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Letter to the Editor

Natural Killer Cells in Chronic Lyme Disease

We appreciate the interest of Marques et al. in the assessment of immune parameters in Lyme disease. The conclusions of their report (2) appear to differ from the findings of our study of $CD3^-$ $CD57^+$ natural killer (NK) cells in patients with persistent symptoms of tick-borne illness (3). Further scrutiny reveals that their analysis employed questionable patient selection criteria and unproven testing and ultimately lacked the power to detect the differences observed in our study.

The two reports have little in common. Our study examined 73 patients with a female/male ratio of 1.6:1, consistent with the gender distribution of patients with chronic Lyme disease (1, 5). Using a flow cytometry test system with an established normal range and well-defined coefficient of variation, we found that the $CD3^-$ $C57^+$ NK subset appears to be a useful immunologic marker in patients with persistent Lyme disease symptoms compared to either normal subjects or 32 disease controls (3). Importantly, factors that appeared to influence the CD57 NK levels were the predominant type of Lyme symptom and response to antibiotic treatment on serial sampling (3).

In contrast, Marques et al. analyzed nine patients with "post-Lyme disease syndrome," a newly described and unvetted diagnostic entity defined by testing that is biased against women (5). These nine patients were selected with a female/male ratio of 2:1 and compared to nine predominantly male controls and 12 patients with unknown serologic test results who had "recovered" from poorly characterized symptoms of Lyme disease. With this small sample size, an excessive discrepancy of 100 cells/μl (corresponding to 2.5 to 5.0 standard deviations in our patient population) would be necessary to detect a significant difference in the NK cell counts. Thus, the study had insufficient power to conclude that there was no difference among these small and poorly matched patient groups. The authors also failed to correlate NK cell numbers with patient symptomatology and/or antibiotic therapy, and serial sampling was not performed.

In terms of NK testing, Marques et al. failed to establish a normal range for the $CD3^-$ $CD57^+$ subset, and they did not report the coefficient of variation of their flow cytometry testing. Thus, the test system itself has no documented consistency or relation to either population norms or other diseases. The scatter plot suggests that the authors were examining a heterogeneous group of patients, making statistical analysis meaningless in this small patient sample.

In summary, Marques et al. have provided questionable data about the $CD3^-$ $CD57^+$ NK subset in an underpowered analysis of a heterogeneous group of patients, and their data are insufficient to reach a meaningful conclusion. As noted in our larger population-based study, which was supported by more recent immunologic evaluation (4), the $CD3^-$ $C57^+$ NK subset appears to be a useful immunologic marker in patients with persistent Lyme disease symptoms.

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Authors' Reply

Dr. Stricker and Winger's letter criticizing our study (9) contains misinterpretations and inaccuracies.

First, their claim that our patient selection criteria were "questionable" is not correct. The patient selection criteria were clear and well described in the manuscript. On the other hand, "chronic Lyme disease" (CLD) is an ill-defined term that includes patients with post-Lyme disease syndrome (PLDS), as well as patients with other conditions (misdiagnosed as, or misattributed to, CLD), with the majority of patients diagnosed with CLD having no evidence of prior Lyme disease (4). In this context, patients with PLDS is the subpopulation of "CLD" patients that is the best defined, requiring patients to actually have had a documentable antecedent infection with *Borrelia burgdorferi*. PLDS has been the subject of the more scientifically rigorous studies (3, 6, 7). As described in our paper, recovered controls had had objective evidence of Lyme disease and had fulfilled the CDC case definition of Lyme disease (1).

Stricker and Winger write that PLDS is an "unvetted diagnostic entity defined by testing that is biased against women," without any real evidence to support this rather inflammatory commentary. The reference cited to support this claim (12) actually shows that there is an equal gender distribution among patients diagnosed with PLDS. Also, the reference cited by Stricker and Winger (5) to support the claim that patients in their study (10) had a gender distribution consistent with CLD

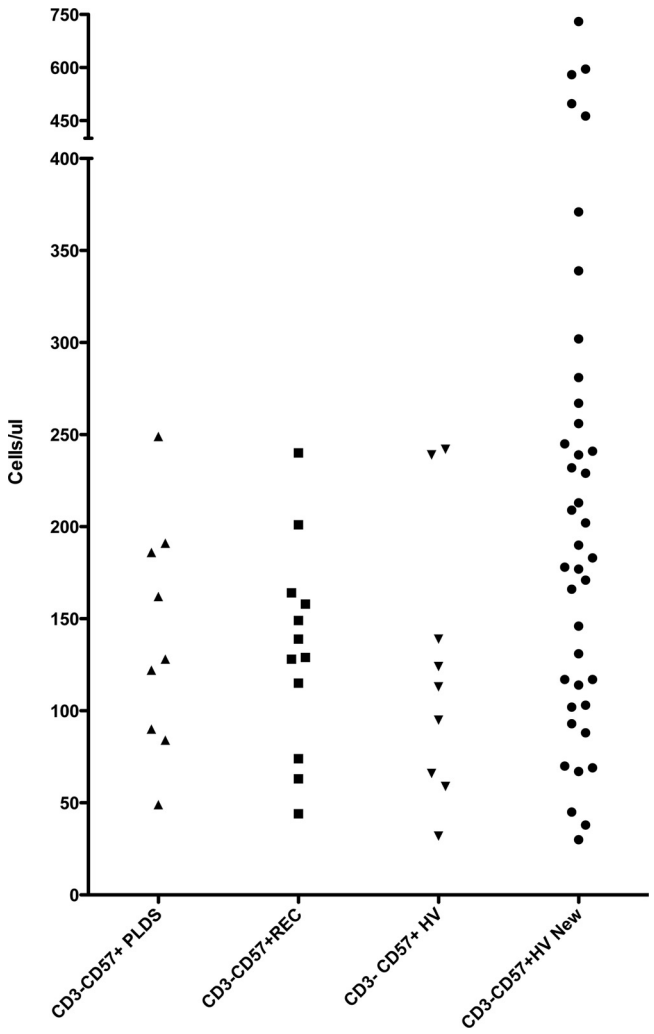

FIG. 1. $CD3^-$ $CD57^+$ cell numbers in PLDS patients, individuals who have recovered from Lyme disease (REC), and healthy volunteers (HV), previously published, and a new group of 40 healthy volunteers (HV New).

has no relevance to CLD whatsoever since it addresses a completely different issue in Lyme disease (reinfection). As for PLDS being "unvetted," this entity is clearly better defined and studied than CLD, as discussed above.

Stricker and Winger claim that the flow cytometry test system used in their study (10) had an "established normal range and well-defined coefficient of variation." We are unaware of any published data to support this claim. Their study only cites a normal range for $CD3^-/CD57^+$ but provides no supportive data, as their study included no healthy volunteers and no repeated measurements from the control donors. Furthermore, we are unaware of any published literature that provides such data specifically on $CD3^-/CD57^+$ cell counts, as this measurement is not used in any other medical context. We are unaware of a flow cytometry laboratory that performs this assay routinely (outside of laboratories offering this test to practitioners using it for CLD). Moreover, as discussed in our study, the measurement of $CD3^-/CD57^+$ cells is not a standard flow cytometry approach for measurement of natural killer (NK) cells; rather, the routine approach for NK quantitation utilizes a combination of CD56 and CD16 surface expression together with negative staining for CD3 (to exclude T cells expressing NK markers). Therefore, for the purposes of our study, healthy volunteers served as the sample source to establish the reference range.

Stricker and Winger criticize that we did not correlate $CD3^-/CD57^+$ counts with patients' symptoms, but in their study the decrease was reported to occur in all patients not receiving antibiotic therapy. None of our patients was receiving antibiotic therapy.

While Stricker and Winger are correct that our study does not have power to look at small differences between the mean numbers of cells of the different groups, the complete overlap between the ranges of values of patients and healthy volunteers indicates that this test is not helpful for evaluating or monitoring the patient groups we studied. For this reason, there is no point in performing serial samples or correlations with patient symptoms.

Furthermore, we have just completed an analysis of an expanded group of healthy volunteers consisting of 40 subjects. In this evaluation of controls, the absolute values for $CD3^-/CD57^+$ cells ranged from 30 to 730 cells/mm^3. These results are shown in Fig. 1, together with the values from PLDS patients, recovered patients, and the group of healthy volunteers previously provided in our paper. We also found that there was a sizeable variation in the numbers of $CD3^-$ $CD57^+$ cells over time based on testing of five healthy volunteers twice, within a 5- to 12-week interval. These data demonstrated that $CD3^-$ $CD57^+$ counts changed in controls over the time interval and that this ranged from a decrease of 124 cells/mm^3 to an increase of 24 cells/mm^3.

Another point, only briefly alluded to in our paper, is that the claimed decrease of $CD57^+$ cells in patients thought to be suffering from a chronic infection is at odds with what has previously been reported regarding CD57. CD57 expression is thought to be a marker of terminally differentiated cells (2), and expansion of $CD57^+$ cells has been associated with chronic antigen stimulation and activation of the immune system (8, 11).

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