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## Reply

*To the Editor:*

We thank Craig and Ledue for their interest and their careful analysis of our study. In the sample series analyzed in the study, we observed that ARD patients had higher ANA titers than healthy individuals, and that some ANA patterns were predominantly observed in patients with ARD whereas some others were almost exclusively observed in healthy individuals. In fact, the nuclear homogeneous and nuclear coarse speckled patterns were solely observed in ARD patients, whereas the nuclear dense fine speckled pattern was observed only in healthy individuals. This striking difference in ANA profile supports the conclusion that the titer and pattern on ANA–HEp-2 testing enhances our ability to discriminate ANA-positive healthy individuals and patients with ARD.

We agree that the nuclear dense fine speckled pattern may be observed also in individuals with diverse clinical presentations including asthma, atopic dermatitis, and interstitial cystitis, and our report actually cited the articles in which these diseases are discussed (1–4). In addition, variability in patient selection criteria and the subjectivity of ANA pattern definition also may account for some discrepancies among reports on the subject. However, the striking reality that we have encountered in our daily practice and that was confirmed in our study is that the nuclear dense fine speckled is rarely observed in patients with ARD. This does not mean that the finding of ANAs with the nuclear dense fine speckled pattern allows summary exclusion of an ARD diagnosis. In this regard, we thank Craig and Ledue for emphasizing this point, which might have been misunderstood in our article. Instead, our data indicate that the isolated finding of ANAs with the nuclear dense fine speckled pattern in the absence of other clinical and laboratory abnormalities provides strong evidence against the presence of an ARD.

Clear-cut recognition of the nuclear dense fine speckled pattern is possible when a serum sample that is positive for anti–lens epithelium–derived growth factor (anti-LEDGF)/p75 does not have other autoantibodies in sufficiently high concentrations to elicit interfering ANA patterns. However, autoantibodies at a low titer may not interfere with the recognition of the dense fine speckled pattern. In this regard, Muro et al detected anti-LEDGF/p75 antibodies by Western blot analysis in 4.4% of samples (22 of 500) from patients with various types of ARD, and 18 of those (82%) had associated disease-marker autoantibodies (against DNA or extractable nuclear antigen) (5). Only 0.8% of the samples (4 of 500) had anti-LEDGF/p75 antibodies in the absence of disease-marker autoantibodies, and 1 of those was from a patient who actually had linear scleroderma. These results indicate that, in the absence of ARD-related autoantibodies, isolated reactivity to LEDGF/p75 in a specific assay (such as in a Western blot or in a newly available enzyme-linked immunosorbent assay [from MBL] [6]) might be considered strong evidence against the presence of an ARD. This scenario corresponds to a positive ANA–HEp-2 test result with an unambiguous dense fine speckled pattern.

Finally, the finding of a positive ANA test result in an individual with no apparent disease is sometimes quoted as being a false-positive result, probably with regard to the diagnosis of systemic lupus erythematosus and related diseases. However, it must be clarified that this is not a false-positive result from the analytical point of view, because the individual does, in fact, have autoantibodies. One interesting point to be addressed in future research is the nature of such vigorous anti-LEDGF/p75 humoral responses in apparently healthy individuals. In our recent study and in previous studies on the subject (2), we observed sustained high-titer ANAs with dense fine speckled pattern over several years in individuals who have no apparent evidence of systemic disease. This humoral response might be an indicator that the immune system is being stressed by a diverse set of conditions, including environmental stimuli, medication, and subclinical infection or neoplastic lesions. These and other related speculations should be investigated in controlled studies in healthy individuals who have high-titer anti-LEDGF/p75 antibodies associated with the ANA dense fine speckled pattern.

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## Individual dosing regimen of mycophenolate mofetil in lupus patients: comment on the article by Zahr et al

*To the Editor:*

Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA), is now known as an effective and safe agent for the treatment of systematic lupus erythematosus (SLE) (1). In a recent study of 71 SLE patients receiving MMF, Zahr et al found large intersubject variability in the total MPA area under the plasma concentration–time curve from 0 to 12 hours ($AUC_{0–12}$), and a strong correlation between SLE activity and total MPA $AUC_{0–12}$ (2). In their conclusion, the authors recommended an individual dosing regimen of MMF, with a target $AUC_{0–12}$ of 35 $\mu$g/hour/ml. Indeed, the data provided by the authors suggest the need for drug monitoring in SLE patients receiving MMF. Never-

theless, in daily practice, physicians should be aware that, under certain conditions, the assessment of MPA exposure based on total MPA $AUC_{0–12}$ can be misleading. Because MPA is restrictively cleared and has a low extraction coefficient, an increase in its free fraction related to decreased albumin binding can result in lower total concentrations but unchanged unbound concentrations, as long as the intrinsic clearance of the drug is unaffected.

Two well-documented studies showed this phenomenon in renal or liver transplant recipients experiencing severe hypoalbuminemia or MPA 7-O-glucuronide (the inactive main MPA metabolite) accumulation related to poor renal function (3,4). Since hypoalbuminemia and renal failure frequently occur in patients with lupus nephritis, a low total MPA $AUC_{0–12}$ might be expected in these patients. Under those circumstances, a low total MPA $AUC_{0–12}$ may be incorrectly interpreted as low exposure to free MPA, leading to the unnecessary recommendation of a higher dosage of MMF and, therefore, to MPA overexposure that might result in MMF-induced toxicity. In this context, the best and safest way to assess MPA exposure in lupus patients experiencing hypoalbuminemia and/or renal failure would be to monitor the free concentration of MPA before modifying the daily dose of MMF. However, measurement of free MPA concentration remains cumbersome and is not routinely performed in most clinical laboratories. Therefore, hypoalbuminemia and/or renal failure should be taken into account in association with clinical end points to rationalize MMF dosing regimens in SLE patients with a low total MPA $AUC_{0–12}$.

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## Reply

*To the Editor:*

We read with great interest the comments by Thomas-Schoemann and colleagues regarding our recent article. Their main concern is that decreased albumin binding may result not only in a lower total MPA $AUC_{0–12}$, but also in unchanged exposure to free MPA, as long as the intrinsic clearance of the drug is unaffected. Thus, the use of a higher dose of MMF not only would improve the total MPA $AUC_{0–12}$, which we believe would be beneficial to the patient, but also would result in increased exposure to free MPA– and MMF-induced toxicity. Consequently, they propose that the safest way to assess MPA exposure in lupus patients who are experiencing hypoalbuminemia and/or renal failure would be to monitor the free concentration of MPA before modifying the daily dose of MMF.

We agree with Thomas-Schoemann et al that the measurement of free concentrations of MPA could be used as an additional tool for monitoring MMF treatment in SLE. However, we also believe that this approach remains largely theoretical, because it has several limitations. First, there are no published data linking free MPA concentrations and MMF efficacy in SLE, while recent reports have emphasized the importance of assessing the total MPA AUC in this disease (1–3). In a post hoc analysis of 61 patients included in our original study (additional data were not available for the remaining 10 patients), free concentrations of MPA measured at 40 and 120 minutes correlated weakly with the SLE Disease Activity Index (SLEDAI) (4) ($r = -0.26$, $P = 0.047$ and $r = -0.37$, $P = 0.003$, respectively), and accounted for only 6.7% and 13.7% of its variability, respectively. Conversely, the total MPA $AUC_{0–12}$ correlated strongly with the SLEDAI ($r = -0.64$, $P < 0.0001$) and accounted for >40% of its variability. Thus, free MPA concentrations cannot be used alone to guide MMF treatment. Second, as stated by Thomas-Schoemann et al, measurement of free MPA concentration cannot routinely be performed in most clinical laboratories. Conversely, measurement of total MPA concentration is more widely available. Third, optimal target values and, to the best of our knowledge, dedicated pharmacokinetic models for assessing exposure to free MPA have not been established in SLE.

Pharmacologic monitoring is a new and rapidly expanding field, with the potential to redefine the treatment of autoimmune diseases. One future challenge will be the identification of a limited number of markers that will allow reliable prediction of both efficacy and toxicity of immunosuppressive agents. Until then, prospective studies with exhaustive measurement of available pharmacologic parameters are needed, so that optimal monitoring strategies can be defined.

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