Review: drug-induced immune hemolytic anemia—the last decade

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I have written three previous reviews on drug-induced immune hemolytic anemia (DIIHA) for this journal.[1-3] The last one was written in 1994.[3] This year, I would like to review what has happened in the last decade.

When Dr. Petz and I published the first edition of our book (Acquired Immune Hemolytic Anemias) in 1980,[4] we found that there was reasonable evidence to support that about 30 drugs could cause DIIHA. One drug, methyldopa, was by far the most common drug to do this. Almost 70 percent of DIIHAs referred to our laboratory in the 1970s were associated with methyldopa. Antibodies associated with methyldopa were drug-*independent* and the patients showed all the serologic and hematologic characteristics of "warm type" autoimmune hemolytic anemia (WAIHA). Twenty-three percent of the DIIHAs were associated with high-dose IV penicillin therapy; only about 10 percent of DIIHAs were associated with other drugs (e.g., quinine, rifampicin, and hydrochlorothiazide).

In the next 20 years, methyldopa and high dose IV penicillin were used less and less; we have not seen a case of DIIHA associated with these two drugs for many years. By 1994 about 71 drugs had been implicated in DIIHA.[5] Recently published results reflect a changing picture in the spectrum of DIIHA in the last 25 years.[6,7] There are now approximately 100 drugs associated with DIIHA (see Table 1), and methyldopa and penicillin have been replaced by a single group of drugs, the cephalosporins (93% of cases), with cefotetan alone accounting for 83 percent of the DIIHAs we have encountered in the last 10 years. Table 2 shows the drugs causing DIIHA that we have encountered in the past 26 years (1978–2003). Methyldopa is probably underrepresented as, by 1978, cases of autoimmune hemolytic anemia (AIHA) in patients taking methyldopa were not usually sent to specialist laboratories such as ours for investigation.

Because of these statistics, I will be emphasizing the cephalosporins in this review.

Cephalosporin-Induced Immune Hemolytic Anemia

There are about 70 individual published case reports of cephalosporin-induced immune hemolytic anemia (CIIHA),[8-64] but many more are contained in reviews or tables without case histories[65-67] (see Table 3). Most patients have had severe hemolytic anemia (HA), often with intravascular lysis, and 40 percent were associated with fatal HA. It is not known if this is the tip of the iceberg and there are many more cases of milder HA or positive DATs that are not reported; the same questions apply to cephalosporin-induced thrombocytopenia. Tables 4 and 5 summarize the clinical and serologic findings associated with cefotetan- and ceftriaxone-induced immune HA. It should be emphasized that cefotetan antibodies always react with cefotetan-coated RBCs and almost always react with untreated RBCs in the presence of cefotetan ("immune complex" method), and about one-third will react with RBCs without the presence of drug (i.e., will appear to be autoantibodies). The latter findings can lead to problems in the blood transfusion service. If a patient receives cefotetan prophylactically for surgery, receives a blood transfusion during or after surgery, and then develops HA 7 to 10 days afterwards, a delayed hemolytic transfusion reaction is often suspected. The hematologic findings can also mimic AIHA. If the HA is due to cefotetan, the DAT will be positive (although we have reported one case where the DAT was negative).[68] Sometimes the serum will react with all untreated RBCs, mimicking an alloantibody to a high-frequency antigen, or a mixture of alloantibodies or autoantibody, and many hours may be wasted investigating these possibilities. If there is a history of cefotetan

Table 1. Drugs causing immune hemolytic anemia and/or positive DATs*

aceclofenac	diethylstilbestrol	nomifensine
acetaminophen/paracetamol	diglycoaldehyde (INOX)	oxaliplatin
aminopyrine/pyramidon	diphenylhydantoin	p-aminosalicylic acid
amphotericin B	dipyrone	penicillin G
ampicillin	erythromycin	phenacetin
antazoline	etodolac	phenytoin
apazone/azapropazone	fenfluramine	piperacillin
apronalide	fenoprofen	probenecid
butizide	fludarabine	procainamide
carbenicillin	fluorescein	propyphenazone
carbimazole	fluoroquinolone	quinidine
carboplatin	fluorouracil (5-FU)	quinine
carbromal	furosemide	ranitidine
catergen/cyanidanol	glafenine	rifampicin
cefamandole	hydralazine	rituximab
cefazolin	hydrochlorothiazide	sodium pentothal/thiopental
cefixime	9-hydroxy-methyl-ellipticinium	stibophen
cefotaxime	ibuprofen	streptomycin
cefotetan	indene derivatives (e.g., sulindac)	sulbactam sodium (e.g., in Unasyn)
cefoxitin	insulin	sulindac
ceftazidime	interferon	sulfonamides
ceftizoxime	interleukin-2	sulfonylurea derivatives (e.g., chlorpropamide)
ceftriaxone	isoniazid	suprofen
cephalexin	latamoxef	suramin
cephalordine	levodopa	tazobactam (e.g., in Zosyn)
cephalothin	mefenamic acid	teicoplanin
chaparral	mefloquine	temafloxacin
chlordiazepoxide	melphalan	teniposide
chlorinated hydrocarbons	mephenytoin	tetracycline
chlorpromazine	methoin	ticarcillin
chlorpropamide	6-mercaptopurine	tolbutamide
cianidanol	methadone	tolmetin
cisplatin	methicillin	triamterene
cladribine	methotrexate	trimellitic anhydride
clavulanate potassium (e.g., Timentin)	methyldopa	zomepirac
cyclofenil	methysergide	
diclofenac	nafcillin	

*Drugs in publications containing reasonable evidence for an immune etiology were the only drugs included (many more are in the literature).

Table 2. Drugs associated with DIIHA investigated at American Red Cross Blood Services, Los Angeles, in the last 26 yrs. (1978–2003)

Drug	Number of patients
cefotetan	74
ceftriaxone	12
piperacillin	5
tazobactam	5
clavulanate	3
fludarabine	2
penicillin	2
probenecid	2
rifampicin	2
cefotaxime	1
sulbactam	1
ticarcillin	1
mefloquine	1
cefoxitin	1
chlorpropamide	1
nafcillin	1
phenacetin	1
procainamide	1
erythromycin	1
tolmetin	1
oxaliplatin	1
Total	119

Table 3. Cephalosporins reported to cause immune hemolytic anemia (up to 2003)

Drug	Number of case reports	References
cephalothin	5	8–11
cefazolin	1	11
cephalexin	2	12, 13
cefamandole	1	14
cefoxitin	2	15, 16
cefotaxime	3	17–19
ceftriaxone	19	20–37
cefotetan	35*	37–59
ceftizoxime	4	60–62
cefixime	1	19
ceftazidime	2	63, 64
Total	75	

*Many other cases are reported, without individual case reports, in references 65 (43 cases) and 66 (85 cases)

administration, I recommend testing an eluate from the patient's RBCs against untreated and cefotetan-coated RBCs to help with the differential diagnosis. If the eluate is reactive with cefotetan-treated RBCs and not the same untreated RBCs, the diagnosis is obvious. Unfortunately, in about 15 percent of cases we have studied, the eluate also reacted with untreated RBCs

Table 4. Clinical* and serologic** findings associated with cefotetan-induced IHA

- Approx. 80 percent of patients received cefotetan for surgery; usually a single dose of 2 g was used.
- A history of previous cefotetan therapy was not common.
- HA was obvious in less than 1 day to 13 days after receiving cefotetan. Only two patients had HA in < 1 day; the mean of the other 29 was 9 days.
- Patients' nadir Hb after receiving cefotetan = 2.6 g/dL (mean = 4.8 g/dL).
- Most patients had signs of intravascular lysis (hemoglobinemia/hemoglobinuria).
- Fatal HA and renal failure occurred in 19 percent of patients.
- Patients always had a positive DAT:
  - 100% had RBC-bound IgG
  - 86% had RBC-bound C3
  - 44% had RBC-bound IgA
  - 7.4% had RBC-bound IgM
- All sera agglutinated cefotetan-treated RBCs (median titer 512) and reacted by IAT (median titer 16,000); all normal sera also reacted with the cefotetan-treated RBCs, but were nonreactive when diluted ≥ 1 in 100.
- All but one of the sera reacted with untreated RBCs in the presence of cefotetan ("immune complex" mechanism).
- 33 percent and 40 percent of sera reacted with RBCs without drug being present, with saline-suspended RBCs or in the presence of PEG, respectively.[65]

*Clincal data from references 38–40, 42–49, 50–53, 56, 57

** Serologic data from reference 65

Table 5. Clinical and serologic findings associated with ceftriaxone-induced IHA

- HA is more acute and severe in children compared to adults. In children, HA started 5 minutes to 7 days after receiving drug; for six children there was a mean of 27 minutes and for three children a mean of 6 days.[22,26–29,31,34,37] In adults, HA started 30 minutes to 34 days. One patient started after 30 minutes; the mean for nine others was 9 days.[20,21,27,28,30,32,33,35,36]
- Fatal HA occurred in 67 percent of 9 children and 30 percent of ten adults.
- There is a history of previous ceftriaxone therapy.
- Positive DATs associated with complement are present in all cases and additional IgG is present in 75 percent; none had detectable RBC-bound IgA or IgM.[65]
- Antibodies are detected only by immune complex method[65] (serum + drug + RBCs). Some patients' sera only react with a metabolite of ceftriaxone (ex vivo antigen).[29,32,35]

(Arndt, Leger, Garratty, unpublished observations). The diagnosis may then rest on the presence of a high-titer (> 100) cefotetan antibody in the patient's serum. Some illustrative case histories associated with such problems have been published in this journal.[36]

Technical hints when investigating CIIHA

1. When preparing cefotetan-coated RBCs, use a buffer with a pH of 6 to 7 (or normal saline) instead of the high pH buffer (pH 8 to 10) used for preparation of penicillin-coated RBCs.[69] The lower pH does not reduce sensitivity but does reduce nonspecific uptake of protein leading to falsely positive antihuman globulin (AHG) tests. Some investigators have suggested that drugs can be solubilized more efficiently in 1% albumin.[70] We do not advise this when testing for cefotetan antibodies, as albumin can cause reduced binding of drug to drug-treated RBCs.[71]
2. Most individuals, including healthy donors, have antibodies in their sera that can directly agglutinate cefotetan-coated RBCs.[72] In addition, almost all sera (including those from healthy individuals) will yield positive IATs with cefotetan-treated RBCs. When testing a patient's serum with cefotetan-coated RBCs, dilute the serum 1 in 100 in saline.[36] This will avoid agglutination and nonspecific protein uptake onto cefotetan-treated RBCs; clinically significant antibodies always have titers > 100 (e.g., in the thousands). Some texts still recommend a dilution of 1 in 20, which is what we recommended when using cephalothin-coated RBCs. We have found that 1 in 20 is sufficient to exclude nonspecific adsorption of proteins by cefotetan-treated RBCs, but is not sufficient to exclude agglutination of cefotetan-treated RBCs by about 10 percent of sera (even from healthy donors).[72]
3. The lesson to be learned by the above is: *never report cefotetan antibodies to be present based on reactions of undiluted sera.* As with penicillin-induced immune HA, the diagnosis should be based on an eluate from the patient's DAT-positive RBCs reacting with cefotetan-coated RBCs, but not untreated RBCs; unlike penicillin, the cefotetan antibody in the eluate may sometimes react with untreated RBCs (see reference 65). Another problem is that the last wash of the patient's RBCs may be reactive.[73] The presence of a high titer (> 100) cefotetan antibody in the serum confirms the diagnosis.
4. Ceftriaxone-coated RBCs cannot be made, thus the serologic diagnosis is based on the results of the "immune-complex" method. Nonspecific adsorption of proteins is not a problem by this method, thus undiluted sera can be used. Sometimes, positive reactions are only obtained by using enzyme-treated RBCs, or metabolites of the drug (e.g., in the presence of urine from a patient taking ceftriaxone).[29,32,35]

DIIHA Associated With Nonimmunologic Adsorption of Proteins Onto RBCs

RBCs treated with the first-generation cephalosporin, cephalothin, were found to adsorb many proteins nonimmunologically when incubated in vitro with normal plasma/sera.[74] Such adsorbed proteins led to a positive AHG test, but it was thought that this was clinically insignificant, and indeed there were only five cases of DIIHA due to cephalothin reported in more than 30 years of use.[8–11] Some other drugs have been found to show a similar phenomenon (see Table 6), but some of these drugs have been thought to cause DIIHA more commonly than cephalothin.

Table 6. Drugs associated with nonimmunologic adsorption of proteins onto RBCs

cephalosporins
cisplatin/oxaliplatin/carboplatin
diglycoaldehyde (INOX)
suramin
sulbactam (contained in Unasyn)
clavulanate (contained in Augmentin and Timentin)
tazobactam (contained in Zosyn)

Causes for the uptake of protein are not always clear. Garratty and Petz[75] suggested that cephalothin changed the RBC membrane in a way that created sites for protein binding. Later this was challenged by Branch and Petz,[76] who suggested that no membrane change occurred and that protein attached to free protein binding sites on the cephalothin that had covalently bonded to proteins on the RBC membrane. Garratty and Leger[77] were stimulated by an old finding, that cefotetan-treated RBCs were "PNH-like" (e.g., had a positive acidified serum test).[78] To reexamine the issue, Garratty and Leger[77] confirmed these old findings by showing, using flow cytometry, that cephalothin- and cefotetan-treated RBCs had markedly decreased amounts of CD55 and CD58 on the RBC membrane; CD59 was only slightly decreased. Thus, the results were different from the changes associated with PNH but confirmed the original hypothesis of Garratty and Petz[75] that cephalosporins can modify the RBC membrane. Whether this is the cause of the nonimmunologic uptake of protein remains to be proved.

Other drugs have been found to cause nonimmunologic adsorption of proteins onto RBCs (Table 6). Some drugs containing aldehyde groups can bind to RBC proteins and adsorb proteins from the plasma, leading to positive AHG tests, but HA has not been associated with such drugs.[79,80]

Drugs belonging to the platinum family (e.g., cisplatin, carboplatin, and oxaliplatin) have been associated with positive DATs and HA.[81–92] Some investigators[81] have suggested that cisplatin causes nonimmunologic adsorption of protein and that the HA is coincidental; others believe the drug can cause DIIHA. We believe that some patients can develop antibodies to the drug and that the DIIHA may occur because of this, regardless of nonimmunologic protein adsorption. We have described a patient who had antibodies to oxaliplatin but no HA.[92]

Nevertheless, I now believe that nonimmunologic adsorption of protein may lead to HA. This is mainly based on work we have performed on another family of drugs, the beta lactamase inhibitors. Examples of these commonly used drugs are sulbactam (contained in Unasyn), clavulanate (contained in Augmentin and Timentin), and tazobactam (contained in Zosyn). They are used together with the beta lactam antibiotics ampicillin, ticarcillin, and piperacillin, respectively.

Lutz and Dzik[93] reported that 39 percent of patients receiving Unasyn developed weakly positive DATs but no HA. We showed later that RBCs treated in vitro with the beta lactamase inhibitors contained in Unasyn, Augmentin, Timentin, and Zosyn would adsorb proteins nonimmunologically, leading to positive IATs, and that this may be the cause of positive DATs associated with such drugs. It should be mentioned that patients may make antibodies to the antibiotics also present in the drug (e.g., ampicillin, piperacillin), and these antibodies can cause positive AHG tests and HA. Garratty and Arndt[94,95] suggested that HA could also occur in patients who have no antibodies to the antibiotics and that this may be associated with nonimmunologic adsorption of protein. This suggestion was supported by case histories and in vitro experiments where it was shown that RBCs treated with the beta lactamase inhibitors, washed, and incubated in normal plasma, washed, and then added to a monocyte monolayer yielded results suggesting that the RBCs would have shortened survival.[95] Broadberry et al.[96] described a DIIHA associated with tazobactam; the HA was thought to be due to nonimmunologic adsorption of protein.

Unfortunately, we cannot prove in the laboratory that this nonimmunologic adsorption of proteins is causing the HA in a particular patient; one can only suggest it to the physicians and refer them to the appropriate literature (e.g., references 6 and 94–96).

Mechanisms of DIIHA

There are two well-accepted mechanisms for DIIHA, namely, that individuals can produce either drug-dependent antibodies or drug-independent antibodies (Table 7). The latter can be classic autoantibodies in that they do not require drug to be present for their reaction with RBCs in vitro or in vivo; drug is only needed to initiate the production of antibodies. It is still unclear how this occurs, but the most popular concept is that certain drugs given to certain patients somehow affect the immune system to produce pathogenic autoantibodies, as do certain infections in certain patients. The mechanisms may turn out to be similar. The AIHA often continues for some time after the drug is discontinued. Some drug antibodies (e.g., cefotetan) sometimes react with RBCs without drug being present, but I do not think that these are "classic" autoantibodies (in contrast to those associated with methyldopa). Drug-independent antibodies (e.g., cefotetan antibodies) are never seen without the presence of drug-dependent antibodies and when the drug is stopped, the "autoantibodies" become weaker, eventually disappearing (see Table 7). I believe that these antibodies are not produced because of the drug's effect on the immune system, but rather are part of the spectrum of antibodies produced as a result of the immune response to a hapten (e.g., antibodies to the drug, carrier [RBC protein], and drug + carrier).[97] See Figure 1.

Table 7. Drugs that have been reported to induce RBC drug-independent antibodies (i.e., autoantibodies)*

Group 1	Group 1a	Group 2
cladribine	catergen	azapropazone
fludarabine	chaparral	carbimazole
levodopa	chlordiazepoxide	ceftazidime
mefenamic acid	cianidanol	cefoxitin
methyldopa	cyclofenil	cefotetan
procainamide	diphenylhydantoin	cefotaxime
	fenfluramine	chlorinated hydrocarbons
	ibuprofen	diclofenac
	interleukin-2	glafenine
	methoin	latamoxef
	methysergide	nomifensine
	rituximab	phenacetin
		streptomycin
		teniposide
		tolmetin
		zomepirac

*Drugs in groups 1 and 1a have been reported to induce drug-dependent antibodies only. More evidence is needed to prove that drugs in group 1a really can induce RBC autoantibodies. Drugs in group 2 induce drug-independent antibodies together with antibodies reacting by different mechanisms.

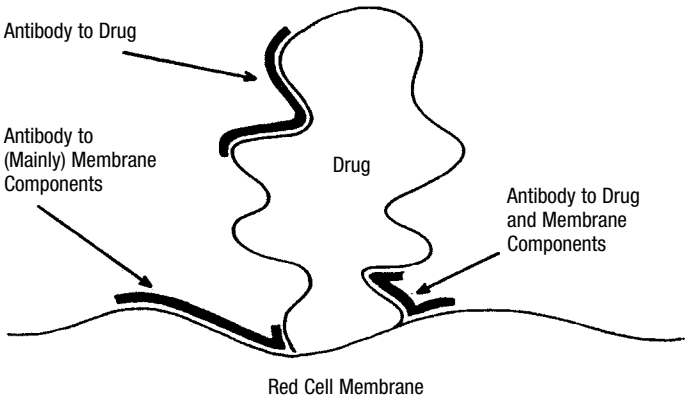

Fig. 1. Proposed unifying theory of drug-induced antibody reactions.[97] The thicker, darker lines represent antigen-binding sites on the F(ab) region of the drug-induced antibody. Drugs (haptens) bind loosely or firmly to cell membranes, and antibodies may be made to the drug (producing in vitro reactions typical of a drug adsorption [penicillin-type] reaction; the 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).

Drug-dependent antibodies can be of two types. One type of antibody can be inhibited by the drug (hapten inhibition) and reacts only with washed, drug-coated RBCs (prototype drug is penicillin). The other type, which relates to most drugs, is not inhibited by the drug and reacts with untreated RBCs in the presence of the drug. We still do not know why. There have been two main suggestions:

- The drug antibody combines with the drug, forming immune complexes which attach to the RBC (transiently) and activate complement.
- The drug changes the RBC membrane, producing a new antigen ("neoantigen") as a new epitope. This neoantigen may be a chemically modified membrane protein or part drug, part RBC membrane protein ("compound" epitope).

The latter part of the latter concept is the basis of the unifying hypothesis originally suggested by Habibi,[98] and supported by Mueller-Eckhardt and Salama,[99] which I feel has much merit. Unfortunately, like other concepts, it has some failings (these are discussed in references 5, 6, and 99). Although the immune complex hypothesis is out of favor, the clinical findings and some serologic data fit much better with the "immune complex" hypothesis. Shulman and Reid[100] tried to take some of the best of both hypotheses, and suggest some explanations for the discrepancies, but personally I do not think that, in 2004, we have any better explanations that we did in 1994!

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