

On a much higher than reported incidence of anti-c in R₁R₁ patients with anti-E

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A previous study involving tube IATs, untreated RBCs, and a low-ionic-strength additive reagent revealed that approximately one-third of R₁R₁ patients with anti-E have a concomitant anti-c. However, the current study finds a much higher incidence of anti-c in such patients, using gel technology in conjunction with ficin-pretreated RBCs. Results of antibody identification studies and transfusion records of 82 R₁R₁ patients with anti-E were reviewed. Serologic test methods included a LISS wash solution for tube IATs (15 min at 37°C, anti-IgG), ficin-tube IATs (30 min at 37°C, anti-IgG + anti-C3), and gel IATs (untreated or ficin-treated RBCs or both, anti-IgG gels). LISS-tube or gel IATs with untreated RBCs revealed anti-c in 32 patients with anti-E. When gel-IAT and ficin-pretreated RBCs were used, 21 additional patients with anti-E were found to have anti-c. In samples from 26 R₁R₁ patients with anti-E, anti-c was not demonstrable by ficin-gel IATs, and in 3 cases, the ficin-gel tests were inconclusive. In five cases in which E- RBCs not tested for c antigen were transfused to patients found by ficin-gel IAT to be without anti-c, all subsequently performed crossmatches with E-, c- untested RBCs were compatible. The incidence of anti-c in R₁R₁ patients with anti-E in this study was 32 of 82 (39%) with untreated RBCs and 53 of 82 (65%) when the ficin gel data were included. The latter is significantly higher than the 32 percent incidence previously reported ($p = 0.0001$). Accordingly, all patients at our facility with an Rh antibody are now tested for those additional Rh antibodies they can make, as predicted from their Rh phenotype. The data from this study strongly support the selection of R₁R₁ RBCs for all c- patients with anti-E. *Immunohematology* 2005;21:94-96.

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The immune response to RhCE protein is variable. For example, R₁R₁ (c-, E-) individuals may make anti-c, anti-E, anti-c plus anti-E, or anti-cE (Rh 27), the latter reacting with a determinant encoded by *c* and *E* within the same haplotype (in *cis*). Some individuals may make all three antibodies, as demonstrated by adsorption-elution studies,¹ or a combination of anti-c plus anti-E.

Shirey et al.² found the incidence of anti-c in R₁R₁ patients with anti-E to be 32 percent when performing IATs by a low-ionic-strength additive technique. They also showed if the RBCs selected for transfusion were E- but not tested for c antigen, 18.5 percent of

recipients developed anti-c. Since their report, gel technology has emerged and has been found to have good sensitivity for Rh antibodies. Further, we have shown that tests performed with anti-IgG gel cards and ficin-pretreated RBCs are exquisitely sensitive for Rh antibodies.³

In this report we present data from ficin-gel tests that demonstrate a higher than previously published incidence of anti-c in R₁R₁ patients with anti-E. These data support the selection of c- RBCs for this patient population.

Materials and Methods

ID-MTS gel technology was from Ortho-Clinical Diagnostics (Raritan, NJ). Reagent RBCs, both untreated and ficin-treated, and LISS (Löw and Messeter⁴ formulation) were from ImmucorGamma, Norcross, Georgia. For gel testing,⁵ untreated reagent RBCs were prepared in ID-MTS Diluent 2 at a concentration of 0.8% and tested on anti-IgG cards according to the manufacturer's product circular. Gel tests with ficin-pretreated RBCs were similarly performed on anti-IgG cards; such testing (previously validated by us³) conflicts with the manufacturer's product circular, which stipulates the use of buffered gel cards. LISS-tube tests⁶ were incubated at 37°C for 15 minutes, washed four times with saline, and tested with anti-IgG (Ortho). Ficin-tube tests⁶ were incubated at 37°C for 30 minutes and tested with polyspecific (anti-IgG + C3) anti-globulin reagent (Ortho). Negative tube tests were validated with IgG-coated RBCs (ImmucorGamma).

Serologic and transfusion records of 82 R₁R₁ patients with anti-E were reviewed. No further testing was performed on 32 patients with concomitant anti-c by routine testing (LISS-tube and ficin-tube). Ficin-gel tests were used to detect the presence or absence of anti-c in samples from the remaining 50 patients.

Results

We found anti-c in 53 out of 82 (65%) R₁R₁ patients with anti-E (Table 1). In 32 cases, the presence of anti-c was evident from the results of LISS-tube or gel IATs. In the other 21 cases (see Fig. 1 for an example), the presence of anti-c was clearly demonstrable only in ficin-gel IATs. However, among these 21 cases, weak reactivity was also seen in two cases with some untreated c+, E- RBCs in gel IATs and suspected from the results of ficin-tube tests in five cases. There were three additional cases in which the presence of anti-c could not be determined due to panreactivity in ficin-gel tests.

Of the 26 R₁R₁ patients with anti-E that did not have anti-c clearly demonstrable by ficin gel, 18 did not require transfusion (five were pregnant, and there were records of previous transfusions on six). A further eight patients were transfused with E- RBCs that were not tested for c antigen. Three of these patients were lost to follow-up. We had the opportunity to test the five remaining patients for anti-c in subsequent antiglobulin crossmatches with E- donor units that had not been selected to be c-; in fact, two patients were transfused with Rh- RBCs that were undoubtedly c+. These crossmatches were performed between 3 and 20 months after anti-E was initially detected in the patients' plasma. All units (n = 10) were crossmatch compatible.

Discussion

The development of alloantibodies to RBC antigens through transfusion or pregnancy is not benign. Patients who become alloimmunized are at risk of hemolytic transfusion reactions and high-risk pregnancies associated with HDN.^{7,8} Further, alloimmunization is sometimes accompanied by autoantibody formation, which may lead to autoimmune hemolytic anemia.⁹ These risks prompted some investigators to recommend the use of phenotypically matched RBCs, especially for sickle cell anemia patients.¹⁰ As shown recently,^{11,12} this recommendation is by no means universally followed.

In the 11th edition of the AABB *Technical Manual*,¹³ it was suggested that R₁R₁ transfusion candidates who have made anti-E should be transfused with R₁R₁ RBCs to prevent formation of anti-c, possible posttransfusion hemolysis, and autoantibody formation. This suggestion prompted Shirey and colleagues² to determine the incidence of anti-c in 100 R₁R₁ patients with anti-E. In their study, using LISS-tube IATs,

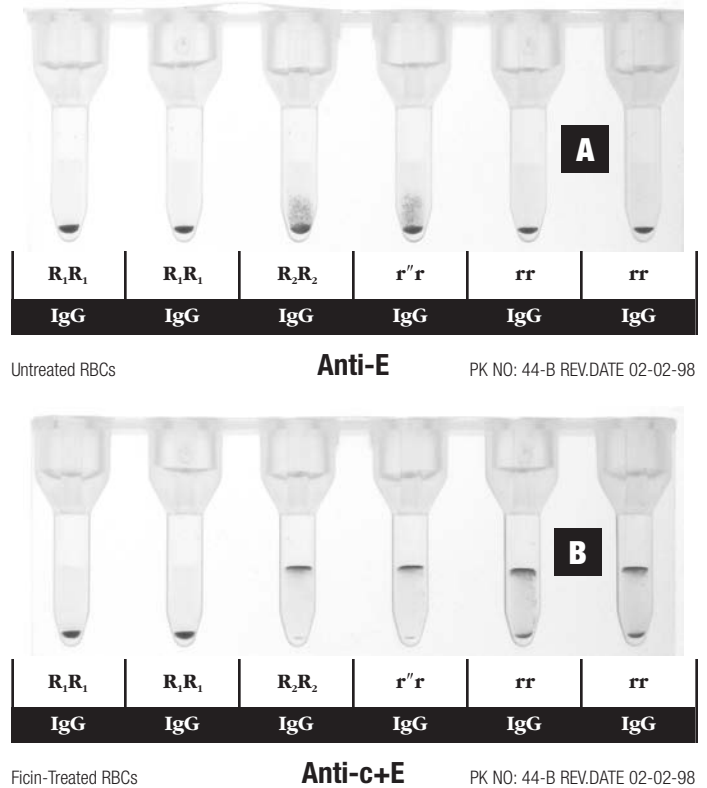


Fig. 1. A. Anti-IgG gel tests with untreated RBCs reveal presence of anti-E. B. Tests with ficin-pretreated RBCs reveal presence of concomitant anti-c.

Table 1. Incidence of anti-c in 82 R₁R₁ patients with anti-E

LISS tube or gel	Ficin gel	Number of patients
anti-E + anti-c	NT*	32 (39%)
anti-E	anti-E + anti-c	21 (26%)
anti-E	anti-E	26 (32%)
anti-E	inconclusive	3 (3%)

*NT = not tested

they found the incidence to be 32 percent. Among the 68 R₁R₁ patients with anti-E alone, 27 were transfused with E- RBCs that were not typed for c antigen; five (18.5%) of these patients subsequently formed anti-c.

Given our past experiences with gel technology,¹⁴ which is exquisitely sensitive for Rh antibodies, we expected to find a somewhat higher incidence of anti-c in R₁R₁ patients with anti-E than was reported by Shirey et al.² However, we did not expect to find a twofold increase (32% vs. 65%; p = 0.0001) through the use of gel technology and ficin-treated RBCs, given that before implementation of gel technology in our laboratory our routine antibody identification protocol included ficin-tube IATs. We postulate that these anti-c antibodies are, for the most part, low-affinity antibodies

that dissociate during the washing phase of tube IATs; gel technology is, of course, a no-wash IAT.

Although we saw no evidence of anti-c development in five R₁R₁ patients with anti-E, as demonstrated by compatible IAT crossmatches with E-, c-untested RBCs, we now select c-blood for R₁R₁ patients with anti-E, regardless of whether or not anti-c is detected. However, due to the comparative rarity of R₂R₂ donors, we do not automatically select R₂R₂ RBCs for patients with anti-C who are e-. Rather, we test for anti-e by ficin gel, if it is not evident by routine studies, and, if it is

present, we crossmatch R₂R₂ donor RBCs. We have a similar policy for R₀ patients with anti-C or anti-E, inasmuch as we select C-, E- RBCs for crossmatching only if ficin-gel tests reveal that both anti-C and anti-E are present (Table 2).

Table 2. Policy for ficin-gel testing and blood selection in D+ patients with Rh alloantibodies

Patient's RBCs	If*	◆	Then [†]	Or, if*	◆	Then [†]
R ₁ R ₁	anti-E	◆	issue c-, E- RBCs			
R ₂ R ₂	anti-C	◆	exclude anti-e	anti-e	◆	issue C-, e- RBCs
R ₀	anti-C	◆	exclude anti-E	anti-E	◆	exclude anti-C

*Antibody found in serum with untreated RBCs.

[†]Exclusion tests performed with ficin-treated RBCs by gel technology.

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