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LETTERS

Infliximab treatment of severe ankylosing spondylitis: one-year followup

To the Editor:

In the first part of an open pilot study, we recently showed that anti–tumor necrosis factor α (anti-TNFα) was very effective in the treatment of severe ankylosing spondylitis (AS) over a 3-month period (1). Three infusions of the monoclonal chimeric anti-TNFα antibody (cA2, infliximab) (kindly provided by Essex Pharma, Munich, Germany), at a dosage of 5 mg/kg were given, at weeks 0, 2, and 6. Eleven patients with AS according to the modified New York criteria (2), with a relatively short disease duration (median 5 years), were initially enrolled in the study. For inclusion, patients had to have active disease as defined by the Bath AS Disease Activity Index (BASDAI) (3) and spinal pain with a score of $\geq$4 on a 10-cm visual analog scale (VAS).

One patient withdrew from the initial study due to an allergic reaction. In the others, fast and sustained improvement of disease activity, function, and pain scores was observed. The median improvement in disease activity after 4 weeks was 70% (range 41–94%). In direct comparisons of week 12 versus week 0, significant improvement was noted for all but 1 parameter, the Bath AS Metrology Index (BASMI) (4). In a pilot study in Belgium, similar results were obtained in a 12-week period in 10 AS patients with disease duration of $>$10 years (4).

Because of the successful results in the preliminary study, we extended the protocol by 3 additional infusions of infliximab in the same dosage. All patients gave written informed consent for their participation in this 1-year extension of the study, which had been approved by the local ethics committee.

The patients were seen at the outpatient clinic every 2–3 weeks, or more frequently if they had worsening of their pain and/or of other symptoms attributable to AS. Information from laboratory studies and patient self-reports was quantified with the assessment tools used in the first part of the study (1): disease activity by BASDAI, function by Bath AS Functional Index (6), metrology by BASMI, spinal pain by VAS, and inflammation by C-reactive protein level. To better determine how long suppression of disease activity is sustained with infliximab infusion, additional infusions were given only if there had been a clear-cut relapse of disease. After the first 3 infusions, a patient was considered to have a first relapse if the BASDAI score reached $\geq$80% of the value obtained in week 0 before the first infusion. Since it later became evident that for most patients, use of this cutoff meant disease activity had to become quite severe before treatment could be resumed, for the fifth and sixth infliximab infusions we redefined relapse as a BASDAI score $\geq$60% of the week-0 value. Patients returned to the outpatient clinic biweekly after each infusion, for assessment of all outcome parameters. The median total observation period in the study was 39 weeks (range 35–41 weeks).

Ten patients were enrolled in this 1-year extension of the study. Six received all 3 additional infusions of infliximab. Four patients dropped out prior to the end of the 1-year extension. Two of the 4 had allergic reactions occurring after the sixth infusion; 1 of the latter developed high-titer antinuclear antibodies (ANAs) and self-limited symmetric arthralgias of the wrists and finger joints. In 1 patient, the AS remained in stable remission after the 3 loading-dose infusions (for 1.5 years as of this writing), eliminating the need for further infusions. The fourth patient was lost to followup after the fifth infusion. Uncomplicated infections not necessitating study withdrawal occurred in 9 patients during the study period (tonsillitis in 2, sinusitis in 2, salmonella enteritis in 1, otitis media in 1 [all resolved after antibiotic treatment], herpes labialis in 1, infectious enteritis in 1, cold in 1).

Nine patients reported first re-experiencing AS symptoms a median of 7 weeks (range 1–17 weeks) after the third of the 3 initial infusions. A relapse according to the 80% BASDAI cutoff, as defined above, occurred after a median of 12 weeks (range 3–21 weeks) in 9 patients, who subsequently received the fourth infusion of infliximab. Subsequent relapses according to the 60% BASDAI cutoff occurred a median of 7 weeks (range 2–15 weeks) after the fourth infusion, a median of 8 weeks (range 2–17 weeks) after the fifth infusion, and a median of 6 weeks (range 2–8 weeks) after the sixth infusion. Figure 1 shows the median BASDAI scores in the extension period compared with the first part of the study. Clear improvement in disease activity was seen, but it did not reach the same magnitude as in the initial period of the study.

This is the first report on 1-year-treatment of active, severe AS with infliximab. The data show that the improvement induced by a loading regimen of 3 infusions of infliximab lasted ~7 weeks before the first symptoms reappeared. The improvement after the loading regimen lasted longer (median 12 weeks) than after the 3 additional single infusions (median 6–8 weeks), despite the lower cutoff used to define relapse. This is probably due to the pharmacokinetics of infliximab:

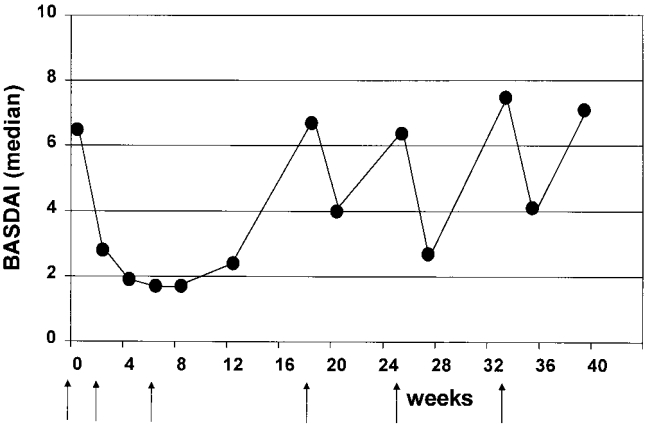

Figure 1. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores during 36 weeks of treatment with infliximab administered in 5-mg/kg infusions. Arrows indicate the times of infusion.

without a loading dosage, serum levels of the drug are lower (7). These findings are being taken into account in terms of the dosage used in our still-ongoing placebo-controlled trial, in which 6-week intervals for infusion of infliximab are being used to maintain suppression of disease activity. The main side effects observed in that study to date have been uncomplicated infections in 90% of the patients. In addition, similar to findings in rheumatoid arthritis (Antoni C: personal communication), 2 patients experienced allergic reactions that did not develop until relatively late in the study, i.e., after the sixth infusion. One patient developed ANAs associated with a transient polyarthritis lasting 4 weeks. None of the side effects has been of longer duration.

In conclusion, these results suggest that sustained improvement of severe AS might be possible with infliximab treatment. Administration of infusion treatment every 6 weeks might be necessary to achieve sustained improvement. Importantly, the AS can even go into remission in some patients, even after long-lasting disease. Some patients develop side effects necessitating discontinuation of therapy.

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Effects of anti–tumor necrosis factor α on synovium in patients with spondylarthropathy: comment on the article by Baeten et al

To the Editor:

The article by Baeten et al describing the effects of anti–tumor necrosis factor α (anti-TNFα) on the synovium of patients with spondylarthropathy (SpA) provides some interesting insights into this form of inflammatory arthritis and indicates several potentially important pathophysiologic differences between rheumatoid arthritis (RA) and SpA (1).

Certain histologic changes in the synovial membrane of peripheral joints, which may help differentiate RA from SpA, have been described previously. In general, the synovium in patients with SpA is characterized by increased vascularity, greater tortuosity of the blood vessels, decreased E-selectin expression, and reduced lining layer thickness in comparison with RA synovium (2). Such abnormalities suggest intrinsic biologic differences between these diseases that may result in different clinical manifestations, functional/radiologic outcomes, and responses to therapy.

Alterations in TNFα expression may also account for some of the differences between RA and SpA. Peripheral blood T cells from patients with SpA may have a decreased capacity to secrete TNFα compared with RA T cells (3). In the synovial fluid, significantly lower TNFα levels and significantly higher ratios of TNF receptor I:TNFα were observed in juvenile arthritis patients with SpA versus those with polyarticular disease (4). Thus, the use of anti-TNFα agents may result in greater TNFα blockade in SpA than in RA, with potentially different physiologic effects.

Baeten and colleagues observed several changes in SpA synovium after anti-TNFα therapy that had not been described in similar studies of RA. First, although lining layer thickness in SpA patients was decreased after treatment with infliximab, this change was due to a selective reduction in the number of type B fibroblasts but not in type A macrophages, despite the fact that sublining layer macrophage numbers were reduced after treatment. This contrasts with previous reports that describe decreases in both synovial lining layer macrophages and fibroblasts in RA patients treated with anti-TNFα (5,6). It is possible that the low number of lining layer cells, characteristic of SpA, contributed to the lack of statistical significance of the decrease in lining layer thickness noted by Baeten et al.

Second, infliximab appeared to induce an increase in synovial B cell and plasma cell infiltration in some of the SpA patients. These cells have been previously found in abundance in the peripheral synovium of SpA as well as RA patients, but since SpA is not particularly associated with increased antibody production, their presence in large quantities in this disease remains enigmatic (7). Anti-TNFα treatment may induce autoantibody formation in some patients (8), although it is not known if increased B cell or plasma cell proliferation in areas other than the synovium contributes to this effect. A previous study examining the effects of infliximab on RA synovial membrane demonstrated a decrease in B cells after 2 weeks of treatment (6). However, it has been suggested that, in TNFα knockout mice, continued absence of TNFα might limit the known homeostatic role of this cytokine in apoptosis, resulting

in exaggeration of the cellular infiltration driven by other inflammation mediators (9). Given that TNFα levels in SpA may be lower than those observed in RA (3), this mechanism might be of relevance in SpA.

The reduction in vascularity noted by Baeten and colleagues is consistent with other reports and with the known effects of TNFα on angiogenesis (10). However, although infliximab treatment resulted in decreased vascular cell adhesion molecule I expression in these patients, no significant difference in expression of intercellular adhesion molecule 1, platelet endothelial cell adhesion molecule 1, or E-selectin was noted. The differences in adhesion molecule expression between RA and SpA after TNFα blockade may reflect the intrinsic vascular differences between these diseases and may account for the increased B cell numbers in SpA after treatment. Since anti-TNFα treatment is known to reduce soluble adhesion molecule levels in RA (10), measurement of these levels in patients with SpA would help determine the true significance of these findings.

One limitation of Baeten and colleagues' study is the small number of patients (8 of 21 from an open-label study), who were chosen because of the presence of knee inflammation. However, several reports indicate that inflammatory changes in RA synovium can occur in the absence of overt disease activity (11,12). Such findings might not necessarily apply to SpA, but they suggest that the other 13 patients in the open-label study may also have demonstrated synovial changes that could have been affected by anti-TNFα. Furthermore, the recent history of disease-modifying therapy in 5 patients and the current use of corticosteroids in 2 patients may have potentially influenced some of the observations.

The spondylarthropathies are a heterogeneous group of diseases with some common characteristics. However, there may be important pathophysiologic differences between patients with predominant axial disease and others who develop peripheral synovitis. Thus, studying the synovial membrane of inflamed knees may not fully answer the question of whether anti-TNFα therapy results in different biologic responses in SpA compared with RA. In contrast, histologic examination of the specific enthesopathic changes recently identified by magnetic resonance imaging in untreated early SpA (13) may yield important information regarding patients being treated with new biologic agents.

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Reply

To the Editor:

We thank Dr. Cunnane for her interest in our recent histologic work and appreciate her positive criticism of our report of the effects of anti-TNFα on the synovial membrane in SpA. Cunnane highlights and discusses elegantly some concepts emerging from this report as well as from previous studies.

A first important concept is that the synovial inflammation is clearly distinct in SpA versus other types of arthritis (1), which may help lead to an understanding of the mechanisms underlying these diseases. Moreover, these differences could be useful in distinguishing the diseases at an early stage (2) and predicting clinical, anatomic, and functional outcome.

Second, not only does the analysis of TNFα/TNF receptors as well as other molecules in the synovial membrane in well-defined disease groups remain essential for the identification of new therapeutic targets, but sequential analysis of synovial histologic features may provide additive information regarding distinct responses to therapy in certain disease groups or individual patients. From this perspective, it is interesting that some histologic changes, such as the reduction in vascularity, are observed in both SpA and RA patients

treated with anti-TNFα (3). However, since the baseline hypervascularization seems particularly prominent in SpA (1,4), a similar biologic effect of anti-TNFα may result in a different immunopathologic outcome. A similar mechanism may apply to adhesion molecule expression and the effect on lining layer hyperplasia.

A third important observation is that some biologic effects seem to be clearly different in the 2 patient groups. More particularly, the increase in numbers of B lymphocytes and plasma cells in SpA, either by infiltration or by local proliferation, contrasts sharply with observations in RA. Although interpretation of this phenomenon remains speculative, it is tempting to relate it to specific B cell activation and (auto)antibody production. Thus, sequential histologic analysis of synovium may also provide some basic insights into the role of target molecules such as TNF in this particular disease and may identify the possible need for further laboratory followup of immunologic (side) effects.

We agree completely with Cunnane that these findings first need to be confirmed in a larger group of SpA patients. In contrast to RA, in SpA it could be more pertinent to include only patients with clinical knee involvement since there are no data supporting the concept that joints not exhibiting clinical involvement may in fact be affected in SpA. Moreover, a major advantage of this small pilot study in SpA was that the patients received no concomitant disease-modifying drugs.

We also share Cunnane's opinion that this pilot study is just a starting point to extend our analysis of the immunologic effects of infliximab to the histologic evaluation of other disease sites (enthesis, gut), and also to a direct comparison with other biologic parameters (cytokine profiles, soluble adhesion molecules, autoantibodies), other disease groups, and other TNFα blocking agents.

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Possible infection with *Brucella* in patients with spondylitis: comment on the article by Brandt et al and the clinical images by Mercié and Leleu

To the Editor:

I read with interest the article by Brandt et al on the use of anti–tumor necrosis factor α in the treatment of ankylosing spondylitis (AS) (1). If an effective treatment for AS becomes available in the near future, it will certainly be a boon.

I have a small suggestion regarding Figures 1 and 2 in Brandt and colleagues' article—magnetic resonance images (MRIs) from a patient with AS. They show osteitis near the endplate of L1, D12, D11, and discitis as evidenced by Schmorl's nodes. Because this patient had AS, he would have sacroiliitis as well. In my experience, such a combination (sacroiliitis, osteitis, and discitis) exists in *Brucella* spondylitis. It would thus be useful to test for IgG, IgM, and IgA *Brucella* antibodies in this patient by enzyme-linked immunosorbent assay (ELISA). Given the 5-year disease duration, agglutination testing would likely yield negative results because of blocking antibodies.

I would like to make the same suggestion with regard to the patient who was the subject of the Clinical Images piece by Mercié and Leleu (2). This patient too had endplate lesions on adjacent vertebrae and sacroilitis, and the HLA–B27 positivity may be incidental. There is currently little that we can offer in the way of treatment to a patient of AS. In contrast, *Brucella* spondylitis is curable and, if left untreated, can have serious complications. In a 48-year-old woman presenting with significant weight loss, increased levels of markers of inflammation, and the MRI and bone scintigraphy findings depicted (2), it is worthwhile to investigate for *Brucella* spondylitis.

Spinal involvement in brucellosis can occur at any stage of the systemic disease, the reported incidence being 6–58% (3–5). The infection seems to start in a superior endplate. Depending upon the size and virulence of the bacterial organism and the immunity of the host, the infection either may regress or may progress to involve the entire vertebra, the disc space, and the adjacent vertebra (6). The disease affects the lumbar, the dorsal, as well as the cervical spine. Involvement of the lumbar spine is most common. Simultaneous involvement of multiple sites is known to occur.

On MRI (7), early lesions are seen as areas of low signal intensity in the anterior aspect of the involved superior endplate (with short repetition time [TR]). With long TR, these areas become hyperintense. These features are characteristic of localized osteitis. The discs and the paraspinal soft tissues show no abnormal signal intensity in early disease. They are, however, affected in advanced disease. Defects in the osseous endplates extend to various depths of the vertebral body. On imaging they are usually rounded, and few have a sclerotic margin. Morphologically they are similar to Schmorl's nodes (7). Sacroiliac joint involvement is reported to occur in 5–30% of patients with brucellosis (6,8,9). It starts as unilateral sacroiliitis but is occasionally bilateral, or involvement of one side is followed by involvement of the opposite side. Sacroiliitis may be associated with lumbar spondylitis with low back pain, mimicking AS.

Patients with *Brucella* spondylitis present with low back

pain, usually in conjunction with systemic manifestations including fever, weight loss, and fatigue. In my experience, many patients presenting in the chronic stage of the disease do not report a history of fever (Gokhale Y: unpublished observation). There are no specific characteristic clinical features of *Brucella* spondylitis. The pain may be severe, leading to difficulty in walking and sometimes to inability to get out of bed; there may be associated radicular pain. It is important to elicit a history of animal contact or ingestion of raw dairy products. In Madkour's series (7), such a history was present in 73% of patients with *Brucella* spondylitis.

The erythrocyte sedimentation rate may be normal or elevated. Positive blood cultures are observed in <20% of patients with *Brucella* spondylitis (10), and culture time is 4–6 weeks. Hence, in a suspected case, the diagnosis should be confirmed by serologic testing. The sensitivity of the standard tube agglutination test decreases if the disease duration is >6 months (11). Determination of antibodies to *Brucella* species by ELISA is a sensitive and specific method for diagnosis of the disease (12). The IgM antibody response decreases after a disease duration of 6 months, whereas IgG- and IgA-class antibodies persist beyond 18 months (13).

The World Health Organization fact sheet on brucellosis states that the disease is underreported by 10–25-fold (14). We are therefore likely to have patients presenting with untreated *Brucella* spondylitis, which is the most common complication of brucellosis. Detection of IgG, IgA, and IgM antibodies to *Brucella* by ELISA in patients with radiologic abnormalities consistent with those seen in *Brucella* spondylitis will be useful in diagnosing the condition.

Cases of brucellosis in animals have been recorded in Germany and France (15) (where the reports by Brandt et al and Mercié and Leleu originated [1,2]). Cases of human brucellosis can occur when the disease exists in animals in a country. I believe the patients described in these reports may benefit from screening for *Brucella*.

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Correlation among macrophage inflammatory protein 1α levels, matrix metalloproteinase 8 levels, and systemic inflammation in rheumatoid arthritis: comment on the articles by Yamanaka et al and Mattey et al

To the Editor:

The articles by Yamanaka et al (1) and Mattey et al (2) demonstrate the value of measuring serum levels of matrix metalloproteinases (MMPs) to assess joint damage in rheumatoid arthritis (RA). MMP-3 (stromelysin), examined by those authors, is one of the best investigated MMPs, but other MMPs are also thought to play a role in the degradation of matrix components. MMP-8, one of the collagenase subfamily members, is mainly produced by macrophages and neutrophils, and there is good evidence that this collagenase is closely involved in the turnover of collagen in joint tissues (3). The MMP synthesis is markedly stimulated by inflammatory cytokines.

Macrophage inflammatory protein 1α (MIP-1α) is an interesting member of the family of chemokines, originally identified as chemotactic cytokines. It has been shown to cause the accumulation of mononuclear cells in the later chronic phase of inflammation (4). Expression and secretion of MIP-1α by mononuclear cells may be indicative of local and systemic inflammation in RA (5).

Using quantitative enzyme immunoassays, we measured MIP-1α, MMP-8, and the acute-phase protein serum amyloid A (SAA) in the serum of 105 patients with RA according to the criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (6). High levels of MIP-1α (mean ± SD 29.1 ± 8.3 pg/ml) and MMP-8 (20.8 ± 10.0 ng/ml) as well as of SAA (187 ± 95 ng/ml) and the radioimmunologically measured immunoactivation marker neopterin (23 ± 15 nmoles/liter) were observed in RA patients with inflammatory activity (clinically and serologically active RA) compared with patients with inactive RA and with a group of healthy controls. A significant correlation ($r = 0.31$, $P < 0.005$) between MIP-1α levels and MMP-8 levels was found; the extent of this correlation was similar to the correlations of MIP-1α and MMP-8 with the inflammation markers SAA, C-reactive protein, and neopterin.

We propose that serum MMP-8 levels in RA depend

on the degree of immunoactivation and inflammation, mainly on the activation of macrophages that are capable of producing the detected high amounts of MIP-1α and neopterin. The correlations we identified suggest that MIP-1α plays an important role in the inflammatory process, accompanied by induction of the neutrophil collagenase MMP-8, which can lead to joint destruction in RA. The precise role of MIP-1α in RA remains to be elucidated.

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Reduced bone mineral density in men with rheumatoid arthritis: comment on the article by Haugeberg et al

To the Editor:

We read with interest the report of the excellent and comprehensive study by Haugeberg and colleagues on bone mineral density (BMD) in male patients with rheumatoid arthritis (RA) (1). The authors indicate that only 1 previously published study focused exclusively on BMD in male RA patients (2). We would therefore like to call their attention to our study on this subject that was published in the *Journal of Rheumatology* in 1995 (3). In that study we evaluated 99 men with RA who were being regularly followed up in our department, in order to analyze their BMD and sex hormone status. The mean $\pm$ SD age of the patients was 57.7 $\pm$ 13.3 years; 74 were currently taking low-dose corticosteroids. A group of 68 healthy age-matched men was used for comparison.

We found significant reductions in lumbar spine BMD, femoral BMD, and levels of salivary testosterone, androstenedione, and dehydroepiandrosterone sulfate in the men with RA. By multiple regression analysis, weight, serum testosterone concentration and cumulative dose of corticosteroids were significant predictors of lumbar spine BMD, and weight, age, androstenedione concentration, and cumulative dose of corticosteroids were significant predictors of femoral BMD.

When our study was performed, no data on BMD status in the general Spanish population were available. Such data are now available, and using these reference-population data, we have now reanalyzed our data in order to establish a comparison with the results obtained by Haugeberg et al. BMD in the lumbar spine at L2–L4 (anteroposterior) and BMD in the hip (femoral neck) were measured with the same dual x-ray absorptiometry equipment (Hologic, Waltham, MA). As suggested by Haugeberg and colleagues, reduced BMD was defined as a Z score of at least 1 SD below the mean in the reference population. The frequency of reduced BMD in the male RA patients was 35.5% for the lumbar spine and 36.5% for the femoral neck, similar to the findings obtained in Norway by Haugeberg et al. We believe longitudinal studies are necessary to establish the magnitude of the problem of bone loss in men with RA.

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2. Garton MJ, Reid DM. Bone mineral density of the hip and of the anteroposterior and lateral dimensions of the spine in men with rheumatoid arthritis: effects of low-dose corticosteroids. Arthritis Rheum 1993;36:222–8.
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Reply

To the Editor:

We thank Drs. Nolla and Mateo for their valuable comments on our report. We regret that their previously published article (Mateo L, Nolla JM, Bonnin MR, Navarro MA, Roig-Escofet D. Sex hormone status and bone mineral density in men with rheumatoid arthritis. J Rheumatol 1995; 22:1455–60) was not included in our list of references; it provides important information, especially on the relationships between reduced BMD and decreased androgen levels in male patients with RA.

The study by Mateo et al (2) differed from ours in terms of selection of control subjects and patients. Their RA patients were recruited from the regular hospital service and were compared with patients with osteoarthritis who had significantly increased body weight. In our study, the patients were recruited from a validated county-based patient registry

(completeness 85%), and they were found to be closely representative of the entire RA registry population. Unlike Mateo and colleagues, we also included patients who had received or were currently receiving medication for osteopenia or osteoporosis.

Despite these differences in patient selection, Drs. Nolla and Mateo, after reanalysis, found the same frequency of reduced BMD as we did, highlighting that bone loss is a frequent problem in male patients with RA. We agree that these cross-sectional observations need further exploration in longitudinal studies, especially to confirm independent associations with corticosteroids and other suggested risk factors for osteoporosis.

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Are cases of rheumatoid arthritis in smokers and lifelong nonsmokers representative of different rheumatoid disease processes? Comment on the article by Harrison et al

To the Editor:

In a recent article in *Arthritis & Rheumatism*, Harrison et al (1) report an association between cigarette smoking and the presence of rheumatoid factor (RF), rheumatoid nodules, and vasculitic complications in patients with polyarthritis. They also observed that, during a relatively short followup time (3 years), joint swelling was significantly reduced in the polyarthritis patients who were smokers. The authors express surprise that, despite a higher rate of seropositivity, the smokers had a lower swollen joint score. This is not, however, an unexpected finding when one considers the pathogenesis of RF in healthy individuals and the consequences of persistent RF in these individuals.

In a study by Heliovaara et al (2), cigarette smoking appeared to be the single most important risk factor for the development of positive RF in healthy individuals. The presence of persistently positive RF in a healthy individual is important because it increases, by 7-fold, the risk for the development of seropositive rheumatoid arthritis (RA) (3). A recent study has demonstrated that heavy cigarette smoking is strongly associated with RA (4), and it was suggested that the mechanism underlying this finding is the association between heavy smoking and RF production.

There are a number of potential mechanisms by which RF may adversely influence the rheumatoid disease process. For example, one of the functions of RF is the induction of neutrophil enzyme release (5). Additionally smokers have primed neutrophils that generate more oxygen free radicals and proteinases (6). Neutrophils are present in large numbers in synovial effusions from patients with erosive RA and contain the proteinases elastase, collagenase, and cathepsin G, which are capable of degrading components of connective tissue matrix (7). Therefore, RA patients who are smokers may have a predominantly neutrophil-driven erosive disease which is independent of synovial proliferation and joint swelling. This mechanism may also explain Harrison and colleagues' finding of an association between smoking and rheumatoid vasculitis, which is also a neutrophil-driven disease (8).

It is possible that, in smokers who develop an otherwise benign and self-limited arthritis, the disease is transformed by the presence of high-titer RF, which, if not for the smoking history, may never have been present. In contrast, RF may occur as a "reactive" phenomenon in response to synovitis, and therefore in these individuals increased RF production may be a reflection of greatly increased disease severity. These individuals thus could have two mechanisms driving their rheumatoid disease: the process responsible for the synovitis, as well as the resulting RF completing a feedback loop and causing subsequent development of sustained severe disease. Therefore, "pound for pound," an individual with RA who has never smoked and who has strongly positive RF is likely to have more inflammatory disease than an individual with RA who has high-titer RF simply as a result of heavy smoking.

A second potential mechanism by which RA patients who smoke may differ clinically from RA patients who are lifelong nonsmokers is the potential interaction between specific cytokines and cigarette smoke. Relatively few studies have investigated the interaction between cigarette smoke and cytokines, but it has been reported that cigarette smoke contains potent inhibitors of both tumor necrosis factor α (TNFα) and interferon-γ (IFNγ) (9).

Celiac disease is associated with both TNFα and IFNγ (10), and it is therefore of interest that a case–control study demonstrated a strong inverse relationship between current cigarette smoking and celiac disease, with a matched odds ratio of 0.15 (95% confidence interval 0.06–0.38) (11). An association between RA and polymorphism of TNF receptor II in familial as opposed to sporadic RA has been reported (12). Another recent study has demonstrated that heavy cigarette smoking is associated with sporadic as opposed to familial RA (4). It is conceivable that cigarette smoking is inversely related to particular cytokine-driven subtypes of RA, further suggesting that RA patients who are smokers and those who are lifelong nonsmokers are distinct groups.

RF may in fact have 2 roles in RA: first, playing a significant part in disease development and second, triggering a "reactive" and disease-worsening phenomenon in particular individuals who develop a cytokine-driven synovitis. This is not to say, however, that smokers develop less severe erosive disease in the *long term*. Wolfe found that erosive disease in smokers is related to pack-years smoked and that there was a direct relationship between pack-years smoked and RF titer (13). This increase in erosive disease was not associated with a higher acute-phase response, suggesting that erosive and inflammatory processes are discordant in smokers.

Certainly more research on the intriguing relationship between smoking and RA is warranted. It will be interesting to see if in fact RA patients who are lifelong nonsmokers and those who are heavy smokers differ genetically and, perhaps more importantly, in their response to treatment.

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Disease activity and survival in vasculitis: comment on the article by Gayraud et al

To the Editor:

The French Vasculitis Study Group is to be congratulated on the great service it has provided to the medical community with the series of studies of treatment of systemic idiopathic vasculitis. Considerable improvement in both morbidity and mortality has been achieved since the first description of these conditions, which were almost uniformly fatal, although much still remains to be learned about their etiopathologic peculiarities (1). I would point out an error in the article by Gayraud et al (2) in Figure 2B, describing survival in vasculitis patients according to their Birmingham Vasculitis Activity Score (BVAS) (3). In the first line, the BVAS should be ≤ 10 rather than ≥ 10, as printed.

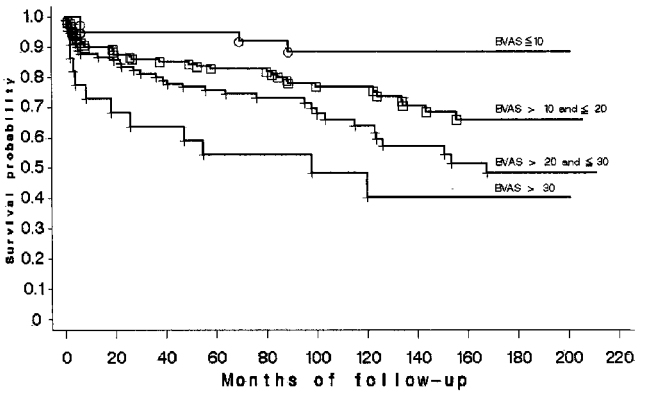

Figure 1. Corrected figure.

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Reply

To the Editor:

We thank Dr. Matteson for his kind comments but, most of all, for his eagle eye. Indeed, in Figure 2B, the top curve should read "BVAS $\leq$ 10." This is corrected in Figure 1 herein.

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Possible association between glucosamine treatment and renal toxicity: comment on the letter by Danao-Camara

To the Editor:

We read with interest the letter to the editor by Danao-Camaro on potential side effects of glucosamine and

Table 1. Renal function–associated laboratory parameters in the glucosamine-treated patient*

	Date				
	3/28/00	6/20/00	6/26/00	7/27/00	9/07/00
Creatinine, mg/dl (normal 0.6–1.3)	1.1	1.4	1.2	1.2	1.1
Blood urea nitrogen, mg/dl (normal 16–45)	44	94	52	52	51
Proteinuria, mg/dl	0	0	ND	0	ND

* Glucosamine treatment was started in February and discontinued in June. ND = not determined.

chondroitin treatment (Danao-Camara T. Potential side effects of treatment with glucosamine and chondroitin [letter]. Arthritis Rheum 2000;43:2853). She reports cases of photosensitization, reversible systolic hypertension, proteinuria, and asymptomatic, reversible elevation of creatine phosphokinase levels in glucosamine-treated patients. We would like to add a report of renal dysfunction characterized by elevation of creatinine as another possible adverse effect of this treatment.

One of our patients, a 79-year-old woman, had myasthenia gravis controlled by low-dose methylprednisolone (4 mg/day) and cyclosporine (200 mg/day). She also had severe, painful knee osteoarthritis that did not respond well to antiinflammatory medications. In February 2000 she began, not on the advice of a physician, to take glucosamine (1 tablet per day). In June 2000, routine blood analysis revealed abnormalities of renal function, with elevation of both blood urea nitrogen and creatinine levels but without proteinuria (Table 1). We suspected glucosamine toxicity and advised her to stop this medication. Regular monitoring after discontinuation of the glucosamine treatment revealed gradual normalization of the blood urea nitrogen and creatinine values. We did not attempt to reintroduce glucosamine later to see if the renal toxicity would recur. Nevertheless, the recovery of renal function after discontinuation of glucosamine suggested a connection between this drug and renal impairment. We cannot exclude the possibility that the concomitant treatment with cyclosporine had an additive nephrotoxic effect.

Physicians should be aware of the possible side effects of glucosamine and should regularly monitor laboratory parameters, including those indicative of renal function, in patients who are taking this medication.

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Fetal cell trafficking and dermal fibrosis: comment on the article by Christner et al

To the Editor:

We read with interest the article by Christner et al on microchimeric cells of fetal origin and dermal fibrosis in vinyl chloride–injected mice (Christner PJ, Artlett CM, Conway RF, Jimenez SA. Increased numbers of microchimeric cells of fetal origin are associated with dermal fibrosis in mice following injection of vinyl chloride. Arthritis Rheum 2000;43:2598–605). Those authors and your readers may be interested to know that we have previously investigated fetal cell microchimerism in pregnant mice, in studies using quantitative polymerase chain reaction to detect cells bearing the Y chromosome (Bonney EA, Matzinger P. The maternal immune system's interaction with circulating fetal cells. J Immunol 1997;158:40–7). In that study we examined 8 C57BL/6(B6) retired breeders that had had at least 3 litters with B6 males. We found that none of them had circulating Y chromosome–bearing cells at the level of sensitivity of our assay, which was $\sim$1 in 10^6 cell equivalents. Moreover, this work showed that $\sim$35% of retired B6 breeders were able to generate a strong cytotoxic T cell response against male cells.

The results of these and other experiments, particularly with SCID mothers, led us to the conclusion that the maternal immune system could clear circulating fetal cells. What was interesting to us in the Christner study was the evidence that fetal cells were not completely cleared from the maternal circulation. Their data showed that all of their BALB/c female retired breeders previously mated to B6 males were positive for cells of the B6 haplotype. The investigators presumed that these cells were of fetal origin. However, although all of the retired breeders in Christner et al's study were positive for these cells, results presented in their report confirm our assertion that if fetal cells persist in the maternal circulation, their numbers are likely to be very low. Three of 7 mice that were tested quantitatively had $<$1 cell equivalent of fetal DNA/100,000 cell equivalents of maternal DNA.

More interesting are Christner and colleagues' findings that fetal cell traffic is increased in response to vinyl chloride and that this increased trafficking is associated with dermal fibrosis. They found that retired breeders given vinyl chloride developed lesions and also had more collagen deposition compared with mice that had never been pregnant or retired breeders not injected with vinyl chloride. There are 3 possible interpretations of the data: 1) after pregnancy, fetal cells may be "sequestered" in maternal tissues away from maternal peripheral blood and this sequestration may be relieved by vinyl chloride injection; 2) treatment with vinyl chloride may activate circulating fetal cells and cause a "graft-versus-host" phenomenon which results in dermal fibrosis; or 3) increased fetal cell trafficking and dermal fibrosis may both occur after injection of vinyl chloride but with the fetal cells not being the direct cause of the lesions seen.

Although Christner et al detected increased fetal cell circulation in the retired breeders that developed dermal fibrosis, they did not assay for fetal cells in the dermal lesions. Therefore, there is no direct evidence that the fetal cells are involved in the lesions. These lesions may be caused by other sequelae of pregnancy. Moreover, their study does not address the possible role played by maternal T cells. The experiments with virgin mice treated with vinyl chloride do not fully address this question because the virgin immune system has not been through pregnancy, whatever its effects. We would suggest that the authors repeat their experiment using SCID mice that have been mated to normal males (so that the fetal cells will be immunocompetent though the maternal cells are not) through

multiple gestations. This would enable one to distinguish the immunologic effects potentially attributable to cells of either (maternal or fetal) origin. Until such a study takes place, it will not be clear whether the fetal cells are involved in the development of dermal fibrosis.

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Reply

To the Editor:

Bonney and Matzinger investigated for fetal cells in the peripheral blood and organs of mice that had been bred 2–3 times (Bonney EA, Matzinger P. The maternal immune system's interaction with circulating fetal cells. J Immunol 1997; 158:40–7). They reported the presence of microchimerism in ~35% of the females that had had pregnancies. Peripheral blood contained the highest number of microchimeric cells. They also reported that the mice were able to clear essentially all the microchimeric cells by 8 weeks post-delivery. They concluded that in the murine system microchimerism occurs in a minority of pregnant mothers and that detectable fetal cells could be eliminated in all animals after 8 weeks.

We do not see any inconsistency in the data from our study published in *Arthritis & Rheumatism* and the data of Bonney and Matzinger, but we do interpret the results differently. We reported that we were able to detect microchimeric cells in >50% of our retired breeder mice. However, we further noted that, whereas >40% of the retired breeders had no detectable microchimeric cells before vinyl chloride injection, we were able to detect fetal cells in these mice after the injections.

Probably because Bonney and Matzinger concluded that fetal cell trafficking is a minor event in mice and that the cells can be cleared by 8 weeks postpartum, Srivatsa and Bonney state in their letter that we found increased fetal cell trafficking in response to vinyl chloride. We disagree with that statement. The introduction of fetal cells into the mother and her treatment with vinyl chloride were clearly separate events. Vinyl chloride treatment began a minimum of 3 weeks after the last litter had been weaned from the retired breeders. Whatever fetal cells were present had trafficked across the placenta during 1 or more of the previous 8–10 pregnancies that the retired breeders had undergone. We do not believe that Bonney and Matzinger's interpretation of their data, that the murine maternal immune system is able to fully remove microchimeric cells after 8 weeks, is supported by our data. We believe that very low numbers of microchimeric cells (perhaps undetectable) remain in the maternal circulation and that vinyl chloride activates the fetal cell(s) and causes them to proliferate and begin attacking the host.

We agree with Srivatsa and Bonney that because we have not yet published data showing that there are fetal cells in the skin and spleen which are causing fibrosis, we cannot yet state for certain that our hypothesis is correct. Experiments that are in progress will determine what types of cells are present in the skin and spleen and whether these cells are of fetal or maternal origin. We also agree that studying SCID mice is a very useful way to distinguish the different roles of maternal T cells and fetal T cells in vinyl chloride–treated retired breeders.

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