A Comparison of Electrochemiluminescence and Flow Cytometry for the Detection of Natural Latex-Specific Human Immunoglobulin E

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In vitro correlates of type 1 hypersensitivity to natural latex (NL) proteins continue to be limited by both sensitivity and specificity. Methods which have detection limits in the picogram range, namely, radioallergosorbent assays (RAST) and enzyme-linked immunosorbent assays (ELISA), are inadequate for the identification of NL hypersensitivity in certain at-risk groups, such as health care workers. A flow cytometry assay (FCA), previously shown to be comparable to RAST and ELISA in the identification of NL-sensitized pediatric patients with spina bifida, was compared with electrochemiluminescence (ECL) in the evaluation of pediatric patients with spina bifida and NL-sensitized adult health care workers. As with RAST and ELISA, ECL is capable of detecting picogram amounts of specific analyte. The ECL assay detected NL-specific immunoglobulin E (NL-IgE) in three of six health care workers with strong histories of NL hypersensitivity. All six patients were negative by FCA. Further, 2 of 11 spina bifida patients found to be NL-IgE negative by FCA were NL-IgE positive by ECL. These findings suggest that in sensitivity the ECL assay is an improvement over the FCA for the identification of NL-sensitive individuals.

Natural latex (NL) allergy has emerged as a significant problem in both patients and health care workers. Initially, most of the NL-allergic patients identified were patients with spina bifida or genitourinary abnormalities. However, as awareness of this problem has increased, other patient groups, including patients with a history of multiple surgical procedures, ventriculoperitoneal shunts, prematurity, and asthma, have been found to be at risk for developing NL hypersensitivity (9). Laboratory or in vitro diagnosis of NL allergy has depended largely on the use of serological assays to measure NL-specific immunoglobulin E (IgE), such as radioallergosorbent assays (15) and enzyme-linked immunosorbent assays (ELISA) (5, 6). These assays have various levels of sensitivity and specificity because of the lack of standardized NL antigen and can require up to 2 days for completion. The use of skin prick testing as a diagnostic test is also made difficult by the lack of a standardized antigen for testing and the risk of anaphylaxis (4, 13, 14). Thus, there is a need for a sensitive and specific test to identify patients and health care workers at risk for allergic reactions to latex so that latex products can be avoided. The use of a flow cytometry assay (FCA) for detecting latex-specific IgE, which was found to be as sensitive as ELISA or radioallergosorbent assays, was recently reported (8). FCA requires only 10 μl of serum or plasma and can be completed in <4 h. However, there remains a subset of patients who do not have detectable NL-specific IgE in their serum but demonstrate clinical hypersensitivity to NL proteins and are found positive by skin prick testing, a more sensitive means of detecting antigen-specific IgE.

We have developed a new method of detecting NL-specific IgE using low-level analyte detection by electrochemiluminescence (ECL). We have evaluated this technique, comparing it with FCA, using sera from a diverse population of known latex-allergic children, children with spina bifida, and adults with a history of latex sensitivity. The ECL assay detected NL-specific IgE in the serum samples of three of six adult health care workers with strong histories of NL hypersensitivity. All six patients were negative by FCA. Further, 2 of 11 spina bifida patients found to be NL-IgE negative by FCA were NL-IgE positive by ECL. These findings suggest that in sensitivity the ECL assay is a significant improvement over the FCA and should be a useful tool for the identification of NL-sensitized individuals in diverse at-risk groups.

MATERIALS AND METHODS

Patient populations. Serum specimens were obtained from 37 pediatric patients with spina bifida who had initially been screened for NL-specific IgE by FCA. Of these 37 samples, 26 were weakly positive by FCA, while 11 were negative by FCA. These serum samples were reevaluated by FCA in parallel with ECL. In addition, parallel studies were performed with serum samples obtained from six adult health care workers with clinical histories of NL hypersensitivity. Finally, parallel studies were performed with serum samples obtained from 13 pediatric asthmatic patients with no known history of NL hypersensitivity; this group of patients served as our negative controls. This study was approved by the Institutional Review Board of the Children’s Hospital of Philadelphia.

NL antigen preparation. NL antigen was prepared from raw ammoniated latex sap (Kilian Inc., Akron, Ohio) as previously described (8). FCA. The solid phase for the FCA consisted of 2.54-μm-diameter carboxylated polystyrene microspheres (Bangs Laboratory Inc., Carmel, Ind.) covalently coupled to 60 μg of NL protein or control human serum albumin (HSA) by carbodiimide coupling (10). Stock microspheres were stored at 4°C as 0.5% solids in PNA buffer (phosphate-buffered saline [PBS] with 10% normal equine serum and 0.1% sodium azide). For the FCA, stock microspheres were diluted 1:20 in PNA buffer (PBS, 10% normal equine serum, 0.1% Na azide, 1% Triton X-100). The patients’ serum samples were diluted 1:50 in PNA buffer. A 10-μl volume of diluted stock microspheres was added to 200 μl of diluted sera, and the mixture was incubated for 1 h at 37°C. All samples were run in duplicate with NL-conjugated and HSA-conjugated microspheres. After being washed with 1 ml of PNA buffer, the microspheres were incubated for 1 h at 37°C with 30 μl of a 1:100 dilution of affinity-purified biotinylated goat anti-human IgE.
After one washing with PNAT buffer, 30 µl were run in duplicate with NL-conjugated and HSA-conjugated microspheres. Microspheres were washed again with PNAT and incubated with 30 µl buffer was added to 200 µl sodium azide. A 0.5-ml sample of the labeled streptavidin was applied to the nm (Bio-Rad, Melville, N.Y.) that had been equilibrated with PBS with 0.1% C in PNAbuffer. The 1.6-8 PBS and finally resuspended in 1 ml of PNAT buffer. The coated microspheres were stored with a standard photomultiplier tube. Results are expressed as ECL intensity, which is a measurement of the total luminescence emitted by the sample. For these experiments, we used an Origen analyzer (IGEN, Inc.).

Preparation of ruthenium-labeled streptavidin. Ruthenium (IGEN, Inc., Gaithersburg, Md.) was conjugated to streptavidin (Sigma Chemical Co., St. Louis, Mo.) at a 20:1 mol ratio. Ruthenium (352 µg) was added to 1 mg of streptavidin in PBS and incubated with agitation for 1 h at room temperature. A 20-µl volume of 2 M glycine was added to stop the reaction, and the mixture was incubated for 10 min. Labeled streptavidin was separated from unconjugated protein by centrifugation. Two types were evaluated: Dynabead M-450 4.5-µm-diameter spheres (DynaI, Norway) and 1.6-µm-diameter paramagnetic spheres (Bangs Laboratory Inc.). The Dynabeads were coated with either HSA or protein by adding 20 µg of protein to 100 µl of uncoupled microspheres with agitation for 24 h. The coated microspheres were washed twice with 1 ml of PBS and finally resuspended in 1 ml of PNA buffer. The coated microspheres were stored at 4°C in PNA buffer. The 1.6-µm paramagnetic microspheres were carbonylated, which allows covalent linkage to NL or HSA by carbodiimide coupling in the same manner as described previously for the 2.54-µm nonparamagnetic microspheres (10). The protein-conjugated microspheres were stored at 4°C in PNA buffer.

Preparation of solid phase for ECL. The solid phase for ECL was prepared in a manner similar to that of the FCA except that ECL requires the use of paramagnetic microspheres. Two types were evaluated: Dynabead M-450 4.5-µm-diameter spheres (DynaI, Norway) and 1.6-µm-diameter paramagnetic spheres (Bangs Laboratory Inc.). The Dynabeads were coated with either HSA or protein by adding 20 µg of protein to 100 µl of uncoupled microspheres with agitation for 24 h. The coated microspheres were washed twice with 1 ml of PBS and finally resuspended in 1 ml of PNA buffer. The coated microspheres were stored at 4°C in PNA buffer. The fluorescence emitted by Ru(bpy)32+ is excited at the surface of the electrode in the flow cell. Ru(bpy)32+ can then reduce Ru(bpy)33+. The activated form of Ru(bpy)33+ is oxidized at the surface of the electrode and then the radical TPA• and can then reduce Ru(bpy)32+. The excited state of the molecule which decays, emitting a photon with a wavelength of 620 nm. The Ru(bpy)33+ can repeat this cycle numerous times as long as there is a continued influx of TPA.

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Evaluation of antigen binding to microspheres. Flow cytometry was used to evaluate the density of antigen bound to individual microspheres. This was accomplished by measuring the binding of excess NL-specific human IgE (NL positive pool) to the various microspheres with subsequent comparisons of net MCF. A previous study optimized antigen binding to a 2.54-µm nonparamagnetic microsphere (10). Therefore, this microsphere was used to compare the densities of antigen bound to the two different paramagnetic microspheres. Figure 1 illustrates that the paramagnetic microspheres bound significantly less NL-specific IgE (0.5%, 4.5 µm; 4.7%, 1.6 µm) than the 2.54-µm nonparamagnetic microspheres (100%). However, subsequent studies (described below) revealed that the 1.6-µm paramagnetic microspheres were adequate for use in the ECL assay, and they were used for all patient evaluations.

Comparison of ECL and FCA. In order to compare the relative sensitivities of ECL and FCA, a serially diluted, pooled positive-control serum was evaluated in parallel using the 1.6-µm-diameter paramagnetic microspheres in both assays, since the 2.54-µm-diameter nonparamagnetic microsphere cannot be used in the ECL assay. In all further experiments, the two assays were compared by using the optimal microsphere for each system, namely, 1.6-µm microspheres for ECL and 2.54-µm microspheres for FCA. Figure 2 illustrates the excellent correlation between the two assay systems (r = 0.995) when the same-size microspheres were used for both assays (1.6 µm).

Figure 3 illustrates a comparison of the relative sensitivity of ECL versus FCA in the endpoint titration of an NL-specific positive-control serum. ECL consistently gave higher endpoint values than FCA.

We were able to improve the sensitivity of the assay by evaluating larger sample volumes of higher dilutions. The detection of NL-specific IgE with various sample volumes of a 1:50 dilution of a moderately positive control serum is shown in Fig. 4. Both assays demonstrated a linear correlation between the amount of NL-specific IgE and the sample volume. On the basis of these results, 200-µl samples of a 1:50 dilution of both control and patient sera were evaluated by ECL and FCA.

Evaluation of patient sera. A previous study (10) established the negative cutoff (22.28 net MCF units) for the FCA by the evaluation of pediatric atopic patients with no known history of latex hypersensitivity. This value represents the mean + 2 standard deviations. An identical approach was taken in the establishment of the negative cutoff for the ECL assay (70,142 net

FIG. 1. Evaluation of NL protein conjugation to different types of solid-phase microspheres by the measurement of selective binding of NL-specific human IgE as determined by flow cytometry. Binding of human IgE to the 2.54-µm-diameter carboxylated microspheres was considered maximum, with the amount of binding of human IgE to the 1.6- and 4.5-µm paramagnetic microspheres indicated as percentages of that bound to the 2.54-µm nonparamagnetic microspheres.
ECL intensity units) by the evaluation of serum specimens obtained from 13 pediatric asthmatic patients with no known history of latex hypersensitivity. In the present study, all 26 pediatric spina bifida patients previously found to be positive by FCA were also positive by ECL ($P < 0.001$ in comparison with the negative-control group) (Fig. 5). Furthermore, 2 of 11 pediatric spina bifida patients found to be negative for NL-specific IgE by FCA were found to be positive for NL-specific IgE by ECL. All 13 pediatric asthmatic (negative-control group) patients who had no history of NL hypersensitivity who were used for determining the negative cutoff for the ECL assay were also negative by the FCA. Additionally, three of six adults with a history of NL hypersensitivity were positive by ECL; this included three subjects who were negative by FCA (Table 1).

**DISCUSSION**

The development of in vitro diagnostic tests which accurately identify individuals who have become sensitized to NL proteins has been difficult. This is attributable, in part, to the fact that NL is made up of a heterogeneous group of proteins which are subjected to various manipulative procedures during the manufacture of different products containing NL components (3, 14). Furthermore, using immunoblotting techniques, a number of studies have shown qualitative differences in the immunological response to NL proteins among different patient groups, such as pediatric spina bifida patients and adult health care workers (1, 7, 12). Two previous studies evaluated sera from pediatric patients with spina bifida and found strong IgE reactivities to 14- and 27-kDa proteins (1, 11). Other minor reactivities were observed with several other proteins with molecular masses ranging from 43 to 66 kDa (3, 7). In contrast, the evaluation of sera from sensitized adult health care workers revealed a more restricted IgE response to NL proteins, with the major response directed at a 43-kDa protein (12). These data may explain the difficulty in detecting NL-specific IgE in sera from sensitized adults by employing immunooassays which use total NL proteins rather than appropriately fractionated proteins for the solid-phase antigen. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the total protein content of NL reveals numerous proteins other than the 14-, 27-, and 43-kDa proteins previously found to be important antigens in either pediatric or adult patients (12). Thus, several studies have been conducted in an effort to identify important antigens in NL which might be isolated and used for the development of standardized assays which will be ef-

![FIG. 2](http://cvl.asm.org/)  
**FIG. 2.** Comparison of ECL and FCA in the measurement of NL-specific human IgE prepared from a serially diluted positive-control serum. The same NL-conjugated microspheres (1.6-$\mu$m paramagnetic microspheres) were used in both assays. The correlation coefficient for the two assays was 0.995.

![FIG. 3](http://cvl.asm.org/)  
**FIG. 3.** Comparison of the detection limits of ECL and FCA in the measurement of NL-specific human IgE in a serially diluted positive-control serum. These assays were performed by using the optimal solid phase for each assay system (2.54-$\mu$m nonparamagnetic microspheres for FCA and 1.6-$\mu$m paramagnetic microspheres for ECL).
effective in identifying NL-sensitized individuals in all patient groups with a high level of specificity and sensitivity. The development of an FCA for the measurement of NL-specific human IgE was reported previously (8). This assay is extremely useful in the identification of NL-sensitized pediatric patients with spina bifida. Furthermore, in both sensitivity and specificity, the FCA is comparable to ELISA and radioallergosorbent assays. However, the FCA is not as sensitive in identifying adult patients with clinical histories of latex sensitivity. For these reasons, the comparative evaluation of FCA and the relatively new technology ECL was undertaken in an effort to improve upon the sensitivity of existing assays in use for the measurement of NL protein-specific IgE. A previous study has found that ECL is capable of detecting soluble analytes, such as digoxin, at levels of <100 pg/ml (2). The present study demonstrated excellent correlation between the FCA and the ECL assay, with 100% concordance in the evaluation of both positive- and negative-control samples. In addition, all pediatric patient samples shown to be positive by FCA were positive by ECL, while two pediatric spina bifida patient samples which were negative by FCA were positive by ECL. More importantly, three of six samples from clinically sensitive adult patients who were negative by FCA were positive by ECL (Table 1). These data suggest that ECL is an improvement upon FCA for the detection of NL-specific IgE. The important antigenic proteins in NL to which adult patients become sensitized probably represent only a small fraction of the total protein composition of the NL protein used in the preparation.

**Table 1. Evaluation of sera for NL-specific IgE by FCA and ECL for adult patients with a clinical history of hypersensitivity to latex.**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Net MCF</th>
<th>Net ECL intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>127,626</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>185,289</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>203,768</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
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<td>−6,594</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>−20,191</td>
</tr>
</tbody>
</table>

**Figure 4.** Influence of sample volume on the detection of NL-specific human IgE in a positive-control serum as determined by ECL and FCA. Each datum point represents the mean ± 2 standard deviations of replicate values.

**Figure 5.** Evaluation of sera obtained from pediatric patients with spina bifida for NL-specific IgE by ECL. Patient sera were grouped as either negative or weakly positive on the basis of an initial screening at a 1/5 dilution by FCA. Negative-control sera were obtained from pediatric asthmatic patients with no clinical history of NL hypersensitivity. Horizontal bars represent the arithmetic means for the groups.
of the solid phase for FCA and the ECL assay. Fractionation of the NL protein preparation into various molecular weight classes, prior to conjugation to the solid phase used in the ECL assay, may lead to identification of important epitopes responsible for NL sensitization in various patient populations (pediatric versus adult).

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REFERENCES