the pathology of murine spondylitis resembles that of SpA in humans is unclear, it is conceivable that the expression of HLA–B27 heavy chains within the testis might also be linked to SpA in humans. Therefore, a closer inspection of the testis and other immune-privileged sites using a panel of mAb that distinguishes between free and β_2m-complexed heavy chains (as in the study by Rehm et al [4]) is warranted, given the strong association of many HLA–B27 alleles with SpA and the possibility that SpA might be initiated at extraarticular locations. The rat strains with different transgenes and transgene numbers that were produced by Taurog and colleagues provide the opportunity to embark on such analyses.

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Reply

To the Editor:

Ziegler et al provide information in support of our recently published hypothesis that dysregulated innate immunity at immune-privileged sites may be an essential mechanism that triggers the onset of SpA. This hypothesis was based on our observation that EO was found to be essential for the pathogenesis of SpA in a particular line of rats transgenic for HLA–B27 and hβ_2m. As part of that study, we carried out radioisotopic in situ hybridization of transgenic rat testes. The results obtained with a probe for HLA–B27 were interpreted to suggest that B27 is not expressed in germ cells. In apparent contrast, Ziegler and colleagues report staining of early spermatocytes in human testis with antibodies to HLA class I heavy chains.

The suggestion that B27 is not expressed in germ cells was inferred from the results of in situ hybridization, which lack the resolution of results obtained by immunohistochemistry. We made the assumption that if B27 was expressed in germ cells, which would be expected to be present in different stages of development in a cross-section containing many seminiferous tubules (Hermo L, Pelletier RM, Cyr DG, Smith CE. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: Background to spermatogenesis, spermatogonia, and spermatocytes. Microsc Res Tech 2010;73:241–78), then the hybridization signal would not be as uniform in distribution or intensity as was actually found. This inference from in situ hybridization is limited by the low resolution of this method, and we cannot completely exclude the possibility of expression of B27 on germ cells, especially near the periphery of the tubules. Additional experiments will be needed to further investigate this point and to resolve the apparent conflict between the results found by Ziegler et al in their study of human testes and the results we found in our study of rat testes. We thank our colleagues for pointing out the need for further work to clarify this interesting question.

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Pathogenic relevance of anti–citrullinated vimentin antibodies: comment on the article by Montes et al

To the Editor:

We read with interest the article by Montes et al (1). The authors describe an independent association between antibodies directed against citrullinated vimentin (anti–Cit-vimentin) and joint erosion prevalence, which they conclude may be of pathogenic relevance. As is mentioned in the Discussion of their article, our group previously did not find an association between anti–Cit-vimentin antibodies and joint destruction in a large study of patients with early rheumatoid arthritis (2). In an effort to reconcile these seemingly conflicting results, we attempted to compare the methods used in both studies. However, the method used to determine the presence of joint erosions in their study was not described. Was a specific validated radiographic score, such as the Larsen score or the Sharp/van der Heijde score used? Neither the method, nor the reliability of the method (whether one or two readers were used and what the interobserver correlation was), nor the cutoff that was applied, is described in the article, making the relevance of this erosive phenotype unclear (3).

Furthermore, it is unclear at what point during followup the radiographs were taken. Do the results represent a cross-sectional study of patients with variable disease duration, and might the increased prevalence of erosive arthritis in anti–Cit-vimentin–positive patients therefore have been due to the longer disease duration? Was there a longitudinal followup that would allow conclusions about the progression of radiographic damage?

Despite the many interesting aspects of antibodies directed against specific citrullinated proteins, such as anti–Cit-vimentin, we feel it is important to answer these questions. Bearing in mind that the in vitro system in which these autoantibodies are measured cannot mimic the way in which

antigens are presented in vivo, we would like to urge caution before drawing conclusions about the possible pathogenic relevance of these autoantibodies.

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Reply

To the Editor:

We appreciate the interest in our article shown by van der Woude and colleagues. Their letter deals with the specific association we found between anti–Cit-vimentin antibodies and the presence of joint erosion in patients with rheumatoid arthritis (RA). This finding is at odds with the results obtained by van der Woude et al in a previous study (1). The letter includes several questions and a recommendation about aspects of the design and interpretation of our study that we would like to address.

We were aware of the conflicting results, and we cited and discussed them in our article. Our group interpreted the contradictory findings as resulting from the clear differences between the disease evolution (early versus established RA), the time of antibody assessment (baseline or after a long disease duration), and the genetic and environmental factors pertaining to the patients in the 2 studies. Any of these differences could justify the contradictory results, as we indicated in our article. This interpretation was reflected in our qualification of the association between anti–Cit-vimentin and joint erosions as observed "in Spanish patients with longstanding disease."

With respect to the questions posed by van der Woude et al, we appreciate the opportunity to provide pertinent details of our methods and analysis of our findings. Assessment of joint erosions was performed by a single reader (an experienced rheumatologist who was a coauthor) following only a qualitative distinction between presence/absence of definite bone erosions. The radiographs we examined were the most recent available radiographs of the hands and feet of each patient. Our results correspond to a cross-sectional evaluation of patients with established disease. Consistent with these methods, no comment on quantitative scores or on the progression of radiographic damage was included in the text. In addition, we stated that other studies, including the study by van der Woude and colleagues (1), "are more valid than ours for assessing the predictive value of [anti–citrullinated protein antibodies] ACPAs." Finally, the association of anti–Cit-vimentin antibodies, including the time since disease onset among the covariates, was determined by multivariate analysis as we described in the article. In this way, variable disease duration was accounted for in the analysis. Therefore, confounding by this factor was excluded in the association between anti–Cit-vimentin antibodies and joint erosions.

Van der Woude and colleagues recommend caution before drawing conclusions about pathogenic mechanisms based on antibody determinations. We agree with this recommendation, and we applied it throughout our study. We did not make inferences about mechanisms or about the pathogenic relevance of our findings; only the possibility that the findings could play a pathogenic role was signaled. In addition, we further applied caution by reporting only the findings of subanalyses that were supported by data from previous studies, including the association between anti–Cit-vimentin and joint erosions (2). Only studies that directly relate antibodies to disease mechanisms, as has recently been reported (3), or studies of animal models will permit conclusions about pathogenesis.

We appreciated the opportunity to review our methods and data analysis, and we think our findings are unchanged by this additional assessment. We hope to have resolved any doubts about the specific associations of anti–citrullinated α-enolase peptide and anti–Cit-vimentin antibodies with the clinical and genetic features of patients with RA. We further hope to have demonstrated the value of pursuing this analysis for a better understanding of RA. In this difficult task, independent and collaborative studies from multiple teams will be needed.

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