Report

Drugs that have been shown to cause drug-induced immune hemolytic anemia or positive direct antiglobulin tests: some interesting findings since 2007

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This review updates new findings in drug-induced immune-hemolytic anemia (DIIHA) since the 2007 review in *Immunohematology* by these authors. Twelve additional drugs have been added to the three tables listing drugs associated with drug-dependent antibodies, drugs associated with drug-independent antibodies, and drugs associated with nonimmunologic protein adsorption. Other updated findings include (1) piperacillin is currently the most commonly encountered cause of DIIHA, (2) new data on blood group specificity of drug-dependent antibodies, (3) drug-dependent antibodies detected in healthy donors, (4) DIIHA associated with transplantation, and (5) DIIHA associated with chemotherapeutic drugs. ***Immunohematology* 2014;30:66–79.**

Key Words: drugs, immune hemolytic anemia, autoantibody, drug-dependent antibody, nonimmunologic protein adsorption

Dr. Garratty has published five previous reviews on drug-induced immune hemolytic anemia (DIIHA) in *Immunohematology*; the last one in 2007 was coauthored by Pat Arndt.[1–5] This issue is devoted to drug-induced immune cytopenias (red blood cells [RBCs], platelets, and white blood cells [WBCs]). If one reads the DIIHA reviews previously published in *Immunohematology,* you will get a view of how many drugs were found to be responsible over each 5-year period (1985–2007), which ones were the most common causes of DIIHA, and what concepts were most commonly suggested to explain various types of DIIHA. The reviews by Arndt[6] and Leger et al.[7] in this issue cover many of these areas in great detail and add new data that were not fully covered in the previous *Immunohematology* reviews. Some other recent reviews by Garratty can update you further.[8–12] For readers who do not have access to these publications,[6–12] we will summarize the most important information that has emerged in the last 10 years, with emphasis on the period since 2007.

Drugs Causing DIIHA

In our 2007 review, we included tables containing 125 drugs that we believed had reasonable evidence of causing DIIHA.[5] Indisputable evidence would require the following findings:

1. A well-defined hemolytic anemia (HA).
2. A temporal relationship to drug therapy and the start of the HA.
3. A positive direct antiglobulin test (DAT) after drug therapy (preferably with a negative DAT result preceding therapy).
4. Results of testing for drug antibodies (e.g., using drug-treated RBCs or testing serum in the presence of soluble drug[7,8]). These results must be accompanied by pertinent controls.[7,8] Laboratory tests cannot prove that autoantibodies are drug-induced, but it is important to show that apparent autoantibodies are truly autoantibodies and not drug-dependent antibodies that are reacting without the in vitro addition of drug because enough drug is present in the patient's plasma or serum.[5–7,13]
5. Patient responds hematologically after drug cessation. Unfortunately, this is not easy to define, as patients are often simultaneously given steroids.

Since 2007, we have added seven new drugs to Table 1 (drug-dependent antibodies): hydrocortisone (2008), pemetrexed (2008), cimetidine (2010), etoricoxib (2013), iomeprol (2013), puerarin (2013), and vincristine (2013). Of these 115 drugs, five (carbromal, cefpirome, furosemide, methadone, and norfloxacin) may have caused positive DATs but no HA. Twenty-seven (23%) drugs were associated with drug antibodies reacting with drug-treated RBCs; 44 (38%) drugs were associated with antibodies reacting in the presence of soluble drug; 41 (36%) drugs were associated

with antibodies detected by both methods. Fifty (43%) drugs were associated with in vitro reactions against untreated cells without any drug being added. These could be associated with drug-independent antibodies, but we believe that many may have been caused by drug or drug complexes being present in the patient's plasma or serum (see footnote in Table 1). It will probably be no surprise that not all of the drugs listed in Table 1 satisfy 100% of our criteria for perfection. Hence, we can only say that there is reasonable evidence that these drugs were the cause of DIIHA.

We added four new drugs to Table 2 that appeared to cause autoimmune hemolytic anemia (drug-independent antibodies): rituximab (2003), alemtuzumab (2007), weidean (2009), and bendamustine (2013). All but one of the drugs in Table 2 (chaparral) may have caused a DAT-positive autoimmune HA (AIHA). It should be noted that 16 of 21 drugs in this group were classified as needing more evidence to support that they belong in this group. One drug (cephaloridine) was added to the nonimmunologic protein adsorption group (Table 3); this was observed in 1967 but was unfortunately left off our 2007 Table 3.

Classification of Drug Antibodies

There are two main types of drug antibodies: drug-dependent antibodies and drug-independent antibodies.

Drug-dependent antibodies need the drug to be present for in vitro reactions; the drug can be bound to test RBCs or soluble drug can be added to patient's plasma or serum (source of antibody) and allogeneic group O RBCs; after 37°C incubation, the tests are inspected for lysis, direct agglutination, and by the indirect antiglobulin test (IAT). Enzyme-treated RBCs should be used in addition to untreated RBCs.[7] Test results are only valid when appropriate controls are used (see Leger et al.[7] for complete details). Salama's group does not advise using enzyme-treated RBCs[169] because of problems with nonspecific reactions, but we believe these can usually be overcome with experience and pertinent controls.

Drug-independent antibodies react without any drug being added in vitro. The results are indistinguishable from RBC autoantibodies. It is thought that such drugs affect the immune system so that true autoantibodies and sometimes AIHA are produced. There are no special in vitro tests to define that a particular drug caused the AIHA. The diagnosis rests on the physician stopping the drug and finding that the anemia resolves, but the serology (e.g., DAT) can remain positive for some time longer.

Positive DATs and DIIHA Caused by Nonimmune Protein Adsorption

Drug-induced positive antiglobulin tests (e.g., DATs) and sometimes HA can occur with no drug antibodies involved, and yet RBCs are destroyed by macrophages in the spleen and liver. Thus, the drug interaction with the RBCs does not involve drug-induced antibodies, but the shortened life of the RBCs is by a cellular immune mechanism. The drugs that can be involved in this mechanism are listed in Table 3. These drugs can change the RBC membrane so that proteins (e.g., immunoglobulins, complement, albumin, etc.) attach to the RBCs, leading to positive antiglobulin tests. This may involve the patient's RBCs in vivo (positive DAT), or may occur in vitro (positive IAT) when the patient's serum or plasma is incubated with drug-coated RBCs (e.g., when testing for drug antibodies). This phenomenon was first observed with the first-generation cephalosporin, cephalothin, and was later found to occur with other cephalosporins (e.g., cefotetan). It was termed the "membrane modification mechanism" or "nonimmunological protein adsorption (NIPA)." It was thought for many years to be an interesting phenomenon that could interfere with interpretation of laboratory tests but had no clinical significance; then β-lactamase inhibitors, which cause NIPA, were shown to possibly cause DIIHA.[162,167] Other drugs (see Table 3) can also cause NIPA and DIIHA (e.g., cisplatin and oxaliplatin). The involvement of NIPA is sometimes hard to define, as drug antibodies (e.g., anti-oxaliplatin) may also be involved.[101] The nonimmunologically adsorbed immunoglobulin (Ig) G (and possibly C3) can react with receptors on macrophages, even though it is not an antibody to the drug or the RBCs. The most useful test to indicate that NIPA is occurring is to show that albumin is present on the RBCs by testing with anti-human albumin by the antiglobulin test.[7] Such antisera have to be standardized carefully in-house, as no U.S. Food and Drug Administration–licensed reagent is available.

New Findings Since Our Last Review in *Immunohematology* (2007)

In the period covered by our 2007 review (2005–2007), the most common drugs to cause DIIHA in patients' samples submitted to our laboratory were cefotetan, ceftriaxone, and piperacillin, in that order. For the period 2008–2013, piperacillin, rose to number 1, followed by cefotetan and ceftriaxone (Table 4). We are starting to see a small increase in DIIHA caused by drugs in the platinum family.

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, or ≥10])	HA	Positive DAT	Method detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Aceclofenac	14	NSAID	Single (1997)	✔	✔		✔		
Acetaminophen (Paracetamol)	15,16	NSAID	Multiple (<10)	✔	✔		✔		
Acyclovir	17	Antiviral	Single (2003)	✔	✔	✔			
Aminopyrine	18	NSAID	Single (1961)	✔		✔			
Amoxicillin	19	Antimicrobial	Multiple (<5)	✔	✔	✔			
Amphotericin B	20	Antimicrobial	Multiple (<5)	✔	✔		✔†		
Ampicillin	21	Antimicrobial	Multiple (<10)	✔	✔	✔	✔		
Antazoline	22	Antihistamine	Multiple (<5)	✔	✔		✔		
Aspirin	23	Analgesic, antipyretic, anti-inflammatory	Single (1984)	✔			✔		
Azapropazone (Apazone)	24	Anti-inflammatory, analgesic	Multiple (<5)	✔	✔	✔			✔
Buthiazide (Butizide)	25	Diuretic, antihypertensive	Single (1984)	✔	✔		✔†		
Carbimazole	26	Antithyroid	Multiple (<5)	✔	✔	✔	✔		✔
Carboplatin‡	27	Antineoplastic	Multiple (<10)	✔	✔	✔	✔		✔
Carbromal	28	Sedative, hypnotic	Single (1970)		✔	✔			
Catechin [(+)-Cyanidanol-3] (Cianidanol)	29	Antidiarrheal	Multiple (≥10)	✔	✔	✔	✔†		✔
Cefamandole	30	Antimicrobial	Single (1985)	✔	✔	✔			
Cefazolin	31	Antimicrobial	Multiple (<10)	✔	✔	✔			
Cefixime	32	Antimicrobial	Single (2000)	✔		✔	✔		
Cefotaxime‡	33	Antimicrobial	Multiple (<5)	✔	✔	✔	✔		✔**
Cefotetan‡	34–38	Antimicrobial	Multiple (≥10)	✔	✔	✔¶	✔		✔
Cefoxitin‡	39	Antimicrobial	Multiple (<10)	✔	✔	✔	✔		✔
Cefpirome	40	Antibacterial	Single (2005)		✔		✔		
Ceftazidime	41	Antimicrobial	Multiple (<10)	✔	✔	✔	✔		✔
Ceftizoxime	42	Antimicrobial	Multiple (<5)	✔	✔	✔	✔		✔**
Ceftriaxone‡	43,44	Antimicrobial	Multiple (≥10)	✔	✔		✔†		✔**
Cefuroxime	45	Antibacterial	Multiple (<5)	✔	✔	✔			
Cephalexin	46	Antimicrobial	Multiple (5)	✔	✔	✔¶			
Cephalothin‡	47–49	Antimicrobial	Multiple (≥10)	✔	✔	✔¶	✔		
Chloramphenicol	50	Antibacterial	Multiple (<5)	✔	✔	✔			✔
Chlorinated hydrocarbons	51	Insecticides	Multiple (<10)	✔	✔	✔	✔		✔
Chlorpromazine	52	Antiemetic, antipsychotic	Multiple (<10)	✔	✔	✔			✔
Chlorpropamide‡	53,54	Antidiabetic	Multiple (<10)	✔	✔		✔		✔**
Cimetidine‡	55	Antiulcerative	Single (2010)	✔	✔	✔	✔		
Ciprofloxacin	56	Antibacterial	Multiple (<10)	✔	✔		✔		✔
Cisplatin (Cisdiamino-dichloroplatinum)	57,58	Antineoplastic	Multiple (<10)	✔	✔	✔¶	✔		
Cloxacillin	59	Antibacterial	Single (1980)	✔	✔			✔	✔
Cyclofenil	60	Gonad-stimulating principle	Multiple (<5)	✔	✔		✔		✔

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

Drug (alternative name)	Reference	Therapeutic category	Number of references (single [year] vs. multiple [<5, <10, or ≥10])	HA	Positive DAT	Method detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Cyclosporin (Cyclosporine)	61	Immunosuppressant	Multiple (<5)	✔	✔	✔			✔
Dexchlorpheniramine maleate (Chlorpheniramine)	62	Antihistaminic	Single (1981)	✔	✔		✔		
Diclofenac‡	63–66	NSAID	Multiple (≥10)	✔	✔	✔	✔†		✔**
Diethylstilbestrol (Stilboestrol)	67	Estrogen	Multiple (<5)	✔	✔		✔		
Dipyrone	68	NSAID	Multiple (<5)	✔	✔	✔	✔		
Erythromycin‡	69	Antimicrobial	Multiple (<5)	✔	✔	✔			
Etodolac	70	NSAID	Single (2000)	✔	✔		✔†		
Etoricoxib	71	NSAID	Single (2013)	✔	✔	✔	✔†		✔**
Ethambutol	17	Antibacterial	Single (2003)	✔	✔	✔	✔		
Fenoprofen	72	NSAID	Single (1988)	✔	✔		✔		✔**
Fluconazole	17	Antifungal	Single (2003)	✔	✔	✔	✔		
Fluorescein	73	Injectable dye	Single (1993)	✔	✔	✔	✔		✔**
Fluorouracil	74	Antineoplastic	Multiple (<10)	✔	✔		✔†		
Furosemide	40	Diuretic	Multiple (<5)		✔		✔		
Glafenine (Glaphenine)	75,76	Analgesic	Multiple (<5)	✔	✔			✔†	✔
Hydralazine	77	Antihypertensive	Single (1977)	✔	✔	✔			
Hydrochlorothiazide‡	78	Diuretic	Multiple (<10)	✔	✔	✔	✔		✔**
9-Hydroxy-methyl-ellipticinium (elliptinium acetate)	79	Antineoplastic	Multiple (<5)	✔	✔		✔		
Hydrocortisone	80	Glucocorticoid	Single (2008)	✔	✔	✔	✔		
Ibuprofen	81	NSAID	Multiple (<5)	✔	✔		✔		✔
Imatinib mesylate	82	Antineoplastic	Multiple (<5)	✔	✔	✔			
Insulin	83	Antidiabetic	Multiple (<5)	✔	✔	✔			
Iomeprol	84	Radiopaque medium	Single (2013)	✔	✔		✔		✔**
Isoniazid	85	Antimicrobial	Multiple (<10)	✔	✔	✔	✔		
Latamoxef (Moxalactam)	75	Antimicrobial	Single (1985)	✔	✔			✔	✔
Levofloxacin (Ofloxacin)	86	Antibacterial	Multiple (<5)	✔	✔	✔	✔		✔
Mefloquine‡	87	Antimicrobial	Multiple (<5)	✔	✔	✔	✔		✔**
Melphalan	88	Antineoplastic	Single (1967)	✔			✔		
6-Mercaptopurine	89	Antineoplastic	Single (2000)	✔	✔	✔			
Methadone	90	Analgesic	Multiple (<5)		✔	✔			
Methotrexate	91	Antineoplastic, antirheumatic	Multiple (<5)	✔	✔	✔	✔		✔
Metrizoic acid	92	Radiopaque medium	Single (1991)	✔	✔	✔	✔		✔
Minocycline	93	Antibacterial	Single (1994)	✔			✔		
Nabumetone	94	Anti-inflammatory, analgesic	Single (2003)	✔	✔		✔†		✔
Nafcillin‡	95	Antimicrobial	Multiple (<10)	✔	✔	✔			
Naproxen	96	Anti-inflammatory, analgesic, antipyretic	Multiple (<5)	✔	✔		✔		
Nitrofurantoin	97	Antibacterial	Single (1981)	✔			✔		

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

Drug (alternative name)	Reference	Therapeutic category	Number of references (single [year] vs. multiple [<5, <10, or ≥10])	HA	Positive DAT	Method detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Nomifensine§	98	Antidepressant	Multiple (≥10)	✔	✔		✔†		✔**
Norfloxacin	99	Antimicrobial	Single (1999)		✔	✔			
Oxaliplatin‡	100, 101	Antineoplastic	Multiple (≥10)	✔	✔	✔¶	✔		✔**
p-Aminosalicylic acid (PAS) (para-aminosalicylsäure)	102	Antimicrobial	Multiple (<10)	✔	✔		✔		
Pemetrexed	103	Antineoplastic	Multiple (<5)	✔	✔		✔		
Penicillin G‡	104, 105	Antimicrobial	Multiple (≥10)	✔	✔	✔	✔		
Phenacetin‡ (Acetophenetidin)	106	NSAID	Multiple (≥10)	✔	✔		✔		✔
Phenytoin (Fenitoine)	17	Anticonvulsant, antiarrhythmic	Single (2003)	✔	✔	✔			
Piperacillin‡	107	Antimicrobial	Multiple (≥10)	✔	✔	✔	✔		✔**
Probenecid‡	108	Uricosuric	Multiple (<5)	✔	✔		✔		✔**
Propyphenazone	109	NSAID	Single (1998)	✔	✔		✔		
Puerarin	110	Chinese herb	Multiple (<5)	✔	✔		✔		
Pyrazinamide	17	Antibacterial	Single (2003)	✔	✔	✔	✔		
Pyrimethamine (Pirimetamine)	17	Antimicrobial	Multiple (<5)	✔	✔	✔			
Quinidine	111	Antiarrhythmic, Antimicrobial	Multiple (≥10)	✔	✔	✔	✔		✔**
Quinine	106	Antimicrobial	Multiple (<10)	✔			✔		✔
Ranitidine	112	Antiulcerative	Multiple (<5)	✔	✔	✔	✔		
Rifabutin	17	Antibacterial	Single (2003)	✔	✔		✔		
Rifampin‡ (Rifampicin)	113–115	Antibacterial	Multiple (≥10)	✔	✔	✔	✔		✔**
Stibophen	116	Antimicrobial	Multiple (<5)	✔	✔		✔		
Streptokinase	117	Thrombolytic	Single (1989)	✔	✔	✔			✔
Streptomycin	118–120	Antimicrobial	Multiple (<10)	✔	✔	✔	✔		✔
Sulfasalazine	121	Anti-inflammatory	Multiple (<5)	✔	✔		✔		
Sulfisoxazole	17	Antibacterial	Single (2003)	✔	✔	✔	✔		
Sulindac	122	Anti-inflammatory	Multiple (<10)	✔	✔	✔	✔		✔**
Suprofen	123	NSAID	Single (1989)	✔	✔		✔		✔**
Tartrazine	124	Colorant	Single (1979)	✔	✔	✔	✔		
Teicoplanin	125	Antimicrobial	Multiple (<5)	✔	✔		✔		✔
Temafloxacin§	126	Antimicrobial	Multiple (<5)	✔	✔		✔		
Teniposide	127	Antineoplastic	Single (1982)	✔	✔		✔		✔
Tetracycline	128	Antimicrobial	Multiple (<10)	✔	✔	✔			
Thiopental sodium	129	Anesthetic	Single (1985)	✔			✔		
Ticarcillin‡	130	Antimicrobial	Multiple (<5)	✔	✔	✔			✔
Tolbutamide	131	Antidiabetic	Multiple (<5)	✔	✔	✔			
Tolmetin‡	132	NSAID	Multiple (≥10)	✔	✔		✔		✔**
Triamterene	133	Diuretic	Multiple (<5)	✔	✔	✔	✔		
Trimellitic anhydride	134	Used in prep of resins, dyes, adhesives, etc.	Multiple (<5)	✔		✔			

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

Drug (alternative name)	Reference	Therapeutic category	Number of references (single [year] vs. multiple [<5, <10, or ≥10])	HA	Positive DAT	Method detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Trimethoprim and Sulfamethoxazole‡	135	Antibacterial	Multiple (<5)	✔	✔	✔	✔		✔
Vancomycin	17	Antibacterial	Single (2003)	✔	✔		✔		
Vincristine	136	Antineoplastic	Single (2013)	✔	✔	✔	✔		
Zomepirac	137	NSAID	Single (1983)	✔	✔		✔		✔

IHA = immune hemolytic anemia; DAT = direct antiglobulin test; HA = hemolytic anemia; RBCs = red blood cells; NSAID = nonsteroidal anti-inflammatory drug.

* 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, not all cases necessarily had drug antibodies reactive 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.

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

‡ Cases of DIIHA or positive DAT caused by these drugs have been identified in Dr. Garratty's laboratory.

§ 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, or ≥10])	HA	Positive DAT	More evidence needed
Alemtuzumab	138	Antineoplastic; immunosuppressant	Multiple (<5)	✔	✔	✔
Bendamustine	139	Antineoplastic	Multiple (<5)	✔	✔	✔
Captopril	140	Antihypertensive	Multiple (<5)	✔	✔	✔
Chaparral	141	Herbal	Single (1980)		✔	✔
Cimetidine	142	Antiulcerative	Multiple (<10)	✔	✔	✔
Cladribine (2-chloro-deoxyadenosine)	143	Antineoplastic	Multiple (<10)	✔	✔	
Fenfluramine	144	Anorexic	Single (1973)	✔	✔	✔
Fludarabine*	145,146	Antineoplastic	Multiple (≥10)	✔	✔	
Interferon	147	Antineoplastic, antiviral	Multiple (≥10)	✔	✔	✔
Interleukin-2	148	Antineoplastic	Multiple (<5)	✔	✔	✔
Ketoconazole	149	Antifungal	Single (1987)	✔	✔	✔
Lenalidomide	150	Immunomodulatory	Single (2006)	✔	✔	✔
Levodopa (L-dopa)	151	Antiparkinsonian	Multiple (≥10)	✔	✔	
Mefenamic acid	152	NSAID	Multiple (≥10)	✔	✔	
Mesantoin (Mephenytoin)	153	Anticonvulsant	Single (1953)	✔	✔	✔
Methyldopa*	154	Antihypertensive	Multiple (≥10)	✔	✔	
Nalidixic acid	155	Antibacterial	Multiple (<10)	✔	✔	✔
Procainamide*	156,157	Antiarrhythmic	Multiple (<10)	✔	✔	✔
Rituximab	158	Antineoplastic	Single (2003)	✔	✔	✔
Tacrolimus	159	Immunosuppressant	Multiple (<10)	✔	✔	✔
Weidean	160	Chinese herbs	Single (2009)	✔	✔	✔

IHA = immune hemolytic anemia; DAT = direct antiglobulin test; HA = hemolytic anemia; NSAID = nonsteroidal anti-inflammatory drug.

*Cases of drug-induced immune hemolytic anemia or positive DAT caused by these drugs have been identified in Dr. Garratty's laboratory.

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, or ≥10])	HA	Positive DAT	Drug-dependent antibody(ies) also detected
Cefotetan*	35,36	Antimicrobial	Multiple (≥10)	✔	✔	✔
Cephaloridine	48	Antimicrobial	Multiple (<5)		✔	
Cephalothin*	47,48	Antimicrobial	Multiple (≥10)	✔	✔	✔
Cisplatin	58	Antineoplastic	Multiple (<10)	✔	✔	✔
Clavulanate potassium* (Clavulanic acid)	161,162	β-Lactamase inhibitor	Multiple (<5)		✔	
Diglycoaldehyde (INOX)	163,164	Antineoplastic	Multiple (<5)		✔	
Oxaliplatin*	101	Antineoplastic	Multiple (≥10)	✔	✔	✔
Sulbactam*	161,165	β-Lactamase inhibitor	Multiple (<5)	✔	✔	
Suramin	166	Antihelminthic, antiprotozoal	Single (1988)			
Tazobactam*	167,168	β-Lactamase inhibitor	Multiple (<5)	✔	✔	

RBCs = red blood cells; HA = hemolytic anemia; DAT = direct antiglobulin test; IHA = immune hemolytic anemia.
*Cases of drug-induced immune hemolytic anemia or positive DAT caused by these drugs have been identified in Dr. Garratty's laboratory.

Table 4. Drug antibodies detected by the Research Lab at the American Red Cross in Pomona, CA, from 1978 to 2013

Years	Ceftriaxone	Cefotetan	Piperacillin	Platinum-based drugs	Other drugs
1978–83	0	0	0	0	7
1984–89	2	0	0	0	4
1990–95	2	20	0	0	7
1996–2001	6	45	2	0	6
2002–07	7	15	6	3	7
2008–13	14	13	30	5	6
Total	**31**	**93**	**38**	**8**	**37**

Drug Antibodies Showing Blood Group Specificity

I (G.G.) have been interested in this area for more than 30 years. The first report (in 1977) concerned a streptomycin antibody having Rh specificity.[118] In 1981, Duran-Suarez et al.[97] described antibodies to three drugs with I specificity. In 1984, we described a chlorpropamide-induced autoanti-Jk^a.[54] In 1983 and 1985, Habibi et al.[129,170] and, in 1986, Salama and Mueller-Eckhardt[171] added more drugs showing this phenomenon. The list has grown quite long (see Table 1 of Arndt[6]), but this is still not a common finding. There are many piperacillin antibodies showing blood group antigen specificity that have been described since 1984.[107,172] Most of these were Rh specificities (especially anti-e or "relative" anti-e specificity). Of 37 piperacillin antibodies we were sent to investigate, the referring laboratories detected 8 anti-e, 1 anti-e–like antibody, and 1 possible anti-f when testing serum without piperacillin added in vitro (in vivo circulating piperacillin was most likely present). Twenty-seven of these were tested with R_1R_1 and R_2R_2 RBCs in the presence of in vitro added piperacillin; 16 of 27 (59%) were nonreactive (7) or weaker (9) with R_2R_2 RBCs (e.g., e or relative e specificity), and 2 (8%) were weaker with R_1R_1 RBCs (relative c specificity).[172]

In 2013, we reported results of tests from our own laboratory during the last 30 years on 53 antibodies to 13 different drugs.[172] We found no blood group specificity in single examples of antibodies to acetaminophen, phenacetin, probenecid, quinidine, teniposide, cefotaxime, cimetidine, or diclofenac, four examples of anti-ceftriaxone, or ten examples of anti-cefotetan. We found some blood group antigen specificities associated with sulfamethoxazole (MNS and Lutheran) and piperacillin (Rh and Lutheran). Some specificities only became apparent when the antibodies were diluted.

Drug Antibodies Detected in Healthy Blood Donors

We have known for many years that healthy humans can have drug antibodies in their plasma or serum. Low-titer, often IgM, penicillin antibodies were detected in blood donors (5%) or patients with DIIHA (6%). This finding did not confuse the serologic diagnosis because patients with penicillin DIIHA had very high titer (>1000), usually IgG, penicillin antibodies. About 30 to 40 percent of blood donors were found to have antibodies to cephalothin and ticarcillin. Problems were encountered when it was noticed that 80 percent of donors and patients with no DIIHA had cefotetan antibodies; luckily, patients with DIIHA had very high titers of cefotetan antibodies compared with patients with no HA. The most interesting results in this area involved piperacillin. In 2008, we reported

that 91 percent of donors' sera and 49 percent of random patients' sera, contained piperacillin antibodies reactive against piperacillin-coated RBCs.[173] Luckily, we determined that antibodies to piperacillin (unlike antibodies to other penicillins) react well by adding soluble drug solution to the patients' sera, and this reactivity correlated with the DIIHA. We no longer use piperacillin-coated RBCs in screening tests. A few other drug antibodies have been detected in healthy and sick individuals with no DIIHA (oxaliplatin,[174] cisplatin,[174] and meropenem[175]).

It is not known why some of the preceding drug antibodies are found in healthy donors or why they sometimes appear more commonly in healthy donors than in random patients. For instance, 91 percent of donors and only 49 percent of random patients have piperacillin antibodies; 16 percent of donors and only 4 percent of patients have oxaliplatin antibodies.

It has been suggested that environmental factors may be responsible for the production of antibodies. For instance, automobile catalytic convertors release platinum into the atmosphere and may explain the production of antibodies to drugs that belong to the platinum family (oxaliplatin, carboplatin, and cisplatin). Another major concept to explain the production of antibodies to antibiotics in individuals with no DIIHA is that, in the United States, antibiotics are routinely fed to cattle and chickens, so we are continually exposed to drugs such as the cephalosporins and penicillins. Many countries in Europe have banned this practice mainly to prevent problems with treatment resistance of many organisms.

DIIHA Associated With Transplantation

Many of the drugs used for transplantation are designed to affect the immune system, so we should not be surprised to see true AIHA develop sometimes. Trying to prove the cause of HA or production of RBC autoantibodies (e.g., positive DAT or IAT) is a difficult task, but DIIHA has to be considered. Examples of drugs said to cause DIIHA after transplantation are cyclosporine, tacrolimus, and the combination of alemtuzumab, mycophenolate, and daclizumab. It is almost impossible to prove that the HA is caused by one of these drugs, so publications (e.g., Elimelakh et al.[138]) often hypothesize that the combination of such drugs has caused the immune aberrations. There are often no demonstrable drug-dependent antibodies but often the presence of drug-independent antibodies (autoantibodies). Some interesting points are (1) some of the AIHAs have not been warm AIHA but are associated with cold agglutinins, (2) there are interesting associations with posttransplant lymphoproliferative disorder (PTLD),[159] and (3) the hemolytic anemia can develop long after the transplant and long after therapy with the suspected drug (e.g., up to 2 years).

DiGiuseppe et al.[159] reported on a child who had a liver transplant at 1 year old; he was successfully maintained on cyclosporine for 4 years and then switched to tacrolimus, which was increased from 2 mg to 8 mg twice daily. The patient developed AIHA; his hemoglobin fell to 3.5 g/dL after transfusion of one unit of RBCs, and he died. Post-mortem examination revealed a clinically unsuspected PTLD. Elimelakh et al.[138] reported on cases of red cell aplasia (RCA) and AIHA after immunosuppression with alemtuzumab (anti-CD52), mycophenolate mofetil (MMF), and daclizumab (anti-Tac/CD25) in pancreas transplant patients. Data from a 2-year period were reported for 357 pancreas transplants. AIHA was detected in 16 patients (7 of these also had RCA; 3 had RCA only). All were DAT+ (all but one had RBC-bound IgG + C3). When MMF was discontinued in the RCA/AIHA group, seven patients recovered from RCA, but only three also recovered from the HA. In the AIHA-alone group (nine patients), MMF was discontinued in two patients with no effect on the HA. Other patients in this group were treated with steroids, rituximab, intravenous IgG, splenectomy, or plasma exchange, with remission seen in only two of the nine patients. The authors suggested that a combination of the drugs may have been involved in the AIHA. It is interesting that the RCA, which is thought to be caused by autoantibody to early RBC precursors, appeared to be caused by MMF.

Drug-Induced AIHA Associated With Chemotherapeutic Drugs

Although several chemotherapeutics have been suggested to cause DIIHA, similar problems to those of the transplant associations make the diagnosis difficult. Often several drugs are involved and no drug-dependent antibodies are detectable; the antibodies are usually drug-independent and the patient appears to have developed a true AIHA. The best examples of such drugs are the purine analogs (e.g., fludarabine and cladribine) used to treat patients with chronic lymphocytic leukemia (CLL). Hemolytic anemia after fludarabine therapy occurred in 14 of 66 (22%),[176] 9 of 52 (17%),[177] 5 of 36 (14%),[178] and 5 of 104 (5%)[146] patients. The analyses of patients with CLL are more complex, with many more confounding factors than the data accumulated in the 1970s on the prototype drug (methyldopa), which inclues RBC autoantibodies and AIHA in patients taking the drug for hypertension. Patients with CLL are a very different group of patients, as CLL is known to be associated with positive DATs and AIHA without any drug involvement. Nevertheless, there are reports of exacerbation

of AIHA that was present before fludarabine therapy. There are fewer reports of AIHA caused by fludarabine in de novo CLL patients receiving fludarabine for the first time compared with patients who have had multiple courses of alkylating agents among whom the prevalence is about 20 percent. There are reports of catastrophic hemolysis (some fatalities) after fludarabine therapy.

In 2008, an interesting study of 777 patients with CLL randomly assigned to receive chlorambucil (C) or fludarabine (F) alone or with cyclophosphamide (FCy) was published.[179] Fourteen percent of these patients had a pretreatment positive DAT. Only 28 percent of patients with positive DATs had an associated HA. Of 249 patients, those treated with F were most likely to become DAT-positive. Patients treated with F or C were twice as likely as those treated with FCy to have AIHA. The authors concluded that a positive DAT was a good prognostic indicator and that FCy combination therapy may protect against AIHA. There is an excellent review on AIHA in patients with CLL by Hamblin.[180]

In one publication, 300 patients with CLL taking fludarabine, cyclophosphamide, and rituximab were followed.[181] Nineteen (6.5%) developed HA. The authors considered these to be AIHA even though 82 percent of the DATs were negative. Such cases of DAT-negative AIHA have been reported in other publications on fludarabine-induced HA.

Recently, there has been an interesting association between fludarabine and bendamustine, a chemotherapeutic drug that is also used for CLL and lymphoma. Several cases of bendamustine-associated AIHA (involving drug-independent autoantibodies) have been described. Goldschmidt et al.[139] reported on five cases of AIHA in 31 CLL patients treated with bendamustine; none of the non-CLL patients developed AIHA; and two of five cases were DAT-negative. All five cases had received fludarabine previously. One single case of bendamustine-associated AIHA was DAT-negative and had received no previous fludarabine.[182] Although fludarabine is a purine nucleoside and bendamustine is an alkylating agent, it contains a purine-like benzimidazole ring. This might explain why the bendamustine appeared to give a more severe "secondary" response in patients who had sometimes had a less severe HA caused by fludarabine.

Closing

As new therapeutic drugs appear, we will need to be aware of their potential for inducing drug-dependent antibodies, drug-independent antibodies, or NIPA. The diagnosis is not always easy, and as stated in previous summaries,[2,4] when studying DIIHA, we typically end up with more questions than answers!

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