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## Role of T Cells in EBV-Infected Systemic Lupus Erythematosus Patients

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## LETTERS TO THE EDITOR

**S100 Proteins in Monitoring Inflammation: The Importance of a Gold Standard and a Validated Methodology**

In the May issue of *The Journal of Immunology*, Abe et al. reported that S100A8, S100A9, and S100A12 are among the genes most profoundly up-regulated during acute Kawasaki disease (1), supporting previously published data (2–4). The authors found profoundly differing serum concentrations using different ELISA kits. They explain these differences with the presence of monomers and complex forms of S100A8 and S100A9. However, there is clear evidence proving that the S100A8/S100A9 complex is the relevant form in vivo (5–7). In our opinion, differing concentrations reported in this article can be simply explained by the use of various Abs detecting different epitopes with different affinities. Especially, quantitative comparison with data generated using the mAb 27E10, which detects a specific epitope formed exclusively by the S100A8/S100A9 complex, is critical. Lack of formation of this unique epitope by the protein standard may explain high concentrations of S100A8/S100A9 reported in this article. We use S100A9-specific Abs and calibrate our ELISA with a protein standard that was extensively characterized by independent methods to ensure correct determination of S100A8/S100A9 concentrations (8, 9). Exact characterization of the gold standard is a crucial step mandatory for any assay before measuring S100A8/S100A9 serum levels for diagnostic purposes.

Proinflammatory S100 proteins are differentially expressed in Kawasaki disease, may contribute to vasculitis, and may serve as biomarkers for monitoring inflammation. However, there are serious pitfalls associated with the detection of these proteins in patient samples. Therefore, both the methodology of detection assays and the interpretation of data have to be validated thoroughly.

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**The Authors Respond**

We appreciate the comments by Drs. Foell and Roth. We reported that plasma S100A8/A9 heterocomplex levels were significantly higher in preintravenous infusion of high-dose IG (IVIG) Kawasaki disease (KD) patients ( $25.3 \pm 1.5 \mu\text{g/ml}$ ) than in post-IVIG patients ( $18.4 \pm 1.7 \mu\text{g/ml}$ ) and febrile controls ( $10.7 \pm 1.0 \mu\text{g/ml}$ ) (1). In the paper, we used an MRP8/14 ELISA kit (Buhlmann Laboratories) that does not cross-react with S100A8 or S100A9 monomer (2). Although we did not measure healthy controls by this kit, the manufacturer's proposed serum cut-off titer in healthy adults was  $12.4 \mu\text{g/ml}$ . In addition, we obtained similar results after we measured the same samples with our ELISA using the mAb 27E10 and the recombinant S100A8 and S100A9 proteins as protein standard ( $r = 0.852$ ,  $p < 0.0001$ , our unpublished data). Thus, we believe the different S100A8/A9 plasma levels observed between the pre- and post-IVIG patients reflect exact changes brought on by IVIG therapy and were not caused by using various Abs detecting different epitopes with different affinities as Drs. Foell and Roth suggested.

With regard to the plasma S100A9 levels, we reported much lower values compared with S100A8/A9 levels for both pre-IVIG patients ( $12.8 \pm 2.6 \text{ ng/ml}$ ) and febrile controls ( $12.5 \pm 5.8 \text{ ng/ml}$ ) by using a MRP14 ELISA kit (Chemicon International) (1). The kit contains two mAbs that detect different epitopes of S100A9 and do not cross-react with S100A8/A9 heterocomplex. These results indicate that S100A9 is mainly present as heterocomplex with S100A8 in human plasma. In this respect, we agree with Drs. Foell and Roth that the S100A8/A9 heterocomplex is the relevant form in vivo (3, 4).

Finally, our oligonucleotide microarray analysis suggests that suppression of an array of immune activation genes underlies the effect of IVIG therapy in KD patients. S100A8/A9 is one of those most significantly down-regulated genes after IVIG. It is important to determine whether plasma S100A8/A9 levels are reliable markers for the responsiveness of patients to the therapy and to elucidate the role of S100A8/A9 in the pathogenesis of KD as well as other autoimmune diseases (5).

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## Role of T Cells in EBV-Infected Systemic Lupus Erythematosus Patients

Gross et al. (1) have provided new data demonstrating that the immunological response to EBV is abnormal in individuals with systemic lupus erythematosus (SLE) and other autoimmune diseases and reviewed previous studies over the past decade with similar observations. Abnormalities of T cell function and increased EBV genomes detected by PCR in lymphocytes are hallmarks of EBV-associated autoimmune diseases including SLE, as noted by the authors (2–6).

We and others have noted the ability of EBV to infect both primary T lymphocytes and T lymphoblastoid cell lines (7, 8), and noted in particular the expression of both the BZLF-1 transcript and the alternatively spliced RAZ in T cells (9, 10). We have also demonstrated in this journal and elsewhere that expression of BZLF-1 protein has potentially important effects upon T cells both through inactivation of signaling by NF- $\kappa$ B (11) and enhanced signaling by p53 (12), both of which would enhance T cell death through apoptosis.

The purification method used by the authors (1) to provide what are described as purified B lymphocytes, a non-B cell specific Ab binding step followed by affinity removal of non-B cells, results in ~90% B cells, and thus by definition ~10% non-B cells. These non-B cells are not characterized further, but it is likely that they are largely T lymphocytes, which are similar in size and physical properties to B lymphocytes, and would aggregate with B lymphocytes.

These T lymphocytes are possibly infected with EBV genomes and expressing a combination of both lytic and latent EBV genes at some small but significant frequency (8) and cannot be ignored. EBV-infected T lymphocytes, in contrast to B lymphocytes, express BZLF-1 transcripts, possibly explaining the presence of these transcripts not normally typical of latently infected B cells.

Furthermore, infection of T cell by EBV in SLE and other autoimmune diseases also may provide some insights into the pathogenesis of these diseases. The receptor for EBV CD21 is expressed primarily upon immature T cells, and declines markedly as the cells mature (13–15). Abnormal expression or func-

tion of CD21 is noted both in human and murine models of SLE (16, 17). If patients with SLE and other disorders release T cells from the thymus at a relatively immature state, these cells would be infected with increased frequency in the periphery. Alternatively, these cells could be infected in the thymus and then migrate to the periphery. In contrast to EBV, CMV does not use CD21 as a receptor and thus CMV levels would not be altered by aberrant CD21 expression on T or B cells as noted experimentally (1).

Increased circulating immature T cells could account for the increased frequency of infected lymphocytes and aberrant expression of BZLF-1 noted (1). This possibility should be commented upon by the authors and if possible addressed through additional studies to differentiate EBV-infected T cells from EBV-infected B cells in their analysis. Whether increased infection of both T lymphocytes and/or B lymphocytes is causally related to the diseases in question or an epi-phenomenon remains to be determined.

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## The Authors Respond

**W**e appreciate the thoughtful comments of Dr. Dreyfus on our work (1). It is interesting to consider that EBV-infected T cells could account for the expression of BZLF we observed in the blood of some patients. However, this is improbable.

On individual patient samples, we generally performed both limiting dilution DNA-PCR to calculate the frequency of infected cells, and RT-PCR to detect EBV gene expression. Therefore, we could calculate the number of infected cells in the samples on which we performed RT-PCR. These samples contained between 8 and 40 EBV-infected cells. The literature does not provide any estimate of the frequency of EBV-infected T cells in the blood; however, in the tonsils of patients with acute EBV infection (infectious mononucleosis), EBV infection of T cells is either a "rare" event (2) or undetectable (3). If one makes the generous assumption that 1% of the EBV-infected lymphocytes in the blood are T cells, rather than B cells, then in a sample of at most 40 infected cells it is quite unlikely that any of the infected cells would be T cells.

The likelihood of these samples containing an EBV-infected T cell falls further when one considers the B cell (and T cell) purity of the samples that were tested. Among the patient samples that contained BZLF expression, the median B cell purity was 92% (range 61% to 99%). For the purposes of this discussion, we will assume that 10% of the cells in these samples were T cells. (It is unlikely that all of the contaminating cells were T cells, but we did not study this issue directly.) Again, assuming that 1% of EBV-infected lymphocytes in the blood are T cells, this would mean that at most 0.1% of the EBV-infected cells in these samples were T cells (T cells account for 10% of the cells in the sample multiplied by 1% EBV-infected cells that are T cells) whereas 99.9% of the infected cells were B cells. Therefore, even if our samples contained 100 infected cells, it still

would have been very unlikely that they contained a single infected T cell, much less that they contained an EBV-infected T cell that also expressed BZLF.

It should also be noted that EBV infection of primary T cells remains a point of contention in the literature. EBV infection of T cells has only been demonstrated in unusual circumstances, such as infectious mononucleosis (2) (which others have refuted (3)), T cell tumors (4), and by infecting cultures of thymocytes in vitro (5). In contrast, T cell infection with EBV has not been demonstrated in the blood or tonsils of healthy individuals. Although SLE patients certainly are not equivalent to healthy individuals, in the absence of any data demonstrating EBV infection of T cells in these patients, it is overly speculative to suggest that the abnormal T cells homeostasis present in SLE facilitates EBV infection of these cells.

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