Antibodies to co-trimoxazole (trimethoprim and/or sulfamethoxazole) related to the presence of the drug in a commercial low-ionic-strength solution

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BACKGROUND: Drug-dependent antibodies have been associated with approximately 10% of acquired immune hemolytic anemia cases. These antibodies are a rare cause of interference in pretransfusion red blood cell (RBC) serologic testing. The aim of this work was to report three cases of subjects developing antibodies against co-trimoxazole, a combination of trimethoprim (TMP) and sulfamethoxazole (SMX).

CASE REPORT AND METHODS: Blood samples of donor/patients were referred to our laboratory for the exploration of a positive antibody detection test. There was no recent history of drug taking. Antibody identification was performed by gel test using an indirect antiglobulin test, with reagent RBCs in low-ionic-strength solutions (LISS) containing co-trimoxazole or not.

RESULTS: All three sera showed positive reactions when RBCs were resuspended in LISS containing co-trimoxazole, but negative reactions when RBCs were resuspended in LISS without antibiotic. We detected antibodies against co-trimoxazole showing three different antibody patterns: anti-TMP plus anti-SMX, anti-TMP alone, or anti-SMX alone. Anti-TMP showed an apparent anti-Ku specificity in the two cases where it was present. Anti-SMX showed an apparent anti-H specificity in one of the two cases described. The drug-dependent antibodies were not associated with acquired hemolytic anemia or other pathologies.

CONCLUSION: Antibodies against co-trimoxazole may only be detected when using a diluent for reagent RBCs containing the drug in question. Antibody pattern (anti-TMP and/or anti-SMX) may vary according to individuals' immune response. Drug-dependent antibodies may react as antibodies against a high-prevalence antigen, supporting the hypothesis of antibodies to drug and membrane components. Drug-dependent antibodies such as anti-co-trimoxazole may be a serologic finding without clinical features.

Antibodies to drugs associated with immune hemolytic anemia were first reported in 1953 by Snapper and colleagues.[1] The patient developed pancytopenia with hemolytic anemia associated with the ingestion of mephenytoin (Mesantoin). Drug-induced immune hemolytic anemia (DIIHA) is a rare event, with an incidence of approximately 1 in 1,000,000 individuals.[2] It has been reported to represent approximately 10% of acquired immune hemolytic anemia cases.[2] The diagnosis is often difficult to be proven. Most DIIHAs are caused by drug-induced antibodies.[2] Drug-induced antibodies are of two types, drug-dependent and drug-independent antibodies.[3] Drug-dependent antibodies (i.e., will only react in vitro in the presence of the drug) that are the most commonly found can be classified into two categories, antibodies reacting with drugs that bind firmly to the red blood cell (RBC) membrane (e.g., penicillin), and antibodies reacting with drugs that do not appear to bind firmly to the RBC membrane (e.g., ceftriaxone). Drug-independent antibodies are antibodies reacting in vitro with RBCs without the presence of drugs, thus appearing to be RBC autoantibodies causing autoimmune hemolytic anemia rather than antibodies to drug (e.g., methyldopa). Some DIIHAs may be due to "membrane modification" of RBCs, leading to a

ABBREVIATIONS: DIIHA(s) = drug-induced immune hemolytic anemia(s); SMX = sulfamethoxazole; TMP = trimethoprim.

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nonimmunologic binding of the proteins to the RBCs (e.g., cephalothin). Different mechanisms involved in DIIHA have been suggested.[4-7] In 1990, Mueller-Eckhardt and Salama[8] proposed an "unifying hypothesis." Antibodies might be directed at the hapten (drug) or a neoantigen (drug and membrane protein). One or more antibodies may be present in one patient. Yet, all suggested mechanisms are controversial, as discussed by Garratty in a general review.[3]

By now, 125 drugs have been described as causing DIIHA.[9] By 1979, α-methyldopa represented the most frequent cause of DIIHA. Since the 1980s, second- and third-generation cephalosporins have been associated with 80% of DIIHAs. Our laboratory first described two cases of donor/patients with antibodies against co-trimoxazole.[10] This antibacterial drug is a combination of two drug products: trimethoprim (TMP), a diaminopyrimidine, and sulfamethoxazole (SMX), a substance belonging to the sulfonamide family. In France, this drug has been marketed under the name of Bactrim (Roche, Neuilly-Sur-Seine, France). In our first report, each case was associated with an antibody against one of the components of co-trimoxazole. The aim of this work was to report a third case of antibodies against co-trimoxazole and to summarize the serologic data related to these drug-dependent antibodies.

CASE REPORT

Case 1

A 57-year-old woman, suffering from multiple sclerosis, was admitted for anemia. She was transfused 7 years previously, but had no history of pregnancy. Her hemoglobin level was 6 g/dL. The diagnosis of aplastic anemia was made. The antibody detection test was positive. The antibody identification was performed using an indirect antiglobulin (polyspecific) test (IAT), gel method, according to the manufacturer's instructions (DiaMed, Cressier/Morat, Switzerland), with native RBCs in a low-ionic-strength solution (LISS) from DiaMed (ID-Diluent 2). Positive reactions with all tested RBCs were recorded. The autologous control was positive. The direct antiglobulin test (DAT) gel card (anti-IgG and anti-C3d) was negative. The reactivity persisted when testing the serum after adsorption onto autologous RBCs.

Case 2

An antibody detection test performed before transfusion on blood sample from an 83-year-old woman was found to be positive. The antibody identification was performed using a polyspecific IAT, gel method, according to the manufacturer's instructions (DiaMed) with native RBCs in ID-Diluent 2. Positive reactions with all tested RBCs were recorded. The autologous control was positive. The DAT gel card (anti-IgG and anti-C3d) was negative.

Case 3

A 28-year-old male blood donor showed a positive antibody detection test on the occasion of a blood donation. The antibody identification was performed using a polyspecific IAT, gel method, according to the manufacturer's instructions (DiaMed) with native RBCs in ID-Diluent 2. Positive reactions with all tested RBCs were recorded. The autologous control was negative. The DAT gel card (anti-IgG and anti-C3d) was negative.

MATERIALS AND METHODS

Study of the three cases

The donor/patients' samples (15 mL of ethylenediaminetetraacetate blood and 15 mL of serum) were referred to the Centre National de Référence pour les Groupes Sanguins for antibody identification. In our laboratory, the initial antibody identification was performed using an IAT (anti-IgG) gel method (DiaMed), with native and papain-treated RBCs. Two different LISS were used: ID-Diluent 2 from DiaMed and a LISS from CDM Lavoisier (Paris, France) containing no co-trimoxazole. DAT using gel method (anti-IgG and anti-C3d separately) was performed using the commercial kit (DC-Screening II, DiaMed), according to the manufacturer's recommendations.

Elution was performed using an acid elution method (Gamma Elut-KitII, Immucor Gamma, Norcross, GA). The kit was used according to the manufacturer's recommendations. The eluate was tested using an IAT gel method (DiaMed), according to the manufacturer's recommendations, with native and papain-treated RBCs. The DiaMed LISS contained co-trimoxazole.

Papain treatment of the test RBCs was performed according to the manufacturer's recommendations (Papain Palerm, Diagast, Loos, France): 1 vol of papain solution was added to 1 vol of washed RBCs. After an incubation of 15 minutes at 37°C, RBCs were washed three times.

Dithiothreitol (DTT) treatment of the serum: 0.01 mol/L DTT was prepared in phosphate-buffered saline (PBS; Sigma-Aldrich Chimie SARL, Lyon, France); 1 vol of serum and 1 vol of 0.01 mol/L DTT were incubated 40 minutes at 37°C. PBS instead of DTT was used as control. The titration tests were performed using an IAT (anti-IgG) gel method (DiaMed).

Drug antibody study

All tests were performed with gel cards (DiaMed), using a LISS from CDM Lavoisier, without co-trimoxazole. Initial

testing for drug-dependent antibody was performed using a co-trimoxazole solution (Bactrim, commercial solution for parenteral administration from Roche: 80 mg TMP plus 400 mg SMX under a volume of 5 mL). A quantity of 25 μL of serum was added to 50 μL of a pool (3 RBC units) of 0.8% (native and papain-treated) RBCs and 25 μL of a co-trimoxazole solution. One control reaction consisting in 25 μL of PBS instead of 25 μL of the co–trimoxazole solution was performed. A pool of AB serum was tested in parallel (co-trimoxazole solution and PBS). The tests were performed using a DiaMed gel test (anti-IgG), with an incubation of 30 minutes at 37°C.

The specific tests for each component (SMX and TMP) were performed using powder obtained from Roche (Basel, Switzerland): 200 mg of each powder was added to 5 mL of PBS. The mixture was mixed vigorously and incubated at 37°C for 2 hours with gentle permanent mixing. After centrifugation to pack and remove the precipitate, the supernatants were used to perform the tests described above, each supernatant being used instead of the co-trimoxazole solution.

Specificity study

A panel of RBCs lacking a high-prevalence antigen was tested: rare RBCs were Rh null, D–, K_0, Fy(a–b–), Jk(a–b–), U(–), Ena(–), Lu(b–), Yt(a–), H(–), Tj^a(–), Ge(–2,–3), Di(a+,b–), Sc(–1,–2), Co(a–b–), Ch/Rg1(–), Vel(–), Lan(–), Jr^a(–), GIL(–), Emm(–), Er^a(–), or PEL(–) RBCs.

When a negative reaction was observed, at least two other RBCs of the same specificity were tested (K_0 and H[–] RBCs). When we observed negative reaction with K_0 RBCs, we tested other RBCs lacking high-prevalence Kell antigens (k, Kp^b, Js^b, KEL22, Mc Leod). The reactions were positive.

RESULTS

We confirmed that all three sera gave a positive reaction against all native or papain-treated RBCs of the Panel National de Référence when suspended in the ID-Diluent 2. In contrast, no reaction against native or papain-treated RBCs of the Panel National de Référence was noted when the RBCs were suspended in our LISS containing no antibiotics (Table 1). The autologous controls for Cases 1 and 2 were positive, but for Case 3, the reactivity was clearly weaker than that of panel RBCs. The DAT using anti-IgG or anti-C3d were negative. The eluates (Cases 1 and 2) gave negative reactions.

Initial testing for drug antibody was performed because the LISS produced by DiaMed (ID-Diluent 2) was known as containing co-trimoxazole, the antibiotic combination of TMP and SMX. In presence of co-trimoxazole, all three sera gave positive agglutination reactions against native or papain-treated RBCs of the Panel National de Référence, demonstrating the presence of antibodies against co-trimoxazole. No hemolysis was noted. After inquiry (subject and medical staff), no data concerning a recent or past intake of the drug could be asserted. The precise specificity of each serum was determined using TMP or SMX solutions. Case 1 produced anti-TMP associated with anti-SMX. Case 2 produced anti-TMP alone, whereas Case 3 produced anti-SMX alone (Table 1). Antibodies were IgG (persistent reactivity after DTT treatment).

These drug-dependent antibodies were then tested against a panel of RBCs lacking a high-prevalence RBC antigen. The antibody against TMP produced by Case 1 and that produced by Case 2 gave a negative reaction when tested against K_0 RBCs, showing an apparent anti-Ku specificity (Table 1). The antibody against SMX produced by Case 3, but not that produced by Case 1, gave a negative reaction when tested against H(–) RBCs. The apparent anti-H specificity explained the weaker reactivity of the autologous control of the subject Case 3 (group B). When other group B RBCs were tested, the reactivity was also weaker than that of the group O panel RBCs.

TABLE 1. Results of testing the serum in the presence of drugs

	Case 1		Case 2		Case 3	
Drug tested	Native serum	Serum after DTT	Native serum	Serum after DTT	Native serum	Serum after DTT
Co-trimoxazole (TMP-SMX) solution						
Normal RBCs	2+	2+	$2+^s$	$2+^s$	3+	3+
K_0 RBCs	2+	NT	–		3+	NT
TMP supernatant						
Normal RBCs	2+	2+	$2+^s$	NT	–	
K_0 RBCs	–		–			
SMX supernatant						
Normal RBCs	2+	2+	–		2+	NT
H(–) RBCs	2+	NT			–	
None (PBS)	–		–		–	

NT = not tested.

DISCUSSION

Our laboratory reported first that antibodies against co-trimethoxazole caused interference in the antibody detection test.[10] The samples were sent to our laboratory for two different reasons. For Case 3, the presence of an alloantibody against a high-frequency antigen was suspected because of the weaker reactivity of the autologous control. For the two other cases, the first laboratory's investigation raised their attention because of the presence of a strong autoantibody with negative DATs and elutions. In terms of serologic testing, the presence of antibodies against co-trimethoxazole may only be assessed when using a diluent for reagent RBCs containing the drug in question. We demonstrated, using the same gel method, that the same serum containing antibodies against co-trimethoxazole gave positive reactions with native or enzyme-treated RBCs when using a diluent containing the drug in question, but negative reactions with native or enzyme-treated RBCs when using a diluent without the drug (Table 1). In the discussion of their case, Arndt and colleagues[11] have described three possible explanations for their results. In our study, only one explanation could be retained because there was no recent intake of the drug (1 week), and we only used the gel method. The positive results were linked, as we stated, to the presence of the drug in the diluent. The role of diluents for reagent RBCs when an initial antibody detection test is positive is likely underestimated.[12] The diluents for commercial reagent RBCs contain different antibiotics, such as chloramphenicol, neomycin sulfate, and gentamycin. The presence of antibodies to a given drug may lead to positive results when performing antibody identification, but only when testing the serum in the presence of the given antibiotic in the RBC diluent used. This has prompted us to use a LISS containing no antibiotic. Laboratories working in the field should pay attention to conflicting results in antibody screening with different techniques of equal sensitivity, presence of autoantibody with negative direct antiglobulin and elution tests, and persistence of the autoantibody after autologous adsorptions. The absence of biologic and clinical data in favor of hemolytic anemia would be of additional value.

In this report, we detailed three different cases where subjects producing antibodies against co-trimethoxazole showed different antibody patterns: antibodies to both co-trimethoxazole components (i.e., antibodies against TMP and antibodies against SMX) or antibodies against one component of co-trimethoxazole only (antibodies against TMP or antibodies against SMX). These different patterns of antibody reactivity, also described by Arndt and coworkers[11] and Gupta and coworkers,[13] support the evidence that immune response elicited by a given drug is an individual response to antigenic challenge governed by many factors.

Above all, our article shows that antibodies against co-trimethoxazole may react as antibodies against a high-prevalence antigen. Antibodies against TMP gave negative reaction against K_0 RBCs, showing an apparent anti-Ku specificity in the two cases where it was present, whereas antibody to SMX showed an apparent anti-H specificity in one of the two cases described. These observations give important information supporting the hypothesis that RBCs act as a surface enabling the drug binding. With regard to the negative agglutination reactions obtained when antibodies against TMP were tested with K_0 RBCs, our hypothesis is that TMP may bind to the Kell glycoprotein and that IgG antibodies against TMP may react with RBC-bound TMP. This drug-induced antibody reaction may correspond to the so-called "antibody to drug and membrane components" of the "unifying hypothesis." Antibodies against SMX may react according to a comparable mechanism. With regard to the negative agglutination reactions obtained when antibody against SMX was tested with H-RBCs in one case (subject group B), our hypothesis is that SMX may bind to a component of H substance, likely a component shared by B antigen. However, other sera of subjects group O, A, or B, containing antibodies against SMX, should be tested to confirm this hypothesis. Our finding that antibodies against co-trimethoxazole may react as antibodies against a high-prevalence antigen was in accordance with previous studies.[2,14-20] In particular, Habibi and Bretagne[16] reported in 1983 an anti-Ku specificity of an antibody against Glafenin. However, antibodies against Glafenin were found in this study to display another specificity (anti-e) in two other cases, suggesting that drug-induced antibodies may recognize different RBC membrane components. At last, some antibodies to chemicals present in the commercial RBC suspension media, commercial antisera, or commercial antibody potentiators have been shown to have a blood group specificity, emphasizing the need to know the formula of media used in medical devices to correctly interpret the serologic data.[12]

The clinical relevance of antibodies against co-trimethoxazole may be questioned. Antibodies against co-trimethoxazole may be a serologic finding without clinical features. These antibodies have been associated with DIIHA and renal failure previously.[11,21] In a general manner, healthy subjects can have drug-dependent antibodies in their sera, as penicillin antibodies notably.[2,22] Despite the absence of clinical events associated with the presence of these antibodies in this report, our recommendation for these subjects is to avoid the use of co-trimethoxazole. If the drug use is imperative, our recommendation is to monitor clinically and biologically the case. Finally, the persistence in the plasma of drug-dependent antibodies has never been assessed. Whether antibodies against co-trimethoxazole may be detected several weeks or months after stopping the drug could be

an explanation to the fact that these antibodies may be a serologic finding only. Further studies with follow-up regarding in vitro detection of drug-dependent antibodies should be performed. In the case of clinical events associated with the intake of co-trimethoxazole, antibody screening with a LISS medium containing co-trimethoxazole, and a LISS medium without the same drug is a very simple informative test. All data taken together, assuming that hemolytic anemia is drug dependent, must be assessed carefully, even if the presence of drug-dependent antibodies is demonstrated.

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CONFLICT OF INTEREST

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript (e.g., employment, consultancies, board membership, honoraria).

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