REPLY TO ROERINK ET AL:
Methods for recruitment, serum separation, and storage were the same for patients and controls

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Roerink et al. (1) raise important and potential methodological biases that could have accounted for our finding regarding elevated TGF-β levels in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (2). Here, we provide additional information as requested by Roerink et al. (1) that supports that the elevation of TGF-β in patients with ME/CFS is most likely to be rooted in the biology of ME/CFS and not due to methodological issues. As stated in the materials and methods of ref. 2, both patients and controls were recruited from Northern California from March 2, 2010 to September 1, 2011. Their peripheral blood was drawn between 8:30 AM and 3:30 PM on the day of enrollment. In addition, as each patient with ME/CFS was being recruited into the study, two corresponding age- and sex-matched controls were contemporaneously enrolled until the target sample size of 200 patients and 400 controls was achieved. This approach resulted in patients and controls being intercalated in their time of entry into the study. As stated in the abstract, discussion, and materials and methods of ref. 2, serum, not plasma, was obtained for this study. The protocol to draw, process, and store blood was identical for both patients and controls. Eight milliliters of blood was drawn into a red-toppled serum tube (Fisher Scientific) by the phlebotomy team of the Stanford Clinical & Translational Research Unit (med.stanford.edu/spectrum/b1_researcher_resources/b1_4_ctru.html). The red tube was stored at room temperature for 40 min to allow clotting. Once clotted, the blood tube was centrifuged in a refrigerated (4 °C) centrifuge (Allegra X-15R; Beckman Coulter) at 2,000 x g for 15 min. Serum was isolated and mixed thoroughly in a tube using a 2-mL sterile serological pipette (Fisher Scientific) to obtain a homogeneous solution before dispensing to storage tubes. Serum was distributed into aliquots per the Stanford Human Immunology Monitoring Center (iti.stanford.edu/himc.html) aliquot guidelines and frozen at −80 °C. For the day of the cytokine assay, matched sets of ME/CFS cases and healthy controls were always mixed in all plates to reduce confounding case status with plate artifacts. In summary, patients with ME/CFS and controls were treated identically in terms of recruitment and sera handling protocols. Thus, it is more likely that TGF-β differences between patients with ME/CFS and controls in our study are due to biological, rather than methodological, differences between the two groups. TGF-β has also been found elevated in patients with ME/CFS in five other studies (3–7). In one study (5), TGF-β bioactivity was found higher in patients with ME/CFS than in controls and patients with major depression, systemic lupus erythematosus, relapsing/remitting multiple sclerosis, and chronic progressive multiple sclerosis as well.


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