conclusions on the kinetics of early reconstitution after rituximab treatment. First, the authors did not specify the time points at which the reconstitution of the naive B cell subset was measured. Rouziere et al found highly mutated memory B cells in the early phase of B cell reconstitution after rituximab treatment in 2 patients with RA (3). Second, the authors did not compare reconstitution of different subsets, but focused on the most prominent subset of naive B cells. The results could be biased because shifts within the different subsets of the B cell population are not interpreted within the context of the “normal” composition in patients with RA.

We believe the frequencies of naive B cells should be compared with the frequencies of memory B cells to gain more insight into the possible mechanisms underlying early B cell reconstitution. Thus, although the majority of reconstituted B cells are naive B cells, our results suggest that the reconstitution of B cells in peripheral blood is derived from 2 different, simultaneously occurring pathways: 1) proliferation and differentiation of immature B cells from the bone marrow and 2) migration of memory B cells out of secondary lymphoid organs. The identification of these 2 mechanisms of B cell reconstitution is consistent with the findings from a study of baboons showing incomplete depletion of CD20+ B cells in the lymph nodes after rituximab treatment (4). Whether tissue depletion of B cells is complete after rituximab treatment in humans is unknown, but the lower expression of CD20 on B cells from bone marrow and lymph nodes versus peripheral blood indicates that further study is clearly needed (5).

Figure 1. Percentages of CD19+/IgD+ (naive) versus CD19+, CD27+ (memory) B cell subsets within lymphocyte gate as determined by flow cytometric analysis of peripheral blood mononuclear cells from 8 patients with rheumatoid arthritis treated with 2 consecutive infusions of rituximab (1,000 mg each). Bars show the median values.

Yoe Kie Onno Teng, MD
Aukje van Tilborg, MD
Jacob M. van Laar, MD, PhD
Leiden University Medical Center
Leiden, The Netherlands

3. Rouziere AS, Kneitz C, Palanichamy A, Dorner T, Tony HP.


Reply

To the Editor:

We thank Dr. Teng and colleagues for their interest in our study. However, most of the points they raise would seem to be covered within the original article. Their graph data show very high frequencies of CD27+ B cells at repopulation, suggesting that CD27high plasma cell precursors were included with CD27+ B cells. The coexistence of naive and memory B cells at repopulation can either reflect the existence of clones resistant to treatment or simply indicate rapid maturation of newly generated cells. Only if memory (CD27+) B cells appeared significantly earlier than naive B cells could we conclude that clones resistant to treatment contribute to repopulation. Since this does not occur, and significantly increased numbers of immature B cells are identified, the interpretation that repopulation is largely dependent on formation of new B cells seems entirely appropriate. There remains an important question about the significance of clones resistant to treatment, but as yet we do not have the answer to this question.

Maria J. Leandro, MD
Geraldine Cambridge, PhD
Michael R. Ehrenstein, MD, PhD
Jonathan C. W. Edwards, MD
University College London
London, UK

DOI 10.1002/art.22066

Replication in genetic association studies: comment on the editorial by Spector et al

To the Editor:

We read with great interest the recent editorial by Spector et al on replication in genetic association studies (1). The authors point out that replication studies should be designed carefully and implemented to a high standard to avoid unreliable results. In this editorial, various aspects of our previously published report (2) are described inaccurately, and
as a result, the findings of our study are interpreted completely out of context.

The errors concern the random sample of 1,369 subjects from the Rotterdam study, a prospective population-based cohort in which the presence of radiographic osteoarthritis (OA) is assessed at 4 different joint sites. Subjects in this study were therefore not, as stated by Spector et al, selected for the presence of primary symptomatic OA at multiple sites. Selection in another hospital-based study, the Genetics, osteoArthritis and Progression (GARP) study, is indeed based on the presence of OA at multiple sites. Spector et al note that no significant association between the R324G variant in the \textit{FRZB} gene and generalized OA was found when the Rotterdam data were combined with data from the GARP study; however, the essence of the article was that we did find a significant association (odds ratio 1.6, \( P < 0.02 \)) with the generalized OA phenotype. Spector et al indicate that we carried out association analysis only in a mixed cohort of men and women. As described in our report, we also stratified for women only, which did not reveal any additional associations.

Subsequently, Spector et al erroneously use our study (2) as an example in which the lack of replication of the original association of the \textit{FRZB} gene with hip replacement in women (3) is due to differences in the phenotypes used. This is compared with the study by Lane et al (4), which is included as an example of true replication. We believe this contrast does not help much in clarifying the discussion on phenotypic criteria in OA research, for the following reasons. In our study, we did not observe association of \textit{FRZB} variants with radiographic hip OA cases in the Rotterdam sample, and we argued that the absence of association may reflect the poor correlation between radiographic and symptomatic hip OA. The absence of association is unlikely to be due to the use of a phenotype other than joint replacement, however, since Lane et al (4) did find association of \textit{FRZB} variants with radiographic hip OA in women in the Study of Osteoporotic Fractures, which recorded no symptomatic data and excluded women with joint replacement. According to the criteria set forth by Spector et al, this should be seen as a confirmation, not a replication, and in our study we simply could not replicate the findings of the Lane study.

We subsequently investigated whether the \textit{FRZB} variants might also be associated with other heritable phenotypes (2). This is a reasonable and relevant question given the central role of this gene in skeletal development. Since we did find association of the R324G variant with a generalized OA phenotype in both the Rotterdam and GARP study samples (2), we stated that although the specific joint sites among patients differ between the study by Loughlin et al (3) and the GARP study, both studies include patients with severe symptomatic disease. We suggested that the R324G variant may predispose to development of severe symptomatic OA that may be expressed at different joint sites.

In summary, our study and the study by Lane et al confirm that carriers of the \textit{FRZB} variant have increased susceptibility to OA development, which may not be restricted to the phenotype criterion of hip replacement in women. It is very relevant that studies and data are correctly described in editorials, such as the one by Spector et al. Indeed, true replication should be based on the same clinical definition of phenotype.


DOI 10.1002/art.22116

\textbf{Reply}

\textit{To the Editor:}

In our recent editorial, we tried to highlight some of the difficulties in interpreting and comparing results from genetic association studies in general. To this end, there was no attempt to criticize the study design, method of analysis, or the conclusions drawn from any particular study. Rather, we used the subject as a practical example of current problems for readers and reviewers interpreting genetic association studies. We limited ourselves to comparing the results from 3 studies investigating the association between 2 \textit{FRZB} polymorphisms and osteoarthritis of the hip, and pointed out that subtle differences between studies could leave the general reader of \textit{Arthritis & Rheumatism} somewhat confused.

For example, we tried to highlight that the results reported by Lane and coworkers (1) are directly comparable with those found in the original study by Loughlin et al (2) given the clinical definitions used, whereas the data presented by Min et al (3) in their Table 2 refer to similar, but not quite the same, traits. We did not use (as suggested in the letter by Min et al) their study as an example of lack of replication due to differences in phenotypes used. Rather, we merely hypothesized that some of the differences in methodology and clinical definitions “could partly explain the differing results from the 3 studies.” We hope we have highlighted the general need for investigators of common disorders to specify clearly the populations used and the specific phenotypes analyzed so as to improve the interpretation of the original study and facilitate further studies.

Tim D. Spector, MD, MSc, FRCP
Kourosh R. Ahmadi, PhD
Ana M. Valdes, PhD
King’s College London
St Thomas’ Hospital Campus
London, UK