Are We Accurately Quantitating Immune Complexes by Radioimmunoassay?

To the Editor:

In the last two decades the role of complexes of antigen and antibody (immune complexes, IC) in the pathogenesis of many diseases has become more and more evident, and numerous tests have been devised for detecting and quantitating circulating IC. Although there appears to be disagreement among results by various methods (1), IC are often assayed in clinical laboratories. Recently it was suggested that plasmapheresis can be effective for treating patients with systemic lupus erythematosus (SLE), presumably by removing IC (2). Here the accuracy of tests for IC quantitation is crucial. In assays for quantitating IC, heat-aggregated human IgG with added complement is used as a standard, and the values obtained with patient's sera are expressed as IgG equivalent. In radioimmunoassays (RIA) for measuring IC, serial dilutions of aggregated IgG are used to construct standard curves, from which the concentrations of IC in sera can be determined. A widely used assay is the Raji cell RIA (3), in which lymphoblastoid cells are used to bind IC via their complement receptors. Radiolabeled anti-IgG (or protein A) is subsequently reacted with the Raji cells.

A criterion of the accuracy of an RIA is the parallelism between the binding curves obtained with serial dilutions of samples and with serial dilutions of the standard. Lack of parallelism of the concentration-reactivity curves leads to inaccurate results when the same sample is assayed at various dilutions. Patients with SLE often have high amounts of circulating IC, and their sera require dilution so that the amount of IC can be read from the standard curve.

We investigated whether the binding of IC from patients with SLE to the Raji cells is similar to that of aggregated human IgG to which complement (fresh human serum) was added. We diluted, with phosphate buffer containing bovine serum albumin, the sera from three patients who had high amounts of circulating IC in an initial screening test and assayed each sample for IC with the Raji cell RIA. The curves obtained with patients' sera were not parallel with the standard curve (Figure 1), as evidenced by the different results obtained with several dilutions of the same serum sample, e.g., 2000 μg of IgG equivalent at 1:4 dilution, 7520 at 1:8, and 10,560 at 1:16 dilution of the same sample. Thus, the binding of complement-containing IC to the Raji cells appeared different from the binding of aggregated IgG to which complement was added. Perhaps there are different kinds of IC in various patients with SLE, which do not react identically with the Raji cells as does the aggregated IgG. The size of IC can vary with time, even in the same patients, and this puts in question the possibility of monitoring IC in patients who are undergoing various therapeutic measures such as hemodialysis (4) or plasmapheresis—i.e., accurate quantitation of IC is not possible. The heat treatment of IgG is known to produce various sizes of aggregates, but separating aggregates by size through gel filtration or ultracentrifugation gave similar findings.

Although RIAs for IC quantitation (e.g., Raji cell or Clq solid-phase assays) are useful tests for detecting small amounts of certain types of circulating IC, it should be remembered that, the value of these assays for quantitating IC with reference to aggregated human IgG is quite limited.

References
1. Lambert, P. M., et al., A WHO collabora-

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LD-1/LD Ratio as a Diagnostic Determinant for Myocardial Infarction

To the Editor:

We are currently conducting a study of the merits of many of the chemical tests used to assist in the diagnosis of myocardial infarction. We, therefore, read with great interest the paper by Bruns et al. (Clin. Chem. 27:1821, 1981) in which they propose the use of the ratio of LD-1 to total LD (lactate dehydrogenase, EC 1.1.1.27) as a diagnostic determinant for myocardial infarction. We too have collected data from many patients in the Coronary Care Unit (CCU) whose diseases have been diagnosed by standard clinical criteria, so it seemed valuable to apply the LD-1/LD ratios to our data.

In our study, patients' sera was collected at first presentation, at 0800 hours the subsequent morning, and thereafter for two days. The data found to be of most value were those from the first two samples. In the cases we