An update on drug-induced immune hemolytic anemia

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Four previous reviews for this journal (1985,[1] 1989,[2] 1994,[3] 2004[4]) and 3 others published more recently[5–7] give a good sense of the changes that have occurred in the last 20 years in the study of drug-induced immune hemolytic anemia (DIIHA). These changes include new drugs that cause DIIHA, incidence of the involvement of certain drugs, revisions to proposed mechanisms involved in DIIHA, and new information on individual drugs. In our review in 2005,[6] we emphasized the changing spectrum of drugs associated with DIIHA. In the 10-year period 1969 to 1979, methyldopa was responsible for 67 percent, and high-dose intravenous penicillin for 23 percent, of the DIIHAs we encountered. In the 1995 to 2004 period, 82 percent of DIIHAs were associated with second- and third-generation cephalosporins (72% of these were associated with cefotetan, and 10% with ceftriaxone).

Drug-induced antibodies are of two types: (1) drug-independent antibodies (no drug is needed to demonstrate the presence of antibody, i.e., react similarly to true autoantibodies); and (2) drug-dependent antibodies, which require drug to be present to demonstrate the antibody. The drug can be covalently bound to the RBC membrane, or exist free in the plasma. Thus, drug-dependent antibodies can be demonstrated by testing the patient's plasma or serum, or an eluate from DAT-positive RBCs with RBCs coated with drug in vitro, or testing the serum in the presence of a drug solution and RBCs or both.

In Tables 1 and 2, we list 125 drugs that we believe have reasonable evidence to support a drug-associated immune etiology for DIIHA or positive DATs.[8–151] We cannot include every reference, so we selected the first report that contained reasonable data and further references that added important extra data. Table 1 contains a list of 108 drugs in which drug-dependent antibodies (i.e., antibodies that only react with RBCs when drug is present, either bound to the RBCs, or when added to the patient's serum, and target RBCs[8–124]) were detected. Twenty-six percent of the drugs were associated with antibodies that only reacted with drug-coated RBCs; 39 percent were associated with antibodies that only reacted in the presence of drug, and 35 percent were associated with antibodies that reacted with drug-treated RBCs or in the presence of drug or both. One surprising result was that 44 percent of these reactions were accompanied by reactions against untreated RBCs without the addition of drugs in vitro. We believe that these reactions are not caused by drug-independent autoantibodies such as those listed in Table 2, but rather are a subpopulation of drug-dependent antibodies reacting with the drug plus RBC membrane proteins and not needing drug for their demonstration, or are caused by circulating drug, or drug–anti-drug complexes (see later).

Figure 1 shows the concept of the unifying hypothesis,[5,67,152,153] based on Landsteiner's work on antibody populations induced by haptens (small molecules such as drugs).[154] One population of antibodies reacts with the hapten (drug) alone; another population reacts with part drug plus carrier (protein on RBC membrane). This epitope may be mostly the carrier as illustrated on the left side of the cartoon. Antibodies that react with drug alone can be detected using RBCs coated with the drug. Some investigators call this the "hapten mechanism." This makes no sense to us as all drugs are haptens (i.e., small molecules that require a carrier such as a protein to be immunogenic). The term was first used to describe the penicillin antibody reactions, but at that time few drug antibodies had been described and little was known of drug immunology. (For further discussion of this see pages 263–7 of Petz and Garratty.[5]) Antibodies reacting with an epitope such as the one illustrated on the bottom right side of the cartoon will react when the patient's serum is mixed with drug and RBCs (this mechanism is sometimes called the "immune-complex" mechanism). When the epitope is mainly composed of the RBC membrane, then the antibody may react with RBCs without any drug being present and appears to be a

Table 1. Drugs associated with cases of IHA or positive DAT or both in which drug-dependent antibodies were detected*

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Method of detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Aceclofenac	8	NSAID	Single (1997)	✔	✔	–	✔	–	–
Acetaminophen (Paracetamol)	9, 10	NSAID	Multiple (<10)	✔	✔	–	✔	–	–
Acyclovir	11	Antiviral	Single (2003)	✔	✔	✔	–	–	–
Aminopyrine (Piramidone)	12	NSAID	Single (1961)	✔	–	✔	–	–	–
Amoxicillin	13	Antimicrobial	Single (1985)	✔	✔	✔	–	–	–
Amphotericin B	14	Antimicrobial	Multiple (<5)	✔	✔	–	✔†	–	–
Ampicillin	15	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	–
Antazoline	16	Antihistamine	Multiple (<5)	✔	✔	–	✔	–	–
Aspirin	17	Analgesic, antipyretic, anti-inflammatory	Single (1984)	✔	–	–	✔	–	–
Azapropazone (Apazone)	18	Antiinflammatory, analgesic	Multiple (<5)	✔	✔	✔	–	–	✔
Buthiazide (Butizide)	19	Diuretic, antihypertensive	Single (1984)	✔	✔	–	✔†	–	–
Carbimazole	20	Antithyroid	Multiple (<5)	✔	✔	✔	✔	–	✔
Carboplatin‡	21	Antineoplastic	Multiple (<5)	✔	✔	✔	✔	–	✔
Carbromal	22	Sedative, hypnotic	Single (1970)	–	✔	✔	–	–	–
Catechin ((+)-Cyanidanol-3) (Cianidanol)	23	Antidiarrheal	Multiple (≥10)	✔	✔	✔	✔†	–	✔
Cefamandole	24	Antimicrobial	Single (1985)	✔	✔	✔	–	–	–
Cefazolin	25	Antimicrobial	Multiple (<10)	✔	✔	✔	–	–	–
Cefixime	26	Antimicrobial	Single (2000)	✔	–	✔	✔	–	–
Cefotaxime‡	27	Antimicrobial	Multiple (<5)	✔	✔	✔	✔	–	✔**
Cefotetan‡	28–32	Antimicrobial	Multiple (≥10)	✔	✔	✔¶	✔	–	✔
Cefoxitin‡	33	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔
Cefpirome	34	Antibacterial	Single (2005)	–	✔	–	✔	–	–
Ceftazidime	35	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔
Ceftizoxime	36	Antimicrobial	Multiple (<5)	✔	✔	✔	✔	–	✔**
Ceftriaxone‡	37, 38	Antimicrobial	Multiple (>10)	✔	✔	–	✔†	–	✔**
Cefuroxime	39	Antibacterial	Multiple (<5)	✔	✔	✔	–	–	–
Cephalexin	40	Antimicrobial	Multiple (5)	✔	✔	✔¶	–	–	–
Cephalothin‡	41–43	Antimicrobial	Multiple (≥10)	✔	✔	✔¶	✔	–	–
Chloramphenicol	44	Antibacterial	Multiple (<5)	✔	✔	✔	–	–	✔
Chlorinated hydrocarbons	45	Insecticides	Multiple (<10)	✔	✔	✔	✔	–	✔
Chlorpromazine	46	Antiemetic, antipsychotic	Multiple (<10)	✔	✔	✔	–	–	✔
Chlorpropamide‡	47, 48	Antidiabetic	Multiple (<10)	✔	✔	–	✔	–	✔**
Ciprofloxacin	49	Antibacterial	Multiple (<10)	✔	✔	–	✔	–	✔
Cisplatin (Cisdiamino-dichloroplatinum)	50, 51	Antineoplastic	Multiple (<10)	✔	✔	✔¶	✔	–	–
Cloxacillin	52	Antibacterial	Single (1980)	–	✔	–	–	✔	✔
Cyclofenil	53	Gonad-stimulating principle	Multiple (<5)	✔	✔	–	✔	–	✔
Cyclosporin (Cyclosporine)	54	Immunosuppressant	Multiple (<5)	✔	✔	✔	–	–	✔

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Method of detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Dexchlorpheniramine maleate (Chlorpheniramine)	55	Antihistaminic	Single (1981)	✔	✔	–	✔	–	–
Diclofenac‡	56–59	NSAID	Multiple (≥10)	✔	✔	✔	✔†	–	✔**
Diethylstilbestrol (Stilboestrol)	60	Estrogen	Multiple (<5)	✔	✔	–	✔	–	–
Dipyrone	61	NSAID	Multiple (<5)	✔	✔	✔	✔	–	–
Erythromycin‡	62	Antimicrobial	Multiple (<5	✔	✔	✔	–	–	–
Etodolac	63	NSAID	Single (2000)	✔	✔	–	✔†	–	–
Ethambutol	11	Antibacterial	Single (2003)	✔	✔	✔	✔	–	–
Fenoprofen	64	NSAID	Single (1988)	✔	✔	–	✔	–	✔**
Fluconazole	11	Antifungal	Single (2003)	✔	✔	✔	✔	–	–
Fluorescein	65	Injectable dye	Single (1993)	✔	✔	✔	✔	–	✔**
Fluorouracil	66	Antineoplastic	Multiple (<5)	✔	✔	–	✔	–	–
Furosemide	35	Diuretic	Multiple (<5)	–	✔	–	✔	–	–
Glafenine (Glaphenine)	67, 68	Analgesic	Multiple (<5)	✔	✔	–	–	✔†	✔
Hydralazine	69	Antihypertensive	Single (1977)	✔	✔	✔	–	–	–
Hydrochlorothiazide‡	70	Diuretic	Multiple (<10)	✔	✔	✔	✔	–	✔**
9-Hydroxy-methyl-ellipticinium (Elliptinium acetate)	71	Antineoplastic	Multiple (<5)	✔	✔	–	✔	–	–
Ibuprofen	72	NSAID	Multiple (<5)	✔	✔	–	✔	–	✔
Imatinib mesylate	73	Antineoplastic	Multiple (<5)	✔	✔	✔	–	–	–
Insulin	74	Antidiabetic	Multiple (<5)	✔	✔	✔	–	–	–
Isoniazid	75	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	–
Latamoxef (Moxalactam)	67	Antimicrobial	Single (1985)	✔	✔	–	–	✔	✔
Levofloxacin (Ofloxacin)	76	Antibacterial	Multiple (<5)	✔	✔	✔	✔	–	✔
Mefloquine‡	77	Antimicrobial	Multiple (<5)	✔	✔	✔	✔	–	✔**
Melphalan	78	Antineoplastic	Single (1967)	✔	–	–	✔	–	–
6-Mercaptopurine	79	Anti-neoplastic	Single (2000)	✔	✔	✔	–	–	–
Methadone	80	Analgesic	Multiple (<5)	–	✔	✔	–	–	–
Methotrexate	81	Antineoplastic, antirheumatic	Multiple (<5)	✔	✔	✔	✔	–	✔
Metrizoate-based radiographic contrast media	82		Multiple (<5)	✔	✔	✔	✔	–	✔
Minocycline	83	Antibacterial	Single (1994)	✔	✔	–	✔	–	–
Nabumetone analgesic	84	Antiinflammatory,	Single (2003)	✔	✔	–	✔†	–	✔
Nafcillin‡	85	Antimicrobial	Multiple (<10)	✔	✔	✔	–	–	–
Naproxen	86	Antiinflammatory, analgesic, antipyretic	Multiple (<5)	✔	✔	–	✔	–	–
Nitrofurantoin	87	Antibacterial	Single (1981)	✔	–	–	✔	–	–
Nomifensine§	88	Antidepressant	Multiple (≥10)	✔	✔	–	✔†	–	✔**
Norfloxacin	89	Antimicrobial	Single (1999)	–	✔	✔	–	–	–
Oxaliplatin‡	90, 91	Antineoplastic	Multiple (≥10)	✔	✔	✔¶	✔	–	✔**
p-Aminosalicylic acid (PAS) (para-aminosalicylsaure)	92	Antimicrobial	Multiple (<10)	✔	✔	–	✔	–	–

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Method of detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Penicillin G‡	93, 94	Antimicrobial	Multiple (≥10)	✔	✔	✔	✔	–	–
Phenacetin‡ (Acetophenetidin)	95	NSAID	Multiple (≥10)	✔	✔	–	✔	–	✔
Phenytoin (Fenitoine)	11	Anticonvulsant, antiarrhythmic	Single (2003)	✔	✔	✔	–	–	–
Piperacillin‡	96	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔**
Probenecid‡	97	Uricosuric	Multiple (<5)	✔	✔	–	✔	–	✔**
Propyphenazone	98	NSAID	Single (1998)	✔	✔	–	✔	–	–
Pyrazinamide	11	Antibacterial	Single (2003)	✔	✔	✔	✔	–	–
Pyrimethamine (Pirimetamine)	11	Antimicrobial	Multiple (<5)	✔	✔	✔	–	–	–
Quinidine	99	Antiarrhythmic, antimicrobial	Multiple (≥10)	✔	✔	✔	✔	–	✔**
Quinine	95	Antimicrobial	Multiple (<10)	✔	–	–	✔	–	✔
Ranitidine	100	Antiulcerative	Multiple (<5)	✔	✔	✔	✔	–	–
Rifabutin	11	Antibacterial	Single (2003)	✔	✔	–	✔	–	–
Rifampin‡ (Rifampicin)	101–103	Antibacterial	Multiple (≥10)	✔	✔	✔	✔	–	✔**
Stibophen	105	Antimicrobial	Multiple (<5)	✔	✔	–	✔	–	–
Streptokinase	106	Thrombolytic	Single (1989)	✔	✔	✔	–	–	✔
Streptomycin	107–109	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔
Sulfasalazine	110	Antiinflammatory	Multiple (<5)	✔	✔	–	✔	–	–
Sulfisoxazole	11	Antibacterial	Single (2003)	✔	✔	✔	✔	–	–
Sulindac	111	Antiinflammatory	Multiple (<10)	✔	✔	✔	✔	–	✔**
Suprofen	112	NSAID	Single (1989)	✔	✔	–	✔	–	✔**
Tartrazine	113	Colorant	Single (1979)	✔	✔	✔	✔	–	–
Teicoplanin	114	Antimicrobial	Single (2004)	✔	✔	–	✔	–	✔
Temafloxacin§	115	Antimicrobial	Multiple (<5)	✔	✔	–	✔	–	–
Teniposide	116	Antineoplastic	Single (1982)	✔	✔	–	✔	–	✔
Tetracycline	117	Antimicrobial	Multiple (<10)	✔	✔	✔	–	–	–
Thiopental sodium	104	Anesthetic	Single (1985)	✔	–	–	✔	–	–
Ticarcillin‡	118	Antimicrobial	Multiple (<5)	✔	✔	✔	–	–	✔
Tolbutamide	119	Antidiabetic	Multiple (<5)	✔	✔	✔	–	–	–
Tolmetin‡	120	NSAID	Multiple (≥10)	✔	✔	–	✔	–	✔**
Triamterene	121	Diuretic	Multiple (<5)	✔	✔	✔	✔	–	–
Trimellitic anhydride	122	Used in preparation of resins, dyes, adhesives, etc.	Single (1979)	✔	–	✔	–	–	–
Trimethoprim and sulfamethoxazole‡	123	Antibacterial	Multiple (<5)	✔	✔	✔	✔	–	✔
Vancomycin	11	Antibacterial	Single (2003)	✔	✔	–	✔	–	–
Zomepirac	124	NSAID	Single (1983)	✔	✔	–	✔	–	✔

* When a drug antibody is indicated to be reactive by two methods, e.g., vs. drug-treated RBCs and when serum + drug + RBCs are mixed together, this does not necessarily mean that all examples of antibodies to that drug were detected by both methods. Using ampicillin for example, four reported antibodies reacted with drug-treated RBCs and were either nonreactive (n = 1) or not tested (n = 3) by the serum + drug + RBCs method, and two antibodies reacted when serum + drug + RBCs were tested but were nonreactive with drug-treated RBCs.

IHA = immune hemolytic anemia; HA = hemolytic anemia; NSAID = nonsteroidal antiinflammatory drug.

† One or more samples only positive or strongest reactions seen with ex vivo (urine or serum) or metabolite.

‡ We have seen cases of DIIHA or positive DAT or both attributable to these.

§ No longer manufactured.

¶ Associated with nonimmunologic protein adsorption (NIPA).

**One or more samples positive possibly owing to the presence of circulating drug or drug–antibody immune complexes.

Table 2. Drugs associated with cases of IHA or positive DAT or both in which only drug-independent antibodies (autoantibodies) were detected

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	More evidence needed
Captopril	125	Antihypertensive	Multiple (<5)	✔	✔	✔
Chaparral	126	Herbal	Single (1980)		✔	✔
Cimetidine	127	Antiulcerative	Multiple (<10)	✔	✔	✔
Cladribine (2-chlorodeoxyadenosine)	128	Antineoplastic	Multiple (<10)	✔	✔	–
Fenfluramine	129	Anorexic	Single (1973)	✔	✔	✔
Fludarabine*	130,131	Antineoplastic	Multiple (≥10)	✔	✔	–
Interferon	132	Antineoplastic, antiviral	Multiple (≥10)	✔	✔	✔
Interleukin-2	133	Antineoplastic	Multiple (<5)	✔	✔	✔
Ketoconazole	134	Antifungal	Single (1987)	✔	✔	✔
Lenalidomide	135	Immunomodulatory	Single (2006)	✔	✔	✔
Levodopa (L-dopa)	136	Antiparkinsonian	Multiple (≥10)	✔	✔	–
Mefenamic acid	137	NSAID	Multiple (≥10)	✔	✔	–
Mesantoin (Mephenytoin)	138	Anticonvulsant	Single (1953)	✔	✔	✔
Methyldopa*	139	Antihypertensive	Multiple (≥10)	✔	✔	–
Nalidixic acid	140	Antibacterial	Multiple (<10)	✔	✔	✔
Procainamide*	141, 142	Antiarrhythmic	Multiple (<10)	✔	✔	–
Tacrolimus	143	Immunosuppressant	Multiple (<5)	✔	✔	✔

IHA = immune hemolytic anemia; HA = hemolytic anemia
* We have seen cases of DIIHA or positive DAT caused by these.

Table 3. Drugs associated with the detection of nonimmunologic protein adsorption onto RBCs

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Drug-dependent antibody(ies) also detected
Cefotetan*	29, 30	Antimicrobial	Multiple (≥10)	✔	✔	✔
Cephalothin*	41, 42	Antimicrobial	Multiple (≥10)	✔	✔	✔
Cisplatin	51	Antineoplastic	Multiple (<10)	✔	✔	✔
Clavulanate potassium* (Clavulanic acid)	144, 145	β-Lactamase inhibitor	Multiple (<5)	–	✔	–
Diglycoaldehyde (INOX)	146, 147	Antineoplastic	Multiple (<5)	–	✔	–
Oxaliplatin*	91	Antineoplastic	Multiple (≥10)	✔	✔	✔
Sulbactam*	145, 148	β-Lactamase inhibitor	Multiple (<5)	✔	✔	–
Suramin	149	Antihelminthic, antiprotozoal	Single (1988)	–	–	–
Tazobactam*	150, 151	β-Lactamase inhibitor	Multiple (<5)	✔	✔	–

HA = hemolytic anemia
* We have seen cases of DIIHA or positive DAT caused by these.

drug-independent antibody (autoantibody). We should emphasize that the latter hypothesis above is our own and has little experimental data to support it. Alternative explanations are that these reactions, without the presence of drug, may be attributable to drug–immune complexes still present in the plasma, or enough drug present in the plasma to cause reactions with drug-dependent antibodies.[48,64,65,77,155–160] The reactions can be differentiated from true autoantibodies by repeating the testing a few days after the drug is discontinued; by then, the drug or drug complexes will no longer be present. If the drug has an unusually long half-life (e.g., mefloquine hydrochloride has a mean half-life of 3 weeks), or if the patient is in renal failure, then the circulating drug may persist longer than expected.

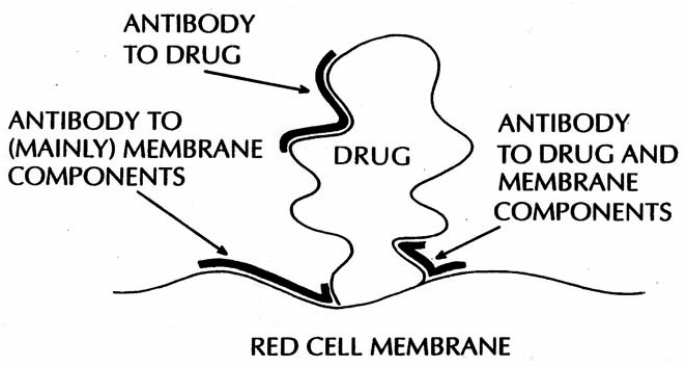

Fig. 1. Proposed unifying hypothesis of drug-induced antibody reactions. The thicker, darker lines represent antigen-binding sites on the Fab region of the drug-induced antibody. Drugs (haptens) bind loosely (or firmly) to RBC membranes, and antibodies can be made to the drug (producing in vitro reactions typical of a drug adsorption [penicillin-type] reaction); membrane components, or mainly membrane components (producing in vitro reactions typical of autoantibody); or part-drug, part-membrane components (producing an in vitro reaction typical of the so-called immune complex mechanism).

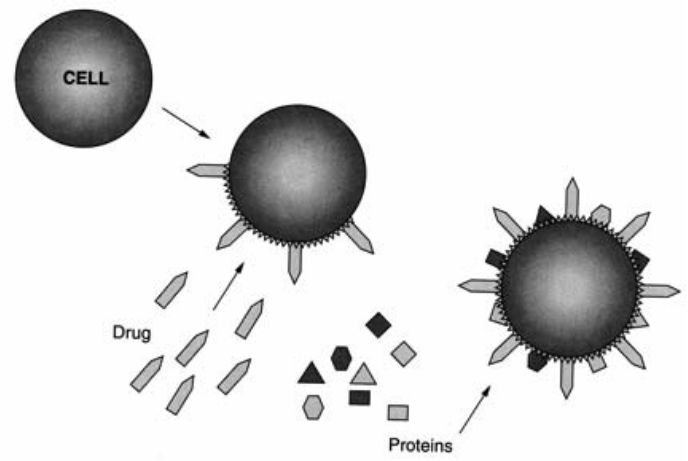

Fig. 2. Nonimmunologic adsorption of proteins onto RBCs. Drugs might change the RBC membrane so that many proteins attach to the membrane, leading to a positive DAT and, possibly, to DIIHA.

Table 2 is a list of 17 drugs that have been associated with drug-independent antibodies (autoantibodies). Many of these associations are not supported by very convincing proof that the drug induced the autoantibodies; idiopathic autoimmune hemolytic anemia (AIHA) is much more common than DIIHA and often is not excluded in the reports. It is not possible to prove the suggestion by laboratory testing as drug antibodies are not demonstrable, so many of the reports are based on a hemolytic anemia or a positive DAT or both developing after drug therapy and a response to stopping the drug. This could be coincidence. If it was a true phenomenon, then giving the drug again should restart the problem. For obvious reasons, this has not been tried often. It was tried, with methyldopa, and the AIHA occurred again, proving that there was a true relationship of drug and AIHA. Petz et al.[161] showed that it was a fallacy in two patients with suspected cimetidine-induced AIHA. When the patients' hemolytic anemia resolved after discontinuation of the drug, they were treated again with cimetidine (55 days in one patient and 24 months in the second patient) and no hemolytic anemia recurred, suggesting the drug had not caused the AIHA. These findings cast doubt on many of the drugs in this table. Often, when the drugs are stopped the patients are also taking steroids, so it is difficult to be certain what caused the recovery.

Positive Antiglobulin Tests Caused by Nonimmunologic Adsorption of Protein

During the last few years, we have become very interested in the nonimmunologic adsorption of protein onto RBCs leading to positive antiglobulin tests, and perhaps DIIHA. The reaction can mislead the investigator into thinking that an antibody to RBC antigens is being detected. The first drug shown to cause this phenomenon was the first cephalosporin (cephalothin). In 1971, Spath et al.[162] showed that cephalothin-treated RBCs adsorbed IgG, C3, albumin, fibrinogen, etc., after incubation in normal plasma; the proteins could be detected by the antiglobulin test. This is because the drug can modify the RBC membrane to adsorb proteins nonimmunologically (see Figure 2). Table 3 shows eight other drugs that cause the same effect. Up to about 1996, we believed that this was an in vitro phenomenon that caused us in vitro problems, but that it had no clinical significance. We now believe this mechanism can cause hemolytic anemia. RBCs having IgG on their membrane, caused by nonimmunologic adsorption, yield positive monocyte monolayer assays (MMA), and we have published data showing that the mechanism can be the probable cause of decreased RBC survival in patients taking drugs that contain β-lactamase inhibitors (clavulanate, sulbactam, tazobactam).[5,150,151]

This mechanism can lead to confusion in the workup of DIIHAs. The patient's serum may react with RBCs coated with the drugs listed in Table 3, but this means nothing as normal sera will also react with these same drug-coated RBCs. This is why it is important to use pooled normal plasma or serum as a negative control in any tests on drug-coated RBCs. Remember,

for five of these drugs, there is no drug antibody involved, so one can only suggest to the physician that this mechanism could be involved and let the physician have the relevant references.

Another complication is that drugs containing β-lactamase inhibitors also contain antibiotics (Zosyn contains tazobactam plus piperacillin; Unasyn contains sulbactam plus ampicillin; Timentin contains clavulanate plus ticarcillin; Augmentin contains clavulanate plus amoxicillin). These antibiotics can also cause DIIHA through more typical mechanisms.[5,163] Piperacillin is an exception to the typical reaction of penicillins in that it is detected best by the "immune complex" method.[7,164,165] An unusual aspect of piperacillin is that most normal donor and patient sera appear to have piperacillin antibodies as they react with piperacillin-coated RBCs, and this is not caused by nonimmunologic adsorption of protein. Normal sera also contain antibodies that react with penicillin- and cefotetan-coated RBCs,[5,166] but fewer sera react with these than with piperacillin-coated RBCs. It seems that we all are exposed to antigens identical, or very similar, to some penicillin-like structures in our environment.

A few technical hints when investigating DIIHA:

1. A positive DAT with a nonreactive eluate should not automatically suggest an investigation of DIIHA. Much more common causes are cytophilic IgG[167,168]; passively transfused anti-A or anti-B or both (e.g., when A, B, or AB patients receive group O RBCs, platelet products from donors not ABO identical, IVIG, or intravenous anti-D immunoglobulin).[169] Remember, drug-induced positive DATs and AIHA are rare.
2. Prepare penicillin-treated RBCs at high pH (e.g., 8–10), but prepare cephalosporin-treated RBCs at pH 7 to 7.4 to reduce nonimmunologic binding of protein.[5]
3. Some drugs do not fit the pattern seen with other members of the same family:
   - It is not possible to prepare ceftriaxone-coated RBCs; the "immune complex" method must be used.
   - Special conditions are needed to prepare nafcillin- and erythromycin-coated RBCs.[62,85,170,171]
   - Piperacillin-coated RBCs will be agglutinated by many normal sera; thus, piperacillin antibodies in serum should only be tested by the "immune complex" mechanism.[165] RBC eluates can be tested with piperacillin-coated RBCs.
4. Some drugs contain more than one chemical, e.g., Zosyn contains an antibiotic piperacillin and a β-lactamase inhibitor, tazobactam. It is important to test each drug separately to determine the specificity of the antibody. In addition to the problem with piperacillin (discussed in an earlier section), there is a special problem with the β-lactamase inhibitors. The patient's serum may react with drug-treated RBCs, suggesting a drug antibody; unfortunately, all normal sera will also react. This is because the drug can modify the RBC membrane to adsorb proteins nonimmunologically (see earlier section). Thus, it is essential to always use a pool of normal sera as a negative control when evaluating any DIIHA serologic results.
5. Antiglobulin test reactivity of a pooled normal sera control with drug-treated RBCs indicates the possible presence of nonimmunologic protein adsorption (NIPA).[5] If this occurs, the normal sera control and the patient's serum should be diluted 1 in 20 in saline and retested (the 1 in 20 dilutions should not contain enough protein to cause detectable NIPA). For testing of cefotetan-treated RBCs, the patient's serum should be diluted 1 in 100 because many normal sera react with cefotetan-treated RBCs, even at a 1 in 20 dilution.[166]
6. If a patient's serum contains a drug-independent antibody, the presence of a drug-dependent antibody can be demonstrated by performing an autologous or allogeneic adsorption to remove the drug-independent antibody and then testing the adsorbed serum by the usual methods to detect drug antibodies.
7. When looking at a patient's drug history, in addition to considering the drugs that the patient is receiving at the time of the hemolytic anemia, it may also be important to determine what drugs the patient may have received 1 to 2 weeks previously (e.g., in surgery).
8. It has been reported that 6% albumin can be used to solubilize drugs that have poor water solubility.[172] If the drug (e.g., cephalothin, cefotetan) bonds covalently to proteins (e.g., albumin), then reduced binding of the drug to RBCs can occur.[173] Thus, this method of

solubilizing drug in 6% albumin should be used cautiously, e.g., only when the drug is known to not be soluble in water and with the knowledge of its protein binding affinity (information about solubility and protein binding can usually be found in the Merck Index[174] or the Physicians' Desk Reference[175] or both).

9. Some drug antibodies have been reported to be detectable in vitro only when ex vivo preparations of the drug, e.g., urine or serum from a person taking the drug, have been used for the testing (by the "serum plus drug plus RBCs" method).[14,19,20,63,84,176–178] This is thought to be caused by the antibody being directed against a metabolite of the drug rather than the native drug and the presence of the appropriate drug metabolite(s) in the ex vivo preparation(s). Drug antibodies that have been reported to only be detected in the presence of an ex vivo preparation have been indicated with a footnote under the "serum plus drug plus RBCs" method in Table 1.
10. One important endpoint in testing for the presence of drug antibodies is hemolysis. A serum sample must be used for this testing (EDTA binds calcium, which is needed for the complement cascade; thus, EDTA plasma cannot be used for the detection of hemolysis). Many blood banks now use EDTA plasma for their routine testing, and the patient will have to have blood drawn again to obtain the necessary serum sample for a proper DIIHA workup.
11. If a patient's serum is nonreactive with drug-treated RBCs and there is no positive control available to confirm the presence of the drug on the drug-treated RBCs, then no interpretation of the negative result can be made.
12. When a drug antibody in the serum is detected by testing drug-treated RBCs, it is important to confirm the presence of that drug antibody in an eluate prepared from the patient's RBCs to conclude that the drug was the cause of the patient's hemolytic anemia.

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