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An Immediate Hemolytic Transfusion Reaction Due to Anti-C and a Delayed Hemolytic Transfusion Reaction Due to Anti-Ce+e: Hemoglobinemia, Hemoglobinuria and Transient Impaired Renal Function

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Abstract. A patient with phenotype R_2r and anti-C had a hemolytic transfusion reaction (HTR) with hemoglobinemia and hemoglobinuria which occurred within 2 h of receiving an R_1r transfusion. Transient impaired renal function ensued. A patient with phenotype R_2R_2 and anti-Ce+e had the same experience on day 4 after receiving three R_1r and one rr units. 2 other patients, 1 R_2r with anti-C who received one R_1r unit and the other R_2R_2 with anti-Ce+e who received two R_1r units, showed no clinical evidence of HTR. Both anti-C antibodies were entirely IgG while both anti-Ce+e antibodies initially were predominantly IgM. IgG subclassing was unsuccessful and red blood cell-mononuclear phagocyte assays were normal. These cases occurred from 1979 to 1981.

Introduction

Anti-C as a cause for immediate or delayed hemolytic transfusion reaction (DHTR) was reported only once prior to 1978 by *Croucher* et al. [5]. That patient had a DHTR without hemoglobinuria due to anti-Ce, e, Fy^a. 5 patients with DHTR occurring over a 14-year period, all presenting with hemoglobinuria at 5–9 days, and all due to anti-C, were reported by *Pickles* et al. [9] in 1978. In 1980 *Moore* et al. reported 2 patients with DHTR due to anti-C and 1 due to anti-C, E. These patients did not have hemoglobinuria. DHTR in which hemoglobinuria is observed are of rare occurrence [6–11]. Of the 24 cases reported, 9 were due solely to Rh antibodies (5 anti-C, 2 anti-$\bar{c}$, 1 anti-$\bar{c}$E, 1 anti-D), and 4 other cases involved multiple antibodies with at least 1 Rh antibody present (1 anti-$\bar{c}$, 1 anti-E, 2 anti-C, E). Renal impairment secondary to HTR, whether immediate or delayed but due to Rh antibodies, is either a rare occurrence or has been rarely reported. In 28 reported DHTR cases due to Rh antibodies [6, 8–11], there was 1 case of renal failure and death due to anti-$\bar{c}$E and 1 case with transient renal impairment in a patient with anti-C, Jk^b, Le^a, Le^b. Of the 6 immediate HTR cases due to Rh antibodies reported in the same articles, transient renal impairment occurred in 1 patient with anti-$\bar{c}$, and in another with anti-C, E, Fy^a, Jk^a.

This report will present the first immediate HTR due to anti-C, a case which exhibited hemoglobinemia, hemoglobinuria and transient renal impairment; a second patient with DHTR due to anti-Ce+e who had hemoglobinemia, hemoglobinuria and transient renal impairment; and 2 matching cases in whom neither signs nor symptoms of HTR occurred.

Methods and Materials

The routine technic used at the reference laboratory for antibody detection, antibody identification, determination of incompatibility of donors' red cells and when determining immunoglobulin class is an incubation of serum and red cells in saline at 26 °C for 15–30 min, addition of polymerized albumin with centrifugation, incubation at 37 °C for 60 min and conversion to indirect antiglobulin phase. Polyspecific antiglobulin reagent is used and there are macroscopic readings at each phase with microscopic readings also done at the final phase. The use of ficin pretreated test cells was done according to the manufacturer's directions (Accugenics Ficin Solution). LISS additives (Biological Corporation of America EM-V®, Gamma LO-ION® and Ortho® Antibody Enhancement Solution) and solutions, Pfizer LISS and a solution prepared at one of the hospitals according to the formula described by *Moore and Mollison* [1] were used according to manufacturers' directions and that hospital's protocol [2]. Eluates were prepared using the rapid acid technic and according to manufacturer's instructions (Gamma Elu-kit®). Commercial red cell panels were used.

Scoring of degrees of agglutination used 12 for $+4^s$ macroscopic reading, 11 for +4, 10 for $+4^w$, 9 for $+3^s$, 8 for +3, 7 for $+3^w$, 6 for $+2^s$, 5 for +2, 4 for $+2^w$, 3 for $+1^s$, 2 for +1, 1 for $+1^w$ and 0.5 for reactions which were positive only on microscopic reading. Because of the weakness of the patients' antibodies particularly in the pretransfusion sera, scores were determined by adding the reactions seen at three phases of the routine technic instead of performing titrations. For example, the serum of patient No. 1 at 28 h postreaction showed +2 agglutination, score 5, at 26 °C, $+1^s$ agglutination, score 3 in albumin at 37 °C and +1 agglutination, score 2, by indirect antiglobulin phase. This gives an accumulative score of 10 (cf. table II).

Immunoglobulin classing was performed using 0.1 *M* 2-mercaptoethanol treatment of sera. Untreated and treated sera were tested in parallel by the routine technic and results at each phase were scored. The accumulated score obtained with the treated serum is divided by the accumulated score of the untreated serum to obtain an approximation of the percentage of the antibody that is IgG.

Direct differential agglutination was performed using reagents anti-C and anti-e on posttransfusion red cell samples and estimating the percentage of agglutinated cells on microscopic examination.

Selected serum samples from the 4 patients were sent to one reference laboratory [3] for IgG subclassing and for human red cell-mononuclear phagocyte assays (RBC-MPA), and two serum samples from the patient with the immediate HTR were sent to another reference laboratory [4] for testing by their RBC-MPA technic.

Case Reports

Case No. 1. An R_2r patient has Crohn's disease. On 7/17/81, he received two packed red cell (PRC) units, one R_2r, one R_1r. A right hemicolectomy was performed on 7/22/81. DHTR due to anti-C with hyperbilirubinemia, unexplained anemia and loss of almost all C cells was evident in retrospect on 7/25/81. Two transfusions, an R_2r followed by an R_1r were given overnight 7/25–7/26, completed at 0500. Hemoglobinuria was present at 0700 and persisted for 2 days. The creatinine level was 1.4 mg/dl on 7/19. It rose to 2.2–2.3 mg/dl on 7/27 through 7/31. BUN, 14 mg/dl on 7/19 peaked at 34 mg/dl on 7/26. It was 28 mg/dl on 7/31. There was no oliguria. The plasma collected on 7/26 was brown. Bilirubin was 6.2 mg/dl on 7/27, 2.7 mg/dl on 7/28, 1.3 mg/dl on 7/31. Haptoglobin was <12 mg/dl on 7/27. There were 25% C cells present in samples collected at 0700 and at 0900 but none were detected in samples collected 28 h after completion of the transfusion. Anti-C was present in samples of 7/17, 7/21, 7/25, 7/26, 7/27, 7/29 and 8/27, but direct antiglobulin tests (DAT) and autocontrols, even with ficin pretreatment, were negative on all samples.

Case No. 2. An R_2r patient had leukemia and was receiving chemotherapy. He had received 11 PRC units

between 2/2/81 and 5/6/81. There were 50% C cells present on 6/8/81 when anti-C was identified. The DAT was positive and anti-C was found in the eluate. On 6/8 and 6/9 he received in sequence one r″r, two rr and one R_1r units. 13 days later there were 35% C cells present, 41 days after the transfusions there were 17% C cells present and on the 85th day no C cells were present. The antibody was weak initially and did not change over the 3-month period of the study. The attending physician was alerted relative to the patient's serologic status on 6/8 and the R_1r transfusion on 6/9. Clinically apparent hemolytic reaction was not reported.

Case No. 3. An R_2R_2 patient had lymphosarcoma and had a splenectomy performed in 1975. She received one R_1r and one rr units on 5/27/80 and two R_1r units on 5/28/80. Back pain, fever and hemoglobinuria occurred on 6/1/80. Hemoglobinuria was observed for 5 days. Oliguria had preceded these signs and symptoms and persisted until 6/5. The BUN reached 52 mg/dl on 6/4, became normal on 6/10. Creatinine, 0.9 mg/dl on admission, ranged from 2.4 mg/dl on 6/2, peaked at 2.8 mg/dl on 6/10, was still elevated at 2.0 mg/dl on 6/16, became normal by 8/9. Plasma hemoglobin was 360 mg/dl on 6/2, total bilirubin was 2.6 mg/dl and haptoglobin 0 mg/dl on that date. There were 40% Ce cells present in the sample 6/2/80, none detectable on 6/5. The DAT was positive on 6/2 but eluates showed no antibody activity. The anti-Ce+e was detected retrospectively in the 5/27/80 sample, easily detected on 6/2 and thereafter for 11 months. The patient subsequently received 57 R_2R_2 units over that 11-month period for treatment of recurrent gastrointestinal hemorrhages without making additional alloantibodies or experiencing HTR.

Case No. 4. An R_2R_2 patient had colon carcinoma with metastases. Anti-Ce+e was present in the sample of 3/6/79 when she received two R_1r units. 9 days later, the DAT was positive and there were 40% Ce cells present in the sample. An eluate was not made. When next studied on day 71, there were no Ce cells present and the antibody was as weak as on initial testing. The hematocrit was 19.5% on 3/6 and 19.1% on day 71 with no values determined in the interim. The attending physician was made aware of the incompatibility of the 3/6 transfusions and clinically apparent hemolytic reaction was not reported.

Table I. Clinical data

Case No.	Sex	Age	ABO and Rh antibody	Prior transfusions	Antigen positive current transfusions	Hemoglobinuria	Renal status
1	M	56	A_1 R_2r Anti-C	2 5 years previous	1 R_1r 7/17 1 R_1r 7/26	none after 2 h for 2 days	BUN↑ creatinine↑
2	M	78	B R_2r Anti-C	11 1–4 months previous	1 R_1r	none	normal
3	F	78	B R_2R_2 Anti-Ce+e	13 5 years previous 2 6 months previous	1 rr, 1 R_1r 5/27 2 R_1r 5/28	6/1–6/5	BUN↑ creatinine↑
4	F	53	O R_2R_2 Anti-Ce+e	6 4–9 months previous	2 R_1r	none	normal

↑ = Elevated.

Results

The clinical data are given in table I and the serologic data in table II. Serum samples of the 4 patients were sent to a reference laboratory [3] for IgG subclassing and for human red blood cell-mononuclear phagocyte assays (RBC-MPA). The sera sent were the 4-hour and 4-day postreaction sera of patient No. 1, the 13-day posttransfusion serum of patient No. 2, the 107-day serum (58% IgG) of patient No. 3, and the 71-day serum (87% IgG) of patient No. 4. The two sera of patient No. 1 were also sent to another laboratory [4] for RBC-MPA. None of the antibodies could be subclassed, three failing to react at all in the subclass testing and one reacting in the capillaries prior to the addition of the subclass antisera. All five sera were normal on RBC-MPA.

Sera of 7 other patients with anti-C and sera of 9 other patients with anti-Ce+e were examined. 5 had been responsible for DHTR without hemoglobinemia, hemoglobinuria or impaired renal function, and eleven were not related to any recent problems. On average, the anti-Ce+e antibodies scored 20 by the routine technic while the anti-C antibodies scored 13. The antibodies involved with the DHTRs on average scored

Table II. Serologic data

Case No.	DAT pre	DAT post	Eluate post	Donor cell survival differential agglutination C+ cells	Ig class antibody	Antibody response accumulative score vs. R_1R_1 cells[1]	
1	–	–	–	25% after 2 and 4 h 0% after 28 h	100% IgG 100% IgG	Pretransfusion after 4 h after 28 h after 4 days after 32 days	10 10 10 12 10
2	+	+	NT	35% after 13 days 17% after 41 days 0% after 85 days	100% IgG 100% IgG 100% IgG	Pretransfusion after 13 days after 41 days after 85 days	4 7 8 11
3	–	+	–	40% after 6 days 0% after 9 days	24% IgG after 6 days 58% IgG after 107 days	Pretransfusion after 6 days after 107 days	4 30 31
4	–	+	NT	40% after 9 days 0% after 71 days	30% IgG after 9 days 87% IgG after 71 days	Pretransfusion after 9 days after 71 days after 113 days	15 25 16 6

+ = Positive; – = negative; NT = not tested.

[1] 3-phase technic: polymerized albumin at 26 °C and at 37 °C converted to indirect antiglobulin.

17, the same score achieved on average by those which had not been involved with recent trouble. The anti-Ce+e sera varied from 4 to 90% IgG and the anti-C sera varied from 24 to 100% IgG using 2-mercaptoethanol. Four of the anti-C antibodies reacted very poorly against test cells suspended in LISS.

Discussion

Two cases of HTR presenting with hemolgobinuria and in whom transient renal impairment existed occurred over a period of 14 months, during 1980 and 1981, in a community with a population of approximately 500,000. The patient with anti-C had an immediate HTR with hemoglobinuria in the first urine sample collected after the transfusion of an R_1r unit. The patient with anti-Ce+e had 'massive' hemoglobinuria which occurred on the 4th day after four antigen positive units were transfused. Two similar cases, one with anti-C who received one incompatible unit and one with anti-Ce+e who received two incompatible units occurred in the same community and in the same approximate time frame. Neither experienced signs nor symptoms of HTR.

The immediate HTR due to anti-C is the first such case to be reported. This and the DHTR due to anti-Ce+e add two cases of HTR due to these antibodies to the nine already reported [5, 8, 9], and they add two cases with hemoglobinuria to the five reported [9]. The cases reported herein also add one DHTR with hemoglobinuria to the 24 reported cases due to all alloantibodies [6–11] and they add two examples of HTR due to Rh antibodies complicated by renal impairment to the four cases already reported [6, 8, 10, 11].

Comparisons of the antibodies in the four cases are outlined in table II. All 4 were present in the pretransfusion samples on retrospective study. All were weak initially but were detected with R_1R_1 cells by the routine technic described on that retrospective study at the reference laboratory. The antibodies had been missed initially at the hospitals for various reasons. The anti-C of case No. 1 did not react by the LISS technic which has been that hospital's routine since 1977 [1, 2]. This antibody also failed to react with LISS solutions and additives at the reference laboratory. The antibodies of the other three cases were missed initially at the two other hospitals perhaps because of failure to use albumin, abbreviated incubations at 37 °C, and questionable reactivities of the reagent red cells used in screens for unexpected antibodies. The HTR cases had negative eluates when incompatible cells were present. Case No. 1 never had a positive direct antiglobulin test nor a positive autocontrol even when the cell samples containing C-positive red cells were pretreated with ficin. Antibody response to the incompatible transfusions was minimal and/or transient in three cases. The strength of all four antibodies was comparable to those reported by *Pickles* et al. [9].

Because we had very similar cases, one of each pair experiencing HTR and the other having no apparent reaction, it was hoped that immunoglobulin classing, IgG subclassing and human RBC-MPA of the four antibodies would provide the answer to their different behaviors 'in vivo'. The two anti-C antibodies were entirely IgG while the two anti-Ce+e antibodies were predominantly IgM initially and mostly IgG later on. These results are similar to those obtained on 16

other examples of these specificities. None of the antibodies could be subclassed, and all four were normal on RBC-MPA. The failure of the antibodies of patients No. 1 and No. 3 to give positive results in the RBC-MPA, whatever the explanation, casts some doubt on the validity of that test to consistently determine biological significance of alloantibodies. These were Rh antibodies which caused severe HTRs with hemoglobinemia, hemoglobinuria, and impaired renal function.

It is obvious that once again no answers are provided although it was hoped initially that IgG subclassing and macrophage assays would give the necessary clues. These four cases add to the evidence that 'in vitro' tests of alloantibodies are not always reliable in predicting the extent, if any, of red cell destruction 'in vivo'.

They also point out that the routine technic used in the reference laboratory for detection of unexpected antibodies and for incompatibility of donors' red cells, involving albumin and 60-min incubations at 37 °C, was successful in detecting the alloantibodies in pretransfusion samples from all 4 patients.

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