Drug-induced immune hemolytic anemia (DIIHA) is rare; it can be mild or associated with acute severe hemolytic anemia (HA) and death. About 125 drugs have been implicated as the cause. The HA can be caused by drug-independent antibodies that are indistinguishable, in vitro and in vivo, from autoantibodies causing idiopathic warm type autoimmune hemolytic anemia (AIHA). More commonly, the antibodies are drug-dependent (i.e., will only react in vitro in the presence of the drug). The most common drugs to cause DIIHA are anti-microbials (e.g., cefotetan, ceftriaxone and piperacillin), which are associated with drug-dependent antibodies. The most common drug to cause AIHA is fludarabine. Finding out which drug is causing the problem and stopping that drug is the first approach to therapy. It is not easy to identify the drug interactions accurately in vitro; laboratories specializing in this area can be of great help.

**1. Introduction**

Drugs were first suspected as a cause of immune hemolytic anemia (IHA) in 1953 when Snapper described a patient who developed pancytopenia with hemolytic anemia (HA), associated with a positive direct antiglobulin test (DAT), following ingestion of mephenytoin (Mesantoin). Harris was the first to document carefully the history and serology of a case of immune hemolytic anemia due to a drug. The drug, stibophen, was used to treat schistosomiasis. The patient had received a course of stibophen 10 years previously with no problems. During the second course, the patient developed acute intravascular hemolysis. The DAT was positive, and the patient’s serum was shown to react with allogeneic RBCs only when the drug was present. The drug was stopped and the patient’s hemoglobin returned to normal in 20 days; the serology became negative after about 60 days.

In 1967, Dausset and Contu reviewed the literature on drug-induced immune hemolytic anemia (DIIHA) and found only 34 published cases due to 15 drugs. In 1969, Worlledge added six more cases but no other drugs to the list. By 1980, we had found reports of approximately 33 drugs as causes of DIIHA. In 1989, the list had grown to over 50 drugs that were reasonably well-documented as causes of IHA. In 2007, we reported that we now found 125 drugs implicated as the cause. The HA can be caused by drug-independent antibodies that are indistinguishable, in vitro and in vivo, from autoantibodies causing idiopathic warm type autoimmune hemolytic anemia (AIHA). More commonly, the antibodies are drug-dependent (i.e., will only react in vitro in the presence of the drug). The most common drugs to cause DIIHA are anti-microbials (e.g., cefotetan, ceftriaxone and piperacillin), which are associated with drug-dependent antibodies. The most common drug to cause AIHA is fludarabine. Finding out which drug is causing the problem and stopping that drug is the first approach to therapy. It is not easy to identify the drug interactions accurately in vitro; laboratories specializing in this area can be of great help.

**2. The immune response to drugs**

To be immunogenic, a chemical usually has to have a molecular weight of more than 1000 kDa, preferably >10,000 kDa. Drugs have molecular weights of less than 1000 kDa (e.g., penicillin has a MW of 300 kDa). Such “haptens” are antigenic but not immunogenic unless conjugated with a large carrier molecule (usually a protein). To elicit an immune response, classical immunological dogma suggests that the hapten needs to form stable covalent bonds with protein, although more recent concepts do not put as much emphasis on a covalent bond. Such conjugates can elicit an immune response composed of antibodies to the drug alone, to an antigen that is part-drug and part-carrier protein, or to the carrier protein alone. Any
combination of these can be detected; antibodies to carrier protein alone, which could appear to be autoantibody if autologous or homologous proteins are involved, are rarer when heterologous proteins are involved. The latter was often the case in some of the classical studies on the immune response to haptens. Some drugs (e.g., most penicillins, cephalosporins) are capable of covalently bonding to proteins (e.g., on RBC membranes), providing a target (e.g., penicillin-coated RBCs) for detection of certain drug antibodies in vitro. Unfortunately, many drugs, especially those that reduce bacterial growth resulting from contamination during frequent sampling for crossmatching purposes. This showed that penicillin-coated RBCs were easy to prepare in vitro and could be used to detect IgM and IgG penicillin antibodies. Soon after, several cases of penicillin-induced IHA were described, and some years later cases of penicillin-induced immune thrombocytopenia and granulocytopenia were described. This second mechanism for DIIHA, called the “drug-adsorption” mechanism by Garratty and Petz,28 suggested that RBCs became coated with penicillin in vivo, and that if IgG penicillin antibodies were present they would react with the RBC-bound penicillin, leading to IgG sensitized RBCs. This situation would lead to a positive DAT and possible destruction of the IgG-coated RBCs by macrophages.

In 1966, it was shown that some drugs (e.g., methyldopa) could cause the production of true RBC autoantibodies, and these could sometimes cause AIHA. This added a third possible mechanism for drug-induced positive DATs and sometimes IHA. A fourth mechanism, originally called the “membrane modification” mechanism by Garratty and Petz,28 was suggested when it was shown that cephalothin, and later some other drugs, can affect the RBC membrane so that proteins become bound to the RBCs immunologically. This mechanism was first thought to lead only to positive antiglobulin tests, but more recently it was proven it could cause DIIHA (see later).

The IgM and IgG antibodies involved in DIIHA are of two main types: the first type is drug-dependent (i.e., will only react with RBCs in vitro in the presence of drug). These antibodies may react in vitro with drug-coated RBCs (if drug can covalently bond to RBC membrane), or if drug-coated RBCs cannot be prepared the antibody may react when added to RBCs in the presence of drug (i.e., in solution not bound to the RBCs). The second type of antibody is drug-independent (i.e., will react with RBCs in vitro without the presence of drugs). Such antibodies appear to be RBC autoantibodies rather than antibodies to the drug; the hematology/serological findings are identical to those of idiopathic warm autoimmune hemolytic anemia (WAHA).

Of the three mechanisms mentioned above, the least controversial is the “penicillin-type” (“drug adsorption”) mechanism. One can demonstrate that some drugs can bind firmly to the RBC membrane in vitro (e.g., penicillin and cefotetan), withstanding washing of the RBCs. Penicillin can also be shown to be present in vivo, on all patients’ RBCs who are receiving high dose intravenous penicillin. One can also show that IgG penicillin antibodies will bind to these drug-coated RBCs in vitro and in vivo and that such IgG-coated RBCs can interact with Fc receptors on macrophages, sometimes leading to extravascular immune destruction in a similar way as in AIHA.

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotetan</td>
<td>36 (4)</td>
<td>43</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>17 (5)</td>
<td>21</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>14 (1)</td>
<td>17</td>
</tr>
<tr>
<td>β-lactamase inhibitors</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Other cephalosporins</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>9b</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>83 (10)</td>
<td>100</td>
</tr>
</tbody>
</table>

*a* Columns contain number (fatalities) of cases associated with each drug.

*b* Oxaliplatin (3), carboplatin (1), rifampin (1), diclofenac (1), cimetidine (1), sulfamerthozole (1), and trimethoprim (1).

3. Suggested mechanisms involved in in vivo hemolysis and associated serological findings

In 1962, Ackroyd20 suggested that a drug [allylisopropylacetylurea (Sedormid/Apronal)] acting as a hapten, conferred new antigenic properties on platelets, leading to antibodies that reacted with drug only when it was bound to the cell membrane. In 1952, Miescher and Miescher21 had suggested an alternative theory: drug antibodies might initially be formed against the drug, and then these antibodies might then react with the drug, forming drug–anti-drug immune complexes. These complexes could attach nonspecifically to platelets, leading to their destruction by macrophages. In later publications, Miescher et al. presented some experimental work in animals to support this hypothesis. Shulman24–26 extended this hypothesis. He proved that drugs such as quinine and quinidine did not bind firmly to platelet membranes because quinidine could be removed by a single washing of the platelets. He reported that concentrations of drug on the order of 1 million times the concentration of membrane sites for antibody fixation did not interfere with antibody reactions. Using equilibrium dialysis, Shulman also showed that the association between cells and drugs was much too weak to account for the large amount of antibody that the same cell adsorbed; however, drug antibodies were shown to combine efficiently with drug in the absence of cells. Thus, he suggested that patients make an antibody against a stable complex of the drug with some soluble noncellular macromolecule; when the drug is received again, drug–anti-drug immune complexes form and these attach to platelets nonspecifically, activating complement and leading to thrombocytopenia. This “immune complex” theory was extended to RBCs to explain drug-induced IHA due to drugs other than methyldopa and penicillin.
The mechanism(s) involved in vivo, and causing the positive in vitro serological results, observed with drugs other than penicillin, are still controversial. The so-called immune complex mechanism suggested by Miescher and Shulman et al. for platelets, and later applied to RBCs, replaced Ackroyd’s hypothesis and reigned supreme for more than 20 years. Nevertheless, during this time several findings did not fit well with the immune complex theory. In 1975, Ackroyd attacked the “immune complex” theory. He related to animal work by Cronin that provided no support for the hypothesis that a drug antibody was stimulated by a stable union of drugs with a soluble macromolecule or that adsorption of drug immune complexes to platelets occurred. In 1985, Aster’s group showed that antibodies from patients with quinine and quinidine-induced thrombocytopenia bound to the platelets by their Fab portion, not their Fc portion, of the Ig molecule. This did not support the “nonspecific” adsorption of drug–anti-drug complexes as suggested by Shulman, and even later suggestions that the attachment was to the Fc receptor on platelets. Shulman’s group, using a different approach, confirmed the Fb attachment of certain drug antibodies to platelets and RBCs.

Because of the deficiencies in the immune complex hypothesis, several other mechanisms were suggested. Many of these “new” hypotheses had a great deal in common with the original suggestions by Ackroyd. Suggestions from Habibi et al. and Mueller-Eckhardt and Salama were based on the early work on the immune response to haptens, as discussed earlier. It was suggested that drugs could combine with RBC membrane proteins forming drug-protein conjugates. Drugs might bind through covalent bonds or perhaps bind loosely but remain on the cell membrane long enough to create a new immunogen/neoantigen composed of drug and protein. Antibodies might be directed at the hapten (drug) or a neoantigen (drug + membrane protein). One or more of these antibody populations may be present in any one patient. Fig. 1 illustrates this concept. Top left “A” illustrates an epitope that would lead to an antibody that would react with the hapten (drug) alone, e.g., a penicillin antibody. “B” and “C” illustrate two epitopes composed of hapten + membrane. The one on the right (“C”) would lead to an antibody that only reacts with a neoantigen composed of drug and membrane protein, thus both are necessary for an immune response and in vitro serological reactions. This concept replaces the requirements needed for the “immune complex” mechanism (i.e., one cannot prepare drug-coated RBCs in vitro; one has to mix serum containing antibody + drug + RBCs to detect drug antibody in vitro). It also replaces the need to suggest that drug–antibody complexes attach to the RBC and explains why the antibody attaches by the Fab portion of the drug antibody. The epitope on the left side (“B”) is similar except the epitope is composed of far more membrane protein than drug. Positive in vitro reactions may not need drug to be added to detect the “drug” antibody. The antibody will appear to be a drug-independent autoantibody. Antibodies to “B” and “C” may show blood group specificity if relevant antigens are on the RBC membrane components acting as immunogens. Such antibodies may be drug-dependent (“C”) and drug-independent (“B”).

Mueller-Eckhardt and Salama suggested that this hypothesis could replace all of the other suggested hypotheses; they called it the “unifying hypothesis.” I like the basic elements of the hypothesis, but do not think it explains everything. For instance, I think that only some drug-independent antibodies may be represented by “B” in Fig. 1. These are the drug-independent antibodies that appear together with drug-dependent antibodies. I think a separate hypothesis is needed for true autoantibodies that appear alone following therapy with certain drugs (see Table 2, and below).

In 1966, a commonly used drug (methyl dopa) for hypertension was reported to be causing a high incidence of positive DATs and even DIIHA. The serological findings were puzzling as the IgG antibodies in the patients’ sera and in eluates from the patients’ RBCs reacted with RBCs in vitro, without any drug being added. The results were indistinguishable from autoantibody found in patients with idiopathic “warm type” AIHA (WAIHA), even in terms of specificity (i.e., directed mainly at the Rh complex). The patients’ clinical findings were also similar to WAIHA; the major difference being that a hematological response was seen within a few weeks of stopping the drug, without any other treatment. Although 10–36% of the patients developed a positive DAT (dose-dependent), only a small percentage (0.5%) of these developed hemolytic anemia. Although there are many hypotheses, it has still not been proven how this drug and others, giving similar results (see Table 2), cause an autoimmune condition. Suggested mechanisms have included: 1) drug adsorption to RBCs changing normal RBC antigens so that they are not recognized as “self” (non-specific) drug causes IgG to aggregate and attach to RBCs; 3) drug stimulates antibody to RBC membrane proteins (i.e., carrier protein for haptenic response) as in “B” of Fig. 1. Drug affects the immune system (lymphocytes). One hypothesis in the latter group of references, by Kirtland et al., purporting to show that methyl dopa affected suppressor T cell function, became the most popular hypothesis (it helped that the paper was in the New England Journal of Medicine, and the concept agreed with the generally-accepted concepts of autoimmunity at that time). I also liked the hypothesis but almost 10 years after publication of the work, we realized no one had tried to confirm these results, so we tried to do this. Unfortunately, we could not confirm the results of the methyl dopa- (or procainamide)-induced suppression of suppressor

![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. (A) Drugs (haptens) bind loosely (or firmly) to cell membranes, and antibodies can be made to the drug (producing in vitro reactions typical of autoantibody); (B) membrane components, or mainly membrane components (producing in vitro reactions typical of autoantibody); (C) part-drug, part-membrane components (producing an in vitro reaction typical of the so-called immune complex mechanism).](image)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 1a</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladribine</td>
<td>Cattergan</td>
<td>Azapropazone</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>Chaparral</td>
<td>Carbimazole</td>
</tr>
<tr>
<td>Levodopa</td>
<td>Chloridiazepoxide</td>
<td>Ceftazidime</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>Clanidanol</td>
<td>Cefoxitin</td>
</tr>
<tr>
<td>Methyl dopa</td>
<td>Cyclofenil</td>
<td>Cefotetan</td>
</tr>
<tr>
<td>Procarbamid</td>
<td>Diphenylhidantoin</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Diclofenac</td>
<td>Glafenine</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>Nomifensine</td>
<td>Phenacetin</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Streptomycin</td>
<td>Teniposide</td>
</tr>
<tr>
<td>Methysergide</td>
<td>Tolmetin</td>
<td>Tolmetin</td>
</tr>
<tr>
<td>Zomepirac</td>
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</tr>
</tbody>
</table>

Drugs in groups 1 and 1a have been reported to induce drug-dependent antibodies (i.e., autoantibodies).
cell activity, using identical assays as those used by Kirtland et al. Nevertheless, I still like the concept that drugs induce true autoantibodies (e.g., methyldopa, procainamide, fludarabine) somehow affect the immune system. With the increased knowledge on the immune system, available since the 1970s, it is a pity that more modern approaches have not been applied to solving the mechanism involved in drug-induced RBC autoantibody formation.

In 1971, Spath et al. showed that RBCs treated in vitro with the first generation cephalosporin, cephalothin, would adsorb many different proteins from plasma, yielding positive antiglobulin tests with appropriate anti-human globulin sera (e.g., anti-IgG, -C3, and -albumin); it was suggested that this might happen in vivo leading to positive DATs. Because very few patients were reported as having a cephalothin-induced hemolytic anemia, this phenomenon was not thought to be clinically relevant until 1998. Table 3 shows other drugs that can cause nonimmunologic protein adsorption (NIPA) onto RBCs. Of special interest are drugs in the platinum family and the beta lactamase inhibitors as we have published evidence that the non-immunological adsorption of protein onto RBCs in vivo may sometimes be involved in shortened RBC survival with or without antibody to the drugs being involved.

4. Diagnosis and treatment of DIIHA

Patients with DIIHA can present with acute intravascular hemolysis soon after receiving the drug (e.g., young children with ceftriaxone-induced HA), or milder extracellular hemolysis, sometimes starting after months of therapy (e.g., methyldopa-induced HA). The clinical signs and hematology are similar to any other immune HA (e.g., AIHA). Patients who have acute hemolysis, after receiving the drug, usually have a history of having received the drugs (e.g., ceftriaxone) previously without a problem and probably had circulating drug-induced antibody present before getting the drug leading to the hemolytic episode. If the patient has no history of receiving the drug before, a careful history should be obtained for any drugs that are biochemically related; the first history may not reveal these as they may have been non-prescription drugs not mentioned by the patient. The above discussion is more complex than stated as the most common drug (cefotetan) to cause DIIHA can cause severe HA within several days of receiving a single dose of the drug (e.g., following prophylactic use in surgery) in patients who have never received cefotetan previously. We believe that this is because weak cefotetan antibodies can be detected in the plasma of approximately 75% of random individuals. These antibodies may be there because, in the US, antibiotics are added to cattle feed and given to chickens and cattle prophylactically. This may cause primary immunization in the American public; because of the increasing cell activity, using identical assays as those used by Kirtland et al. Nevertheless, I still like the concept that drugs induce true autoantibodies (e.g., methyldopa, procainamide, fludarabine) somehow affect the immune system. With the increased knowledge on the immune system, available since the 1970s, it is a pity that more modern approaches have not been applied to solving the mechanism involved in drug-induced RBC autoantibody formation.

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<table>
<thead>
<tr>
<th>Drugs that can cause nonimmunologic adsorption of proteins onto RBCs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotetan</td>
</tr>
<tr>
<td>Cephalothin</td>
</tr>
<tr>
<td>Cisplatin/oxaliplatin/carboplatin</td>
</tr>
<tr>
<td>Diglycoaldehyde (INOX)</td>
</tr>
<tr>
<td>Suramin</td>
</tr>
<tr>
<td>Sulbactam (contained in Unasyn)</td>
</tr>
<tr>
<td>Clavulanate (contained in Augmentin and Timentin)</td>
</tr>
<tr>
<td>Tazobactam (contained in Zosyn)</td>
</tr>
</tbody>
</table>

5. Characteristics of the most common DIIHAs

Of the 127 drugs we selected as having reasonable evidence reported to support that they caused DIIHA, 42% were anti-microbials;
16% were non-steroidal anti-inflammatory; 13% were anti-neoplastics, and 6% were anti-hypertensives/diuretics. The most common drugs to cause DIIIHA, in the last 32 years of studies in our laboratory, are all antimicrobials; they are cefotetan (53%), ceftriaxone (16%), piperacillin (9%), and beta lactamase inhibitors (5%). The drug most commonly associated with fatal HA was cefotetan (8%), closely followed by ceftriaxone (6%), particularly in young children. Table 1 shows the results of the last 10 years. The most common drug, for the last two decades, to produce drug-independent antibodies and AIHA is fludarabine.

5.1. Cephalosporins

The first generation cephalosporins (cephalothin, cepahlexin, and cefazolin) caused positive DATs but rarely caused DIIIHA. Of the second generation cephalosporins (cefamandole, cefoxitin, cefaclor, cefuroxime, cefonicid, cefotetan, and cefmetazole), cefotetan has led the pack in causing DIIIHA by a wide margin. Of the third generation cephalosporins (ceftaxime, cefoprazone, cefotixaime, ceftriaxone, and cefixime), cefotetan is a far more common cause of DIIIHA than the others. So far there have been no reports of DIIIHA due to the fourth generation cephalosporins.

5.2. Cefotetan

More than 50% of cases of DIIIHA we encounter are due to cefotetan. Table 4 shows the characteristics of cefotetan-induced DIIIHA. An FDA group published data on 85 cases of cefotetan-induced HA.62 There was a mean hemoglobin decrease of 6.7 g/dL, with a final mean hemoglobin of 5.2 g/dL. There were 18% fatalities and 8% renal failures. Fifty-nine percent of the cases were associated with prophylactic use of cefotetan (60% associated with surgery). Only 18% of the patients had received cefotetan previously. This seemingly unusual finding may relate to findings that it is common to find cefotetan antibodies in random patients and blood donors.53 We have published a report of 10 cases of cefotetan-induced DIIIHA where a single dose of cefotetan was used prophylactically for gynecological/obstetric surgery.63 The hemoglobins fell to 3.5–7.6 g/dL, 9–14 days following surgery. This was often intravascular lysis. We know of some cases where more cefotetan was given leading to fatal HA; the correct diagnosis of DIIIHA was made retrospectively.

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

- Cefotetan is the most common drug to cause DIIIHA.
- Most 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 less than 1 day; the mean of the other 29 was 9 days.
- 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% and 19% of patients, respectively.
- Patients always had 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 by antiglobulin test, but were nonreactive when diluted 1 in 20.
- Almost all sera reacted with untreated RBCs in the presence of cefotetan (“immune complex” mechanism).
- 33% and 40% of sera reacted with RBCs without drug being present, with saline-suspended RBCs or in the presence of a potentiator [polyethylene glycol (PEG)]; respectively, thus reacting like an autoantibody (see text).

An important hint is to remember that many patients with cefotetan-induced HA only received one dose of the drug during surgery. We receive consultations where the attending physician does not know about this administration of cefotetan, and is asking us to investigate other drugs they gave or investigate a hemolytic transfusion reaction if the patient was transfused during or after surgery. We always ask them to check the surgical notes for cefotetan administration (it may be a single dose) and if administered, this would be the primary suspect we would concentrate on, trying to demonstrate the presence of cefotetan antibodies (see reference64 for educational case studies).

5.3. Ceftriaxone

Ceftriaxone is the second most common drug in our series.7,58 Table 5 shows the characteristics of ceftriaxone-induced DIIIHA. Some children have dramatic intravascular HA (e.g., hemoglobin dropping to 1 g/dL within 1–2 h of receiving ceftriaxone); approximately 50% have a fatal HA. The children have always received ceftriaxone previously, the DAT is usually positive (all have RBC-bound complement and many have IgG in addition), and ceftriaxone antibodies are detectable in the patient’s serum. The HA is usually not as dramatic in adults; the fall in hemoglobin is much less and does not occur within a few hours of receiving the drug; fatalities are rare. Recently, Quillen et al.65 reported on the incidence of ceftriaxone-induced RBC antibodies in sickle cell disease and HIV-infected pediatric patients. They found 8/24 (12.5%) had antibodies; two of these patients had HA; one of these patients (11 years old) had catastrophic fatal hemolysis.

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

- Ceftriaxone is the second most common drug to cause DIIIHA.
- Usually HA is more acute and severe in children [HA started 5 min to 7 days after receiving drug (for 5 children a mean of 44 min. and 2 children a mean of 5 days)] compared with adults [HA started 30 min to 34 days (1 patient was 30 min, the mean for the other 7 was 12 days)].
- Fatal HA occurred in 38% cases (75% of these were children).
- All had received ceftriaxone therapy previously.
- Positive DATs associated with RBC-bound complement in all cases and IgG in 56%.
- Sera and eluted antibodies were only detected by “immune complex” method (serum + drug + RBCs); a few patients’ sera only reacted with a metabolite of ceftriaxone.
coated RBCs but not with untreated RBCs in the presence of penicillin. The clinical/hematological course is also different (see Table 6). Penicillin-induced HA only occurs when millions of units of penicillin are given intravenously (rare nowadays, explaining why we do not see penicillin-induced HA anymore). Penicillin antibodies causing the HA are usually IgG and cause moderate extravascular in vivo destruction of RBCs. Piperacillin antibodies are usually IgM + IgG, activate complement, and cause intravascular lysis.

5.5. Purine nucleoside analogues

Fludarabine and cladribine have both caused production of RBC autoantibodies and AIHA.67 There are many single case reports, but there are four reports describing a series of more than 30 chronic lymphatic leukemia (CLL) patients. Hemolytic anemia following fludarabine therapy occurred in 5/104 (5%),68 9/52 (17%),69 5/36 (14%),70 and 14/66 (22%)71 patients. It is unclear why the largest series of 104 patients yielded far fewer DIIHA than the 3 smaller studies.

The analysis of data on AIHA due to fludarabine is far more complex than data gathered in the 1960s and 1970s for methylldopa. Methylldopa was given for hypertension, thus most of the patients involved were relatively healthy individuals with no hematological/immunological abnormalities, this is in contrast to the patients with CLL receiving fludarabine. In the study by Dearden et al.,72 14% of the 777 CLL patients had a positive DAT before treatment; only 28% of the DAT + patients developed HA. Thus, unless data on pretreatment DATs are available, it is very difficult to know if the drug caused autoantibody production, or whether it had already started. Fludarabine appears to exacerbate any hemolytic anemia present before treatment; there are some reports of catastrophic hemolysis leading to fatalities. AIHA rarely occurs with de novo CLL patients receiving fludarabine for the first time. Following multiple courses of alkylating agents, fludarabine causes AIHA in about 20% of CLL patients.

In 2008 an interesting study of 777 CLL patients, randomized to receive chlorambucil or fludarabine alone, or with cyclophosphamide, was published.72 Fourteen percent of their CLL patients had a pretreatment positive DAT; only 28% of positive DATs had an associated hemolytic anemia. Of 249 patients, those treated with fludarabine were most likely to become DAT +. Patients treated with fludarabine or chlorambucil alone were twice as likely as those treated with fludarabine + cyclophosphamide to have AIHA. The authors concluded that a positive DAT was a good prognostic indicator and that combination therapy may protect against AIHA.

It has been mentioned before how hard it is to prove that any drug is the cause of AIHA, as the antibodies produced are drug-independent and thus show no difference to those found in idiopathic WAIHA. The best proof is to see if stopping the drug leads to a hematological response and starting the drug again causes IHA again. This has been done in at least 13 patients69,73 and the HA started again after fludarabine was readministered. It is not wise to perform this experiment as it has led to a much more severe HA.

6. DIIHA following transplantation

Hemolytic anemia is relatively common following transplantation, and some of them have an immune etiology and some have non-immune causes (e.g., hemolytic uremic syndrome). Immune hemolytic anemias can be alloimmune (e.g., hemolytic transfusion reactions and passenger lymphocyte syndrome13,74); autoimmune [e.g., AIHA, red cell aplasia (probably autoimmune)], and DIIHA. In the DIIHA group, both drug-dependent and drug-independent antibodies have been found. Many of the AIHA group are probably drug-induced. I say probably because the most popular hypothesis to explain drug-induced AIHA is that some drugs interfere with the immune system. Thus, it should come as no surprise that when immunosuppressive drugs are given we can expect to see increased autoantibody formation (e.g., involving circulating blood cells).

Table 7 shows some immunosuppressive drugs that have been suspected of causing DIIHA, but there are still not definitive data to support the suggestions. Most patients appear to have developed AIHA with RBC autoantibodies in the plasma and positive DATs. As emphasized in this review several times, it is difficult to prove that a particular drug caused the AIHA; many of the reports are single case reports. Some patients appear to have DIIHA associated with drug-dependent antibodies (i.e., antibodies react with RBCs in vitro only when drug is present, thus no antibody reactions are seen with routine blood bank antibody detection methods other than the DAT will be positive; an eluate from the DAT + RBCs will only react when drug is added).

Some interesting points are: 1) some of the AIHAs have not been WAIHA but associated with cold agglutinins75,76; 2) there are interesting associations with post-transplant lymphoproliferative disorder (PTLD)77; 3) the hemolytic anemias can develop long after the transplant and long after therapy with the suspected drug (e.g., up to 2 years).

Two reports that contain more useful information than some others are references 77 and 78. DiGiuseppe et al.77 reported on a child who had a liver transplant at 1 year old; he was successfully maintained on cyclosporine for 4 years then switched to tacrolimus, which was increased from 2 mg to 8 mg bid. He developed AIHA; hemoglobin fell to 3.5 g/dL after transfusion of one unit RBCs, and the patient died. Post mortem revealed a clinically unsuspected PTLD. Elinezalak et al.78 reported on cases of red cell aplasia (RCA) and AIHA following 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 7 patients recovered from RCA but only 3 also recovered from the HA. In the AIHA alone group (9 patients), MMF was discontinued in 2 patients with no effect on the HA. Other patients in this group were treated with steroids, rituximab, IVIG, splenectomy, or plasma exchange with remission seen in only 2 of the 9 patients. The authors suggested that a combination of the drugs may have been involved in the AIHA. I find it interesting that

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<tr>
<th>Table 6 Characteristics of piperacillin-induced immune hemolytic anemia.</th>
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<td>• Piperacillin is the 3rd most common drug causing DIIHA. Most of the cases have been associated with Zosyn, which contains piperacillin and a beta-lactamase inhibitor, tazobactam; about 50% of the patients had cystic fibrosis.</td>
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<td>• Piperacillin is a semi-synthetic penicillin but gives different in vivo and in vitro reactions to other penicillins (including other semisynthetics).</td>
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<td>• Patients often show signs of complement-mediated intravascular hemolysis; 6% of cases referred to us had fatal hemolytic anemia.</td>
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<td>• Antibodies to piperacillin are usually IgM + IgG.</td>
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<td>• Positive DATs are usually due to RBC-bound IgG + C3.</td>
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<td>• The antibodies react with piperacillin-coated RBCs but unlike penicillin G antibodies, they also react with untreated RBCs in the presence of piperacillin.</td>
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<td>• The latter approach is the best diagnostic test as a high percentage of random healthy donors and patients’ sera react with piperacillin-coated RBCs but not by the so-called immune complex mechanism detection tests (i.e., patient’s serum + drug + untreated RBCs).</td>
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<td>• Many are misdiagnosed as AIHA because in vitro reactions are seen with RBCs without adding drug in vitro. This is usually due to drug and/or drug-anti-drug complexes present in the serum. Within 48 h of stopping the drug, this reactivity will disappear, which contrasts with AIHA where the reactions will continue.</td>
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<th>Table 7 Drugs associated with post-transplant immune hemolytic anemia.</th>
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<td>• Alemutzumab (anti-CD52)</td>
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<td>• Cyclosporine</td>
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<td>• Daclizumab (anti-Tac/CD25)</td>
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<td>• Mycophenolate mofetil</td>
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<td>• Tacrolimus</td>
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the RCA, which is thought to be due to autoantibody to early RBC precursors, seemed to be caused by MMF.

7. Conclusions

DIIHA is rare. Even though rare, hematologists should be aware of the most common drugs and characteristics causing the often severe and sometimes fatal hemolytic anemia. Unfortunately, arriving at the correct diagnosis is not easy, even when there is a good temporal relationship to a specific drug. A positive DAT is the first clue, but help from a laboratory that has experience in this area is often needed to confirm that an immune event to a particular drug is occurring. The reward is that the treatment is often simple. Often, stopping the incriminated drug is all that is necessary, although in severe cases, transfusion and perhaps plasma exchange may be necessary. If the drug (e.g., fludarabine) has caused an autoimmune HA, then steroids or other drugs usually used for WAIHA may help after stopping the drug. There are little data (if any) proving that steroids help when the HA is caused by drug-dependent antibodies (e.g., the cephalosporins or piperacillin).

Conflict of interest

Have consulted to Novartis, Cerus, Cardian, Astra-Zeneca.

Practice points

- Most common drugs causing DIIHA are cefotetan, ceftriaxone, piperacillin, beta-lactamase inhibitors, and oxaliplatin.
- Most common drug to cause autoimmune hemolytic anemia is fludarabine.
- DIIHA can be confused with idiopathic warm type AIHA or hemolytic transfusion reactions.
- Stopping the suspect drug is the most important first step. This may be the only treatment needed as the hematological response may be quite fast.
- A thorough serological evaluation is required to confirm that an immune reaction is involved and to differentiate DIIHA from the above two similar conditions.
- A laboratory experienced in investigating DIIHA is recommended as appropriate serological tests may not be available in hospitals or large community reference laboratories.

Research Agenda

- Why do some (very few) patients have such severe hemolysis after receiving some commonly used drugs?
- Could a laboratory test be devised to prevent these severe reactions, before starting a patient on a course of certain drugs?
- Why does the same drug cause immune hemolytic anemia in one patient and immune thrombocytopenia in another patient?

References

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