Evaluation of a Cordia-IC Enzyme-Linked Immunosorbent Assay Kit for the Detection of Circulating Immune Complexes

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A commercial kit (Cordia-IC) from Cordis Laboratory, Miami, Fla., was compared with the Raji cell radioimmunoassay for its ability to detect circulating immune complexes (CIC) in sera from 30 control subjects and 118 patients with infectious diseases. The 118 patients were categorized into the following groups: (i) 23 patients with bacterial endocarditis, (ii) 41 patients with bacteremia from an infected intravascular catheter or access device, and (iii) 54 patients with Staphylococcus aureus bacteremia related to a deep tissue infection. The Cordia-IC was comparable to the Raji cell radioimmunoassay in intraassay variability (4.0 versus 8.0%) and interassay reproducibility (8.7 versus 20.0%). Neither assay found CIC amounts above 12.5 μg equivalents (eq) of aggregated human gamma globulin (AHG) per ml in any of the 30 control individuals. In group 1, Cordia-IC detected 19 of 23 positives (mean, 73.6 μg eq of AHG per ml), whereas the Raji cell detected 16 of 23 positives (mean, 54.8 μg eq of AHG per ml). In group 2, Cordia-IC detected 19 of 41 positives (mean, 20.6 μg eq of AHG per ml), whereas the Raji cell detected 16 of 41 positives (mean, 15.1 μg eq of AHG per ml). In group 3, Cordia-IC found 38 of 54 positives (mean, 28.0 μg eq of AHG per ml), whereas the Raji cell found 32 of 54 positives (mean, 23.9 μg eq of AHG per ml). Statistically, these findings were not significantly different in any of the three patient groups (P > 0.15), and there was an overall good correlation between the results obtained by the two assays (r = 0.64, P < 0.001). The Cordia-IC provided a suitable assay for the detection of CIC and might find application in routine clinical laboratories.

The significance of circulating immune complexes (CIC) in patients with chronic antigenic stimulation related to infections, autoimmune diseases, or certain malignancies is now well established (1, 3, 6, 7). The detection and quantitation of these CIC are important for diagnosis as well as for monitoring the response of patients to therapy (2). Unfortunately, the current assays for the detection of CIC are not applicable for widespread use in routine clinical laboratories. Such disadvantages as the lack of automation and standardization and the use of radioactive compounds restrict the availability of these assays to specialized centers. Recently, Cordis Laboratory, Miami, Fla., developed a commercial kit (Cordia-IC) for CIC measurement by an enzyme-linked immunosorbent assay (ELISA) to quantitate the binding of immune complexes to Clq. The purpose of our investigation was to compare the Cordia-IC with the Raji cell radioimmunoassay (RIA) that is currently used in our clinical immunology laboratory. We evaluated the ability of both methods to detect and quantitate CIC in patients with infectious diseases including endocarditis, intravascular catheter-related and arteriovenous access device infections, and deep tissue infections.

MATERIALS AND METHODS

Patient clinical categorization. Patients were divided into three major infection categories based on a review of clinical records and microbiologic findings. A diagnosis of endocarditis (group 1) was based on the following criteria: bacteremia and pulmonary infiltrates or vegetations demonstrated by echocardiogram in abusers of intravenous drugs, bacteremia with the appearance of valvular insufficiency or peripheral manifestations of endocarditis, and bacteremia and positive valve cultures at surgery. Intravascular catheter-related and arteriovenous access infections (group 2) were diagnosed in patients with bacteremia and an intravascular device in whom no other focus of infection was identified and evidence of catheter infection was found either by culture of the catheter or by purulent drainage from the catheter site. A bacteremia secondary to a deep tissue infection (group 3) was diagnosed when a deep visceral or extensive infection could be identified. A normal control group included healthy blood donors without any evidence of infection. Sera were stored at −20°C until tested for CIC.

Raji cell RIA. Raji cells were cultured for 72 h in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) enriched with 10.0% fetal calf serum and then harvested by centrifugation at 2,000 × g for 15 min. The assay was performed by the modification of Irvine et al. (4) of the original assay described by Theofilopoulos et al. (8). Serum samples diluted 1:4 with RPMI 1640 medium were incubated with 2.0 × 106 Raji cells for 45 min at 37°C. After being washed three times with the same medium, 25.0 ng of 125I-labeled protein A was added to the cells and incubated for another 30 min at 4°C. The cells were again washed, and the radioactivity in the cell pellet was determined in a gamma scintillation counter. Each serum sample was tested in duplicate, and the uptake of radioactivity was averaged. A standard curve was constructed with dilutions of known quantities of aggregated human gamma globulin (AHG) in RPMI 1640 medium. Results from patients were expressed in...
microgram equivalents (eq) of AHG per ml by reference to the standard curve.

**Cordia-IC ELISA.** The Cordia-IC ELISA was performed according to instructions included with the kit. Supplied in the kit were goat Clq-coated plastic disks, alkaline phosphatase-conjugated goat anti-human immunoglobulins G and M, strong- and weak-positive controls, AHG, negative control, p-nitrophenyl phosphate, and buffers. Serum samples were diluted 1:100 in 0.01 M phosphate-buffered saline containing 0.05% Tween 20 and 6.0% bovine serum albumin. Simultaneously analyzed with the test samples were dilutions of the strong-positive (1:1, 1:4, 1:16, and 1:64), weak-positive (1:100), and negative controls. All samples were tested in duplicate. A Clq-coated disk was added to each sample and incubated for 45 min at 37°C in a shaking water bath. The disk was then removed and washed five times with phosphate-buffered saline. Enzyme conjugate was added to the disk, and the same incubation was repeated. The disk was removed, washed another five times, and then added to 0.01% p-nitrophenyl phosphate substrate. The reaction was terminated after 20 min by the addition of 3.0 M sodium hydroxide, and A405 was determined. A standard curve was constructed from the absorbance of the dilutions of the strong-positive control and its known microgram eq of AHG. Results were obtained by reference to the standard curve and were expressed as microgram eq of AHG per ml.

**Statistical analysis.** The coefficients of variation (CVs) were calculated for intraassay variability and interassay reproducibility of the ELISA and Raji cell tests. The correlation between the Raji cell RIA and the Cordia-IC was tested with Pearson's correlation coefficient. Analysis for differences in the amount of CIC among clinical groups was performed with a two-tailed Student's t test for unpaired data.

**RESULTS**

Patients. A total of 118 serum specimens was collected from individual patients. An additional 30 serum specimens were obtained from a group of healthy blood donors.

The endocarditis group (group 1) consisted of 23 patients. Endocarditis was related to *Staphylococcus aureus* in 14 patients, to *Staphylococcus epidermidis* in 2 patients, to viridans streptococci in 2 patients, to Escherichia coli in 2 patients, and to *Candida albicans* in 1 patient. Also included within this group were two additional patients, one with *S. epidermidis* infection of a ventriculoatrial shunt and one with *S. epidermidis* infection of a LeVeen shunt. Intravascular catheter-related and arteriovenous access infections (group 2) were diagnosed in 41 patients. In 32 patients, the bacteremia was related to an infected intravascular catheter, whereas in 9 patients, the bacteremia was related to an infected arteriovenous access device. In 54 patients with *S. aureus* bacteremia, a deep tissue focus of infection was found (group 3). Osteomyelitis was identified as the source of bacteremia in 11 patients, pneumonia was the source in 10 patients, septic arthritis was the source in 8 patients, and burn eschars or other wound infections were the sources in 25 patients.

**Characteristics of the Cordia-IC ELISA and the Raji cell RIA.** The Cordia-IC ELISA intraassay CV for an identical sample was 4.0%. The equivalent intraassay CV for the Raji cell RIA was 8.0%. The CV for the interassay reproducibility for 10 consecutive days was 8.7% for the Cordia-IC ELISA and 20.0% for the Raji cell RIA.

**CIC in patient groups.** CIC amounts detected by both the Raji cell RIA and the Cordia-IC ELISA were less than 12.5 μg eq of AHG per ml in all 30 serum specimens from healthy blood donors. Among the 23 patients with endocarditis (group 1), CIC values greater than 12.5 μg eq of AHG per ml were detected in 19 patients (82.6%; mean, 73.6 μg eq of AHG per ml) by Cordia-IC ELISA and in 16 patients (69.6%; mean, 54.8 μg eq of AHG per ml) by the Raji cell RIA. There was no statistically significant difference between the amounts of CIC detected in the patients from group 1 by the two assays (*P* = 0.44).

In the 41 patients with infected intravascular catheters or access devices, an elevated CIC value (>12.5 μg eq of AHG per ml) was detected in 19 patients (46.3%) by the Cordia-IC ELISA and in 16 patients (39.0%) by the Raji cell RIA. The mean CIC values were 20.6 μg eq of AHG per ml by the Cordia-IC ELISA and 15.1 μg eq of AHG per ml by the Raji cell RIA. There was no significant difference in the CIC amounts detected by the two assays (*P* = 0.19). The Cordia-IC ELISA detected elevated CIC values (mean, 28.0 μg eq of AHG per ml) in 38 (70.4%) of the 54 patients with *S. aureus* bacteremia related to a deep tissue focus (group 3). The Raji cell RIA detected elevated CIC values (mean, 23.9 μg eq of AHG per ml) in only 32 (59.3%) of these 54 patients. Again, there was no statistically significant difference between the amounts of CIC detected by the Cordia-IC ELISA and the Raji cell RIA (*P* = 0.44).

**Correlation of Cordia-IC ELISA and Raji cell RIA.** The CIC amounts detected by both assays in the 118 patients were significantly different from the CIC amounts found in the 30 control subjects (*P* < 0.001). Figure 1 compares the CIC values obtained by the Cordia-IC ELISA and the Raji cell RIA. A statistically significant correlation was found between the two assays (*r* = 0.64, *P* < 0.001).

**DISCUSSION**

Although important diagnostic and therapeutic information may be gained by the detection of CIC, reliable techniques for CIC quantitation are not readily available in most routine clinical laboratories. Certainly, as Jacob et al. (5) mentioned, with more than 40 different assays now reported for the detection of CIC, more widespread application of this technology is possible. Much of the confusion results from an inherent variability among the tests related to the different principles and reactivities associated with each method. This variability has led to the recommendation by some investigators that serum suspected of containing CIC be screened by more than one methodology (9).

The most common assays rely upon the binding of immune complexes by either the Clq component of complement or the Raji cell surface receptors (9). The Cordia-IC ELISA used a solid phase (plastic disk) coated with Clq for the binding of the CIC. An enzyme-conjugated antibody was then used to quantitate the amount of CIC bound to the immobilized Clq. In the Raji cell RIA, the amount of CIC bound to the cell surface receptors is quantitated by 125I-labeled protein A. Both the Cordia-IC ELISA and the Raji cell RIA used dilutions of AHG as a control. The CVs of the Cordia-IC ELISA were less than those of the Raji cell RIA in both intraassay variability (4.0 versus 8.0%) and interassay reproducibility (8.7 versus 20.0%).

Both the Cordia-IC ELISA and the Raji cell RIA were used to quantitate CIC. There were no significant differences between the two assays in CIC amount found in the three groups of patients with infectious diseases. The Cordia-IC ELISA detected more patients with CIC values above 12.5 μg eq of AHG per ml in each of the groups. The mean CIC
value for each group was also higher in the Cordia-IC ELISA. None of these differences, however, proved to be statistically significant. The sensitivity of the Cordia-IC ELISA in our hands was 5 μg eq of AHG per ml. When the results obtained in the three groups were analyzed, there was a good overall correlation between the Cordia-IC ELISA and the Raji cell RIA (Fig. 1).

Jacob et al. (5) previously reported an ELISA for the detection of CIC with C1q adsorbed to microtiter plates. They found that considerable interference resulted from the binding of the enzyme conjugate to the adsorbed C1q. This interference could be inhibited by the addition of 5.0 to 10.0% dextran sulfate. We did not find any appreciable interference in the Cordia-IC ELISA, perhaps because of the addition of 6.0% bovine serum albumin to the serum samples, although we used only serum samples with added bovine serum albumin and followed exactly the instructions of the manufacturer. We did not investigate the effects of storage and of freezing-thawing of the serum on the results obtained with Cordia-IC ELISA. Samples tested with this kit were kept at −20°C, which seemed not to influence the sensitivity of the test. We also did not analyze extensively with the Cordia-IC kit serum samples from patients with connective tissue diseases. Preliminary results showed that the number of patients with cancer and CIC detected by the Cordia-IC ELISA was similar to the number detected by the Raji cell RIA.

In summary, the Cordia-IC ELISA had many features that might recommend it for routine clinical laboratories. The assay is now commercially available, and the ELISA technique avoids the precautions necessary with the use of radioactive compounds. The assay showed intraassay variability, interassay reproducibility, and CIC amounts comparable with those of the popular Raji cell assay.

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LITERATURE CITED


