Response to citrullinated fibrinogen (cit-fib), and 5 different linear citrullinated peptides, including C2 (vim), C3 (vim) derived from human vimentin, C4 (fb) and C5 (fb) derived from human fibrinogen, and C6 (en) derived from human enolase, were assessed by ELISA with 2 non-citrullinated peptides as controls. Cutoff level was established on the basis of the mean + 2 standard deviations of the values obtained from testing 30 healthy Caucasian controls. RF and acute-phase reactants remained negative throughout the study period. IgG1-ACPA was positive the first visit (151 AU), with increasing
ACPA in suspected early RA

titers during followup (Figure 1). IgA-ACPA was initially negative, but became positive with increasing titers after symptom onset. IgM-ACPA, IgG2, IgG3, and IgG4 remained negative throughout the study period. After being initially negative, she developed increasing titers of antibodies against citrullinated fibrinogen, but remained negative for anti-Sa and all of the linear citrullinated peptides tested.

These data suggest that onset of clinical synovitis was heralded by an expansion in ACPA isotype usage, along with epitope spreading to nonlinear cit-fibrinogen-associated antigens. Interestingly, as shown in Figure 1, the autoantibody levels continued to rise despite amelioration of the clinical symptoms with pregnancy. This observation implies that the clinical improvement typically seen during RA pregnancy may not necessarily reflect an attenuation of the underlying autoimmune mechanisms, at least at this early stage of the process.

Figure 1. Development of ACPA response over a 2-year period in relation to clinical features in suspected early RA. Levels of all ACPA isotypes and specificity are shown as arbitrary units (AU), with levels of IgA-ACPA and anti-citrullinated-fibrinogen (cit-fib) shown on Y1 axis, and levels of IgG1-ACPA and anti-CCP2 shown on Y2 axis. Approximate cutoff level determined using 30 healthy controls is indicated by broken line. There is an increase in the titers of all ACPA over the study period, with IgA-ACPA and anti-cit-fib both becoming positive after onset of articular symptoms.
In contrast to the findings reported in studies of early RA, the synovium demonstrated normal sublining architecture and minimal microvascular changes. The synovial lining layer was markedly abnormal throughout multiple samples, with the lining cells appearing to float in an amorphous extracellular matrix (Figure 2A). Immunoperoxidase staining revealed the presence of an occasional CD3-positive cell without aggregates, but no CD19 cells. CD68 and CD55 staining were present in the lining layer, and CD86-positive cells were scattered throughout the sublining. Staining using a polyclonal anti-citrulline antibody (AP064, GemacBio) to detect citrullinated proteins demonstrated intense staining of the synovial lining cells (Figure 2B). Immunofluorescence studies showed staining of the lining layer for IgG, IgA, C3, and fibrin (Figure 2C), suggesting that the synovial lining layer may have been the target of an antibody-mediated immune response with complement activation and deposition. Although the specific target of this immune response was not directly tested, the colocalization of citrullinated antigens in lining cells with the immune deposits is consistent with the possibility that the response is directed towards a citrullinated antigen(s). TdT-mediated dUTP-biotin nick-end labeling (TUNEL) demonstrated extensive staining of the synovial lining cells (Figure 2D) compared to that seen in a normal synovial membrane (data not shown), indicating that the lining cells were undergoing widespread apoptosis.

We describe a case where there was a transition from asymptomatic autoimmunity to clinically evident synovitis. At this point, it is not possible to determine with certainty whether this patient’s synovitis represents the earliest clinical evidence of RA, since she has yet to meet the American College of Rheumatology criteria set. On the other hand, it is possible that the very early use of antimalarials may indeed serve to prevent the full expression of the clinical RA syndrome. This has been shown with the use of methotrexate in anti-cyclic citrullinated peptide-positive patients with undifferentiated arthritis\(^6\). The serological evolution and synovial features are consistent with the hypothesis that isotype expansion and epitope spreading of ACPA responses to synovial autoantigens are associated with the onset of synovitis in an individual who is genetically susceptible to RA.
Figure 2. Synovial tissue analysis of biopsy material from a symptomatic knee joint shortly after onset of articular symptoms. A. H&E light microscopy showing marked disruption of the synovial lining cell layer. Lining cells appear to be floating in an amorphous extracellular matrix. Sublining stroma is relatively unremarkable with minimal evidence of inflammatory infiltration. B. Intracellular citrullinated antigens are detected by intense staining of the synovial lining cells using a polyclonal anticitrulline antibody. There is less intense staining of the surrounding matrix. C. Immunofluorescence staining of synovial tissue for C3 showing positive staining in lining layer. Similar results were seen with IgG, IgA, and fibrin staining. D. Synovial lining cells exhibit evidence for widespread apoptosis as detected by TUNEL staining. Original magnification ×200 for A, and ×400 for B, C, D.
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