(1–3). In the first report by Barton et al (1), the IVIG infusion was followed by a decrease in IgG and IgM serum levels within 72 hours, and a typical biopsy-proved cryoglobulinemic membranoproliferative glomerulonephritis. In a case described by Odum et al (2), IVIG infusion was followed within 48 hours by diffuse purpura, a rise in plasma creatinine levels, a microscopic hematuria, and high-level proteinuria strongly suggestive of glomerulonephritis. Yebra et al reported a flare of hepatitis C virus–related cryoglobulinemic vasculitis 4 hours after the first IVIG infusion, an increase of cryoglobulin precipitation, and depletion of the monoclonal IgMκ after in vitro addition of IVIG, and suggested that this simple method could help to predict the risk of cryoglobulin–IVIG immune complex formation and should be performed before starting IVIG in patients with mixed cryoglobulinemia (3). As with infliximab and rituximab, we have reported in our article that polyvalent exogenous human immunoglobulins were also recognized in vitro by RF-positive IgMκ.

Taken together, these results strongly suggest that the recognition of monoclonal or polyclonal immunoglobulin by RF-positive IgMκ is not specific and that treating RF-positive IgMκ cryoglobulinemic vasculitis with either monoclonal immunoglobulins (e.g., rituximab or infliximab) or polyvalent immunoglobulins is associated with a risk of increased cryoprecipitation and vasculitis flare shortly after treatment initiation.

We believe that, in the presence of RF-positive IgMκ type II cryoglobulinemic vasculitis, any treatment with IVIG should be used with caution. IVIG does not have the clear benefit of rituximab in cryoglobulinemic vasculitis, and there is not a rationale for the use of monoclonal anti–tumor necrosis factor α antibodies. The use of rituximab, should, as well, be proposed cautiously in patients with a RF-positive IgMκ type II cryoglobulinemic vasculitis. We recommend that plasma exchanges should be performed to reduce high serum cryoglobulin levels, and that rituximab should be given in low doses. This precaution should also be recommended for other treatments that are based on B cell–depleting monoclonal antibodies, which have not yet been used in cryoglobulinemic vasculitis, such as veltuzumab (anti-CD20), inotuzumab ozogamicin, and epratuzumab (anti-CD22).

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Enhanced NF-κB activation with an inflammasome activator correlates with activity of autoinflammatory disease associated with *NLRP3* mutations outside of exon 3: comment on the article by Jéru et al

To the Editor:

We wish to comment on a recent report regarding functional consequences of *NLRP3* mutations (1). Based on the genetic analysis of a family with atypical autoinflammatory symptoms, Jéru et al identified a mutation in the leucine-rich repeat (LRR) domain of *NLRP3*. *NLRP3* is a 9-exon gene comprising 3 major domains: an amino-terminal pyrin domain, a nucleotide-binding oligomerization domain (NOD), and carboxyl-terminal LRR. More than 50 disease-associated mutations have been described for *NLRP3*; most were found within the centrally located NOD encoded by exon 3.

Jéru et al reported that NLRP3 was an inhibitory molecule, although the function of NLRP3 in NF-κB signaling remains controversial. We previously reported that *NLRP3* mutations showed spontaneous ASC-dependent NF-κB activation, and this was clearly associated with disease severity in patients with cryopyrin-associated periodic syndrome (CAPS) (2). However, among 11 mutations identified from our recruited patients (3), G755R located in exon 4 did not show spontaneous NF-κB activation (Figure 1A), even though a patient carrying G755R had severe disease manifestations. To date, 2 other *NLRP3* mutations located outside of exon 3 have been reported: G755A (4) and Y859C (5). G755A was identified in a typical CAPS patient (4). In contrast, a patient with Y859C had only 1 episode of a transient rash and, of note, absence of fever (5). A family member with Y859C (1) also did not manifest skin eruptions and showed a relatively mild phenotype. As seen in Figure 1A, neither G755A in exon 4 nor Y859C in exon 6 exhibited NF-κB activation.

For *NOD2*, gain-of-function mutations associated with granulomatous disorders are recognized in the centrally located *NOD* and exhibit similar spontaneous activation of NF-κB without the *NOD2* ligand (6). Interestingly, the amino acids affected by an R260W mutation in *NLRP3* and an R334W mutation in *NOD2* are at analogous positions, suggesting a common molecular mechanism for development of autoinflammatory disease. In contrast, *NOD2* mutations at LRRs, related to Crohn's disease, show defective responses to the *NOD2* ligand. In the presence of R837, an NLRP3-inflammasome activator, a G755R mutation located outside exon 3 of *NLRP3* showed remarkably enhanced NF-κB activation with an activity level that was higher than that observed with the R260W mutation (Figure 1B). With regard to 2 other mutations located outside of exon 3, G755A showed slightly increased NF-κB activation with R837, whereasY859C, which was identified in the atypical CAPS family whose members had mild phenotypes, did not. Thus, the enhanced NF-κB activation after stimulation with R837 correlates with disease activity, including mutations outside of exon 3. However, we still do not know how R837 activates the NLRP3 inflammasome.

We agree that *NLRP3* mutations, especially those identified from de novo cases, should be carefully evaluated by functional analyses. We believe that, in addition to excess production of interleukin-1β (IL-1β), enhanced NF-κB activa-

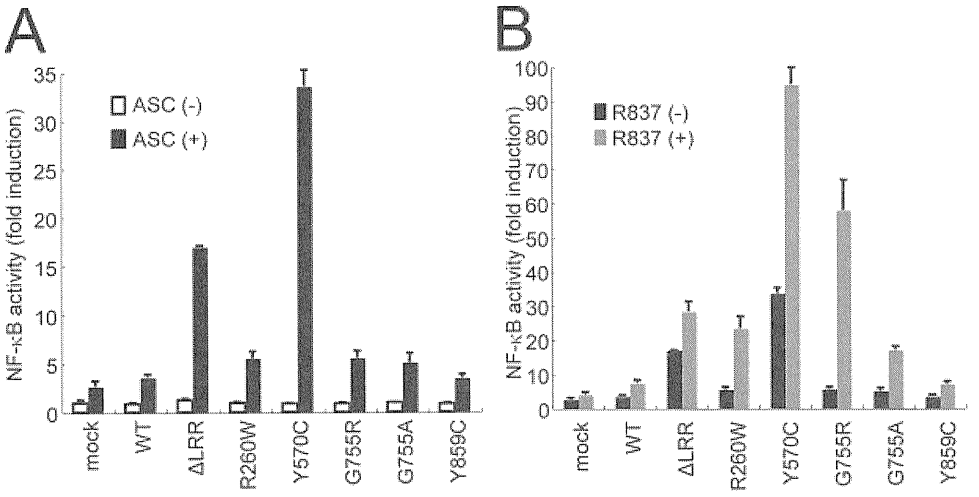

Figure 1. NF-κB activity in disease-associated mutations of *NLRP3*. **A,** Spontaneous NF-κB activation occurs in disease-associated mutations of *NLRP3*, but not in mutations located outside of exon 3. Expression plasmids for NLRP3, its deletion mutation lacking leucine-rich repeats (ΔLRRs), and ASC in the pEF-BOS vector background have been previously described (2). Mock is an empty vector. WT is wild-type NLRP3. R260W and Y570C are disease-associated mutations located within exon 3. G755R, G755A, and Y859C are the mutations outside of exon 3. **B,** Addition of R837 induces appreciable NF-κB activation in G755R mutations compared with WT *NLRP3*. ASC-dependent activation of NF-κB in the presence and absence of 10 μg/ml R837 was assessed. Values are the mean and SD of normalized data (in relation to mock with ASC [**A**] or R837 [**B**], set at 1), from triplicate cultures. Representative data from 3 independent analyses with similar results are shown.

tion may be associated with the accumulation of the IL-1β proform (7), which may also contribute to disease onset and the clinical manifestations of CAPS. In addition, careful observations of patients who bear *NLRP3* mutations outside of exon 3, who sometimes present with atypical symptoms of CAPS as in the cases described by Jéru et al, may provide an opportunity to better understand the physiologic and pathologic functions of NLRP3.

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Reply

To the Editor:

We thank Dr. Kambe and colleagues for their comments. As mentioned by Kambe et al and in our article, research on the function of NLRP3 in NF-κB signaling has led to many conflicting data. Several studies have shown an activating effect of NLRP3 in the presence of ASC (1–5), while