bjh review

Management of Evans syndrome

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Summary

Evans syndrome is an uncommon condition defined by the combination (either simultaneously or sequentially) of immune thrombocytopenia (ITP) and autoimmune haemolytic anaemia (AIHA) with a positive direct antiglobulin test (DAT) in the absence of known underlying aetiology. This condition generally runs a chronic course and is characterised by frequent exacerbations and remissions. First-line therapy is usually corticosteroids and/or intravenous immunoglobulin, to which most patients respond; however, relapse is frequent. Options for second-line therapy include immunosuppressive drugs, especially ciclosporin or mycophenolate mofetil; vincristine; danazol or a combination of these agents. More recently a small number of patients have been treated with rituximab, which induces remission in the majority although such responses are often sustained for <12 months and the long-term effects in children are unclear. Splenectomy may also be considered although long-term remissions are less frequent than in uncomplicated ITP. For very severe and refractory cases stem cell transplantation (SCT) offers the only chance of long-term cure. The limited data available suggest that allogeneic SCT may be superior to autologous SCT but both carry risks of severe morbidity and of transplant-related mortality. Cure following reduced-intensity conditioning has now been reported and should be considered for younger patients in the context of controlled clinical trials.

Keywords: Evans syndrome, autoimmune cytopenias, autoimmune thrombocytopenia, autoimmune haemolytic anaemia, immunosuppression.

Evans syndrome is defined by the combination (either simultaneously or sequentially) of autoimmune haemolytic anaemia (AIHA) and immune thrombocytopenia (ITP), sometimes together with immune neutropenia, in the absence of known underlying aetiology. Thus, by definition true Evans syndrome is a diagnosis of exclusion and other confounding disorders should not be present (Evans *et al*, 1951). In this article we briefly review the epidemiology, pathophysiology and clinical features of Evans syndrome, focussing on mainly on the management of this very difficult disorder.

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History

Evans syndrome was first described in 1951 when Robert Evans presented evidence of a spectrum-like relationship between acquired haemolytic anaemia and primary thrombocytopenic purpura (Evans *et al*, 1951). He studied 24 patients (age range 3–78 years): four with haemolytic anaemia accompanied by thrombocytopenia but no purpura, six with primary thrombocytopenic purpura with red cell sensitisation but no haemolysis and four with both autoimmune haemolysis and thrombocytopenic purpura (the remaining patients described had acquired haemolytic anaemia ($n = 10$) or thrombocytopenic purpura ($n = 5$) alone). These observations, and the similarity of the response to splenectomy, led Evans to suggest the disorders were likely to have an identical aetiology. Acquired haemolytic anaemia had already been shown to be due to autoantibodies; Evans suggested that thrombocytopenia was similarly due to an autoantibody directed against platelets, a hypothesis supported by the presence of a platelet-agglutinating factor in their serum. In the original patient group, four were also neutropenic (as part of leucopenia rather than a selective neutropenia). The anaemia and thrombocytopenia were characterised by great variability in onset, course and response to treatment and both spontaneous remissions and frequent exacerbations were observed.

Epidemiology

Evans syndrome is a rare diagnosis although the exact frequency is unknown. A review of adult patients with immunocytopenias from 1950 to 1958 included 399 cases of AIHA and 367 cases of thrombocytopenia; only six of these 766 patients had Evans syndrome (Silverstein & Heck, 1962). The first described series of Evans syndrome in children reported seven children with Evans syndrome out of 164 cases of ITP and 15 cases of AIHA (Pui *et al*, 1980). No sex predilection is known and Evans syndrome has been described in all ethnic groups and at all ages (Pui *et al*, 1980; Wang, 1988; Mathew *et al*, 1997; Savasan *et al*, 1997). In four reported series of children with Evans syndrome, the median age at presentation ranged from 5·5 years to 7·7 years (overall age range

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0·2 –26·6 years; Pui *et al*, 1980; Wang, 1988; Mathew *et al*, 1997; Savasan *et al*, 1997).

Pathophysiology

Although Evans syndrome appears to be a disorder of immune regulation, the exact pathophysiology is unknown. Most studies have involved small numbers of patients and interpretation of the findings is made more difficult by the recent recognition that some cases of Evans syndrome may instead have autoimmune cytopenias secondary to autoimmune lymphoproliferative syndrome (ALPS) (Teachey *et al*, 2005). However, taken as a whole, there is evidence to support abnormalities in both cellular and humoral immunity in Evans syndrome.

In a study of six affected children, Wang *et al* (1983) found decreased percentages of T4 (T-helper) cells, increased percentages of T8 (T-suppressor) cells and a markedly decreased T4:T8 ratio compared with normal controls and patients with chronic ITP; these abnormalities persisted over the mean follow-up period of 1 year. Similarly, Karakantza *et al* (2000) found a decreased CD4/CD8 ratio in a 12-year-old boy with Evans syndrome although in this patient the numbers of both CD4 and CD8 lymphocytes were reduced; interestingly, the reduced CD4/CD8 ratio persisted postsplenectomy. They also found increased constitutive production of interleukin-10 and interferon-γ that, they postulated, caused activation of autoreactive, antibody-producing B cells. However, the significance of these abnormalities of cellular immunity is unclear as they are seen in other autoimmune conditions and in association with viral infection and are not specific to Evans syndrome.

Despite the frequency of haemopoietic cell-specific autoantibodies in patients with Evans syndrome, there is very little information about the identity of target antigens. Early work showed that the autoantibodies are specific to their target cells and, as shown by absorption and elution, do not cross-react (Pegels *et al*, 1982). Alterations in serum immunoglobulin levels in Evans syndrome have been reported in a number of studies but these are neither consistent or specific (Wang *et al*, 1983; Wang, 1988; Savasan *et al*, 1997) and the number of circulating B cells appears to be in the expected range (Pegels *et al*, 1982).

Clinical presentation

Patients may present with AIHA or ITP either separately or concomitantly. Neutropenia occurs in up to 55% of patients at presentation (Evans *et al*, 1951; Pui *et al*, 1980; Wang, 1988; Mathew *et al*, 1997; Savasan *et al*, 1997), or pancytopenia (14% in a national survey of 42 patients; Mathew *et al*, 1997). The development of the second cytopenia may occur months to years after the first immune cytopenia and may delay diagnosis (Mathew *et al*, 1997).

Clinical presentation includes the usual features of haemolytic anaemia: pallor, lethargy, jaundice, heart failure in severe cases; and thrombocytopenia: petechiae, bruising, mucocutaneous bleeding. Examination may reveal lymphadenopathy, hepatomegaly and/or splenomegaly (Pui *et al*, 1980; Savasan *et al*, 1997; Teachey *et al*, 2005). The lymphadenopathy and organomegaly may be chronic or intermittent and in some cases may only be apparent during episodes of acute exacerbation (Savasan *et al*, 1997; Teachey *et al*, 2005).

The role of childhood immunisations in the development of ITP or AIHA has been investigated by a number of authors (Seltsam *et al*, 2000; Chen *et al*, 2001; Johnson *et al*, 2002; Black *et al*, 2003) although the specific association of Evans syndrome and immunisations has not been reported. Thrombocytopenia has been demonstrated following the measles, mumps and rubella (MMR) vaccination, with Black *et al* (2003) estimating the relative risk for ITP within 6 weeks after MMR vaccination to be 6·3 [95% confidence interval (CI) 1·3–30·1] and the attributable risk of developing ITP within 6 weeks of vaccination to be 1 in 25 000 vaccinations. In contrast Nieminem *et al* (1993) reported the frequency of ITP to be 1 in 40 000 following the MMR vaccination. In the case of AIHA, life-threatening AIHA has been described in a 6-week-old girl, 5 days following her first diptheria–pertussis–tetanus (DPT) vaccination (Johnson *et al*, 2002). AIHA may also develop after the MMR vaccination (Seltsam *et al*, 2000). The two children in the report by Seltsam *et al* (2000) also demonstrated AIHA following revaccination [following the third oral polio vaccine in one case and following a combination of six vaccines (diptheria, pertussis, tetanus, haemophilus influenzae type B (Hib), polio and hepatitis B] in the other case), thus reflecting a secondary immune response. Taken together, these reports suggest that immunisations may provide a trigger for the development of disease in susceptible individuals and may also lead to a sustained increased risk in some of them (Chen *et al*, 2001).

Laboratory investigations

A full blood count will confirm the presence of cytopenias and a blood film should be examined for features of AIHA (polychromasia, spherocytes) and to exclude other underlying diagnoses (malignancies, microangiopathic haemolytic anaemia, congenital haemolytic and thrombocytopenic conditions). Features of haemolysis should be sought including a raised reticulocyte count, unconjugated hyperbilirubinaemia and decreased haptoglobins. The direct antiglobulin test (DAT) is almost invariably positive (although often weakly so), even in the absence of haemolytic anaemia, and may be positive for IgG and/or complement (C3) (Pui *et al*, 1980; Pegels *et al*, 1982; Wang, 1988; Mathew *et al*, 1997; Savasan *et al*, 1997). The indirect antiglobulin test may also be positive (52–83% patients; Pegels *et al*, 1982; Mathew *et al*, 1997).

Assays for antiplatelet and antigranulocyte antibodies have shown varied results. Fagiolo (1976), in a report of 32 adult patients with AIHA, showed antiplatelet antibodies in 91% (demonstrated by thromboagglutination and indirect anti-

globulin consumption tests) and leucocyte antibodies in 81% (demonstrated by a cytotoxicity test). In Pegels' characterisation of responsible autoantibodies in Evans syndrome (Pegels *et al*, 1982), all patients with neutropenia and/or thrombocytopenia demonstrated the relevant granulocyte and/or platelet antibodies, but mostly only on the patients' own cells as demonstrated by the direct immunofluorescence test. In only a few patients were autoantibodies demonstrable in the patients' sera. Pui *et al* (1980), however, found platelet autoantibodies in only two of six patients tested by the ^{14}C serotonin release assay and granulocytotoxic antibodies in three of four patients. Thus, autoantibody testing for platelets and granulocytes may be positive but a negative result does not exclude the diagnosis and routine testing at presentation may not be helpful.

It is advisable to measure serum immunoglobulins and immunoglobulin subclasses in all patients; not only to exclude differential diagnoses, such as common variable immunodeficiency (CVID) and IgA deficiency, which have been reported to develop acquired cytopenias (Hansen *et al*, 1982; Sneller *et al*, 1993), and also as a baseline prior to immunomodulatory therapy. In addition, other autoimmune conditions, particularly systemic lupus erythematosis (SLE), should be sought by measuring antinuclear antibody (ANA), double-stranded DNA (dsDNA) and rheumatoid factor. The most important differential diagnosis is ALPS. Therefore measurement of peripheral blood T-cell subsets by flow cytometry is essential in all cases of Evans syndrome. The presence of double negative ($CD4^-$/$CD8^-$, $CD3^+$, $TCR\alpha\beta^+$) T cells has been found to be the most sensitive first-line screening test for ALPS (and allows differentiation from cases of Evans syndrome) (Teachey *et al*, 2005), (see below and Table I).

Bone marrow investigation may be of use in evaluation of Evans syndrome where it is necessary to exclude infiltrative processes in patients who present with pancytopenia. Otherwise it is not usually helpful as the findings are non-specific and may be normal or show trilineage increased cellularity (Pui *et al*, 1980; Mathew *et al*, 1997).

Differential diagnoses

Evans syndrome is a diagnosis of exclusion and by definition other confounding disorders should not be present (Evans *et al*, 1951). Therefore, before accepting a diagnosis of Evans syndrome other causes of acquired immune cytopenia should be excluded, in particular SLE, IgA deficiency, CVID, acquired immunodeficiency syndrome and ALPS as all require different management (Teachey *et al*, 2005). Other conditions that cause concurrent haemolytic anaemia and thrombocytopenia and may mimic Evans syndrome include paroxysmal nocturnal haemoglobinuria (PNH), acquired thrombotic thrombocytopenic purpura, inherited ADAMTS-13 deficiency, haemolytic uraemic syndrome and Kasabach–Merritt syndrome (Schneppenheim *et al*, 2004).

Evans syndrome may also develop as a secondary syndrome; a number of case reports describe Evans syndrome secondary to multicentric Castleman's disease (Marsh *et al*, 1990; Quinn *et al*, 2004), to recombinant interleukin-2 therapy for renal carcinoma (Abdel-Raheem *et al*, 2001) or following autologous (Kamezaki *et al*, 2004) or allogeneic (Urban *et al*, 2004) stem cell transplantation (SCT).

Differentiating ALPS from Evans syndrome

The ALPS (originally known as Canale-Smith syndrome; Canale & Smith, 1967) is a disorder of defective lymphocyte apoptosis, usually presenting in early childhood, where the primary underlying defect occurs in the Fas–Fas ligand apoptotic pathway with a consequent chronic lymphoproliferation and persistence of autoreactive cells. The National Institutes of Health (NIH) ALPS group criteria for the diagnosis of ALPS requires the triad of (i) chronic non-malignant lymphoprolif-

Table I. Characteristics of Evans syndrome compared with ALPS.

Disease manifestation	Evans syndrome	ALPS
Autoimmune cytopenias	Required for diagnosis	Not required for diagnosis
	AIHA and ITP +/− neutropenia (simultaneous or sequential)	Lifelong risk (increases with age)
		Frequently seen (not invariable)
	Exacerbations and remissions	Exacerbations and remissions
Non-malignant lymphoproliferation	Not required for diagnosis	Required for diagnosis
	Lymphadenopathy/hepatosplenomegaly in some cases	Lymphadenopathy and/or splenomegaly common
		50% will have hepatomegaly
Defective lymphocyte apoptosis *in vitro*	Not seen	Required for diagnosis
≥1% α/β^+ $CD4^-/CD8^-$ T cells (in peripheral blood and /or lymphoid tissue)	Not seen	Required for diagnosis
Molecular basis	Not known	76% have mutations in *FAS*, Fas-ligand, caspase 8 or caspase 10
Neoplastic risk	Not defined	Lifetime incidence of 10% (especially lymphomas)
Serum immunoglobulins	Variable	Polyclonal hypergammaglobulinaemia
		Increased IgG/IgA

eration; (ii) an increased percentage (>1%) of α/β^+ $CD4^-/CD8^-$ (double negative) T cells; and (iii) defective *in vitro* Fas-mediated lymphocyte apoptosis (Straus *et al*, 1999). Patients with true Evans syndrome would not fulfil these criteria (Table I), although increasing awareness of ALPS means that many cases hitherto considered as Evans syndrome may in fact have had ALPS (Teachey *et al*, 2005). The majority of patients with ALPS have mutations in the *FAS* gene, but mutations have also been found in other components of the pathway including Fas ligand, caspase 8 and caspase 10 (Rieux-Laucat *et al*, 1995; Drappa *et al*, 1996; Wu *et al*, 1996; Wang *et al*, 1999; Chun *et al*, 2002). By contrast, patients with Evans syndrome, by definition, do not have these mutations (Table I).

The clinical presentation of ALPS frequently includes lymphadenopathy and autoimmune manifestations and therefore has similarities with that of Evans syndrome (see Table I), as recently highlighted in a study by Teachey *et al* (2005). The authors hypothesised that a subset of patients diagnosed as Evans syndrome may in fact have ALPS and tested their hypothesis by screening 12 children with Evans syndrome for double-negative (DN) T cells ($CD4^-/CD8^-$, $CD3^+$, $TCR\alpha\beta^+$) and Fas-mediated apoptosis. Elevated DN T cells were found in seven (58%) of 12 patients, suggesting a diagnosis of ALPS, which was confirmed functionally in six of these seven patients with the demonstration of defective *in vitro* Fas-mediated apoptosis. Hence, routine screening for ALPS is suggested in patients presenting with symptoms suggestive of Evans syndrome, with flow-cytometric testing for DN T cells; it is important to note that although this is a very sensitive and specific first-line screening test that will identify the vast majority of cases of ALPS, some cases fall into a grey area between ALPS and Evans syndrome, which will only become clearer with further studies (Teachey *et al*, 2005).

Treatment

The management of Evans syndrome remains a challenge. The syndrome is characterised by periods of remission and exacerbation and response to treatment varies even within the same individual. Most patients require treatment although occasional spontaneous remissions have been recorded: one patient of 42 patients with Evans syndrome in the national survey by Mathew *et al* (1997). Indications for treatment have not been established by evidence-based studies. However, it is usual and reasonable to treat symptomatic patients with low counts; as with ITP, not all asymptomatic patients with low counts require treatment and the decision to treat has to be taken on a case-by-case basis.

There have been no randomised-controlled trials in Evans syndrome and the few trials of treatment regimens contain small numbers of patients. Therefore the evidence presented here largely reflects data from case reports and retrospective surveys. The available treatment options are reviewed below; we then describe our personal approach to treatment of Evans syndrome although we believe there is insufficient evidence to produce a treatment algorithm appropriate for all children and adults and at present the use of one modality over the other will come down to individual clinician and patient choice and will reflect the previous experience of the centre. In most cases the regimens suggested will be appropriate for patients of all ages (adults and children); any differences in approach for different age groups are discussed in the text.

First-line therapy

The most commonly used first-line therapy is corticosteroids and/or intravenous immunoglobulin (IVIG). In the acute setting, blood and/or platelet transfusions may also be required to alleviate symptoms although their use should be minimised. It is our practice to use steroids as initial therapy and to add IVIG if patients fail to respond or are steroid dependent (Fig 1).

Corticosteroids Despite the lack of controlled trials demonstrating their effectiveness, corticosteroids remain the mainstay of treatment for control of the acute, symptomatic cytopenias with good initial results. Pui *et al* (1980), describing the clinical features and long-term follow-up of seven children with Evans syndrome, found that in all six children requiring treatment, prednisolone at a daily dose of 1–2 mg/kg resulted in remission; however this response was lost upon dose reduction and/or during acute viral infections. In the review of 10 children with Evans syndrome by Wang (1988) all nine patients treated initially with prednisolone responded; however, in all but one patient the cytopenia(s) recurred on cessation or tapering of the steroid dose. It is impossible from the reported studies to clearly summarise the average duration of response to steroids; either these data are not included or, more commonly, the results are confounded by the concomitant use of other immunosuppressants.

The dose of prednisolone used has generally varied from 1 mg/kg/d to 4 mg/kg/d (Pui *et al*, 1980; Mathew *et al*, 1997); although a good initial response to megadose i.v. methylprednisolone (30 mg/kg/d for 3 d then 20 mg/kg/d for 4 d, subsequently 10,5,2,1 mg/kg/d, 1 week each) has also been reported (Özsoylo, 2000). Our experience is similar to that of Pui *et al* (1980) and Wang (1988); we have found that most children respond promptly to prednisolone at a daily dose of 1–2 mg/kg but that relapse during weaning is common. Nevertheless, we would recommend that steroids continue to be used as first-line therapy in both adults and children as not only is there the greater experience with this form of therapy compared with the newer immunosuppressive agents, but also responses continue to be seen in the acute setting and, on occasion, complete remission (CR) is achieved.

Intravenous immunoglobulin For those patients for whom steroids are ineffective or who require unacceptably high doses to remain in remission or in whom toxicity results, the most commonly used first-line therapy is IVIG. The proportion of

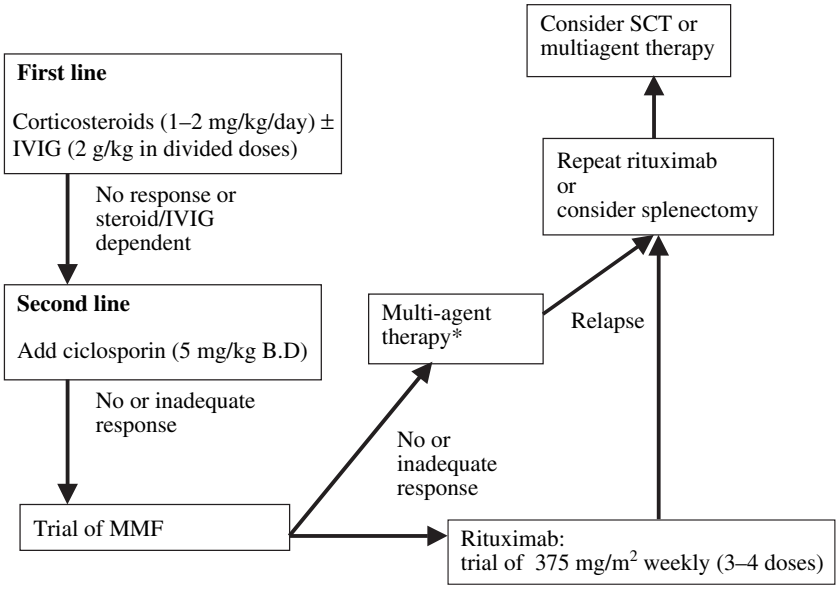

Fig 1. Management of Evans syndrome: a sequential approach. *Multiagent therapy: steroids/IVIG/vincristine/danazol/ciclosporin (Scaradavou & Bussel, 1995); vincristine/methylprednisolone/ciclosporin (Williams & Boxer, 2003).

patients who respond to IVIG is variable and, for those who do respond, normalisation of all or only some of the cytopenias may be achieved. Various doses have also been suggested, most commonly 0·4 g/kg/d for 4 d, with some authors recommending higher doses (up to 5 g/kg) to improve response in AIHA (Hilgartner & Bussel, 1987).

No reported studies have examined the role of IVIG as single first-line treatment for Evans syndrome; instead it has been used concomitantly with steroids or after steroid failure. The first use of IVIG for AIHA in Evans syndrome was described in a 5-month-old boy whose haemolysis and thrombocytopenia were refractory to steroids, but in whom a platelet response was noted with cyclophosphamide (Oda *et al*, 1985). IVIG (0·4 g/kg for 4 d, repeated 2 weeks later) together with prednisolone (1 mg/kg/d) resulted in remission of the AIHA and a negative DAT. Despite subsequent prednisolone withdrawal, remission was maintained after 6 months follow-up. Nuss and Wang (1987) then described three patients with Evans syndrome and thrombocytopenia refractory to splenectomy and prednisolone who were treated with modified immunoglobulin at a dose of 0·4 g/kg/d for 5 d (prednisolone therapy was also continued in two patients). A complete response was achieved in one patient, which persisted even after steroid withdrawal, but the other two patients failed to respond. Subsequently, the national survey of 42 children by Mathew *et al* (1997), found that single or multiple courses of IVIG were given to 40 patients with varied outcome: CR was seen in nine patients lasting up to 2 years; two patients initially responded but then became refractory after two courses; 24 had transient responses lasting up to 4 weeks; and five patients had no response.

Taken together, the data support a role for IVIG in the first-line management of Evans syndrome. It is not clear whether it is important for steroids to be administered at the same time although this is common practice. A total dose of 2 g/kg in divided doses appears to be sufficient for most cases that are going to respond. Repeated courses (either alone or with steroids) may also be successful. There may also be a role for first-line use of IVIG in preference to steroids in the acute setting in very young children (i.e. <2 years) in whom the risks of corticosteroid use, in terms of infection and growth suppression, may outweigh the benefits. The majority of patients, unfortunately, relapse as therapy is tailed off and second-line therapy will be required.

Second-line therapy

The range of options for second-line therapy is shown in Table II. These include immunosuppressive agents [ciclosporin, mycophenolate mofetil (MMF) and danazol], the monoclonal antibody rituximab and chemotherapy (vincristine). Splenectomy may also be considered a second-line treatment. Most of the data are anecdotal and inconclusive with interpretation difficult because of the concomitant use of corticosteroids and other modalities (Mathew *et al*, 1997; Kotb *et al*, 2005). The choice of which second-line agent to use depends upon clinical criteria, particularly the age of the patient, severity of the disease and its natural history because all of these treatments have significant short- and long-term side effects. It is our practice to start with a trial of ciclosporin, followed by MMF (Fig 1). In recent years, we have used rituximab for patients who fail to respond to these agents or who remain dependent upon high-dose steroids as well as ciclosporin/MMF. A promising alternative is to employ the multi-agent protocol, which includes vincristine and danazol, described by Scaradavou and Bussel (1995).

Table II. Options for second and third-line therapy of Evans syndrome.

Immunosuppressive agents
Ciclosporin
Mycophenolate mofetil
Chemotherapy
Vincristine
Cyclophosphamide
Danazol
Splenectomy
Therapeutic antibodies
Rituximab
Alemtuzumab
Other uncommonly used modalities
Azathioprine
Antilymphocyte globulin
6-thioguanine
Tacrolimus
Anti-D
Plasmapheresis

Ciclosporin The first report of ciclosporin for Evans syndrome used 5 mg/kg ciclosporin twice daily on alternate days together with prednisolone for the treatment of a 6-year-old girl with severe, life-threatening haemolysis refractory to multiple therapies including steroids, IVIG, anti-lymphocyte globulin (ALG) and splenectomy (Rackoff & Manno, 1994). Within 8 weeks of commencing therapy, the patient's blood counts improved and the steroids were successfully reduced from 2 mg/kg/d to 0·5 mg/kg/d. After nearly 2 years of this continued regimen the patient remained free of serious episodes of haemolysis or thrombocytopenia and her severe steroid-related myopathy had resolved.

Since this initial report, other authors have described success with similar regimens combining corticosteroids and ciclosporin (initial doses of ciclosporin ranging from 5–10 mg/kg/d) in refractory Evans syndrome patients. Responses have been noted in both adult and paediatric patients for ITP and/or AIHA (Emilia *et al*, 1996; Uçar *et al*, 1999; Williams & Boxer, 2003). In one report (Uçar *et al*, 1999) CR was maintained for more than a year after discontinuation of ciclosporin and prednisolone. Liu *et al* (2001) have also reported on the effects of ciclosporin in a large series of patients with AIHA and Evans syndrome (44 cases in total). They found greater response rates in Evans syndrome patients treated with ciclosporin in combination with prednisolone and danazol (89%) compared with either prednisolone alone or in combination with danazol (Liu *et al*, 2001); they also reported a reduced rate of relapse in the ciclosporin-treated group (Liu *et al*, 2001,2003).

Ciclosporin has also been used as part of a multi-agent approach (Scaradavou & Bussel, 1995; Williams & Boxer, 2003). Scaradavou and Bussel (1995), using a 'stepwise' protocol: (i) steroids + IVIG; then (ii) pulsed i.v. steroids, IVIG, i.v. vincristine and oral Danazol; and finally (iii) addition of oral ciclosporin (5–6 mg/kg/d) for non-responders, demonstrated a promising result in a 6-month-old boy with Evans syndrome whose AIHA had responded to first-line treatments but who required the addition of ciclosporin before partial remission of his prolonged thrombocytopenia was achieved. The one adult patient (age 27 years) in this study failed to respond to oral ciclosporin for thrombocytopenia (no patients in this study required ciclosporin for refractory haemolytic anaemia).

More recently, Williams and Boxer (2003) have treated four children with Evans syndrome (refractory to prior immunosuppressive therapies including steroids and IVIG) with a combination of vincristine, methylprednisolone and oral ciclosporin. The protocol was fairly intensive as it involved weekly vincristine (1·5 mg/m^2/week) and methylprednisolone (100 mg/m^2/week) until the platelet count was $>50 \times 10^9$/L together with oral ciclosporin 5–7 mg/kg twice daily (Williams & Boxer, 2003). Three of the four had a complete response (after 4–12 months of therapy) which was sustained for >9 months off therapy at the time of reporting. These are probably the best results with conventional immunosuppression in Evans syndrome and compare favourably with the recent data using rituximab (see below).

Although no significant toxic side effects have been reported at the doses used in these reports, careful monitoring is essential as long-term ciclosporin use, at least in the transplantation setting, is also associated with an increased risk of malignancy (Ades *et al*, 2004). Thus, ciclosporin (used alone or in the context of a multi-agent protocol) is probably best reserved as second-line therapy where steroids and IVIG have failed or need to be continued at unacceptably high doses.

Mycophenolate mofetil Mycophenolate mofetil, a potent inhibitor of inosine monophosphate dehydrogenase, inhibits lymphocyte proliferation and has been used in AIHA, as well as in a few cases of Evans syndrome (Howard *et al*, 2002; Hou *et al*, 2003; Kotb *et al*, 2005). A study by Howard *et al* (2002) described two adult patients with refractory ITP and AIHA who were commenced on MMF at a dose of 500 mg twice a day, increasing to 1 g b.i.d. after 2 weeks. Both patients were taking prednisolone at the start of the study and this was continued. At 13–15 months follow-up one patient had a sustained partial response allowing reduction of her prednisolone dose, the other patient had a complete response despite prednisolone discontinuation (MMF treatment continued in both at follow-up). In the study by Kotb *et al* (2005), the patient with Evans syndrome (previously refractory to high-dose steroid therapy and IVIG) had a complete response to MMF (1 g/d); at the time of reporting she had been weaned off prednisolone (2 mg/kg/d) and was maintained on MMF alone 6 months after starting therapy. These studies suggest that MMF is well tolerated and may be useful in Evans syndrome; therefore, although further studies are required to establish its role, we believe it is a useful agent to try if ciclosporin fails.

Vincristine In one review of 10 children treated for Evans syndrome (Wang, 1988), four children refractory to corticosteroids and splenectomy received treatment with vincristine (1·5 mg/m^2/week i.v.) for 3 weeks. A transient improvement in ITP was seen in all patients but further treatments were subsequently required. Vincristine has also been used as part of multi-agent therapy along with other immunosuppressants (Scaradavou & Bussel, 1995; Williams & Boxer, 2003), as discussed above, and it is on this basis that we have included it among the second-line treatments for Evans syndrome (Fig 1). We would suggest that vincristine should be considered as therapy for Evans syndrome as part of such a multi-agent protocol rather than as a single agent, although it is our preference to proceed to a trial of rituximab before using this approach.

Danazol There is only anecdotal experience of danazol in Evans syndrome, usually in combination with corticosteroids; however, it is included in the list of second-line options because of its relative lack of serious long-term side effects. Pignon *et al* (1993) used danazol (600–800 mg) and prednisolone (1 mg/kg/d) as first-line treatment for adults with AIHA. The two patients with Evans syndrome in this study failed to respond, although one patient previously refractory to treatment had a partial response and maintained a normal haemoglobin for 77 months on continuing therapy (10 mg prednisolone and 400 mg danazol daily). Wang (1988) described a 7-year-old patient with Evans syndrome and severe haemolysis who was refractory to treatment with steroids, splenectomy, vincristine and IVIG. She normalised her blood counts on danazol 200 mg three times daily with prednisolone and remission was maintained on a lower dose (200 mg/d) even after the prednisolone was discontinued. Danazol was also included in the multi-agent approach proposed by Scaradavou and Bussel (1995). Lack of data means that it is difficult to make any recommendations about the role of danazol in Evans syndrome. It is not our practice to use danazol as second-line therapy as we find it poorly tolerated in children. However, it may be worth considering danazol in adults either combined with prednisolone or as part of a multi-agent protocol (Scaradavou & Bussel, 1995) before resorting to therapy with potential for more serious toxicity, such as splenectomy or SCT.

Rituximab Rituximab, a chimaeric human/mouse monoclonal antibody which targets CD20 on B lymphocytes, is increasingly used in the management of a variety of autoimmune disorders, including Evans syndrome (Abdel-Raheem *et al*, 2001; Quartier *et al*, 2001; Seipelt *et al*, 2001; Galor & O'Brien, 2003; Shanafelt *et al*, 2003; Zecca *et al*, 2003; Knecht *et al*, 2004; Mantadakis *et al*, 2004; Quinn *et al*, 2004; Urban *et al*, 2004; Jubinsky & Rashid, 2005). Studies of the use of rituximab in Evans syndrome are summarised in Table III. The current evidence, although mostly from anecdotal reports, is encouraging with sustained CRs of up to 17 months and the possibility of achieving a second or third CR with repeated courses of treatment reported.

There are two series included more than one patient with Evans syndrome treated with rituximab (Shanafelt *et al*, 2003; Zecca *et al*, 2003). Shanafelt *et al* (2003) treated four adult patients with a variable number of infusions (3–8) of rituximab (375 mg/m^2). Two patients had an improvement in their AIHA or ITP but not both; the remaining two patients did not respond (one of these was an elderly lady with hepatocellular carcinoma).

Zecca *et al* (2003) evaluated the efficacy of rituximab in a prospective multicentre study for children with refractory AIHA, five of whom had Evans syndrome. Their ages ranged from 0·3 to 12·5 years and all had received from two to three courses of immunosuppressive treatment previously (including corticosteroids, IVIG, azathioprine and ciclosporin); none had had a splenectomy. Treatment consisted of weekly rituximab i.v. (375 mg/m^2 /dose) for three doses in four patients and four doses in one patient. The majority of patients were also receiving concomitant therapies of steroids +/− ciclosporin +/− azathioprine. All five patients responded within 72 days with at least a 1·5 g/dl increase in Hb level and a simultaneous platelet rise from a median of 27×10^9/l pretreatment to 140×10^9/l two months post-treatment. In all patients other concomitant immunosuppressive drugs were tapered and stopped within 25 weeks. At follow-up two patients had experienced a relapse (at 7 and 8 months respectively). In both these patients a subsequent treatment course of rituximab resulted in a second disease remission; one patient required four courses of rituximab in total for disease relapse but achieved a positive response after each treatment. In the remaining three patients follow-up at a mean of 13 months revealed continued remission after just one course of therapy. In this study, all children received IVIG (0·4 g/kg) every 3 weeks for 6 months post-rituximab to prevent therapy-induced hypogammaglobulinaemia.

Single case reports of the use of rituximab in Evans syndrome As shown in Table III, of the eight patients described in the case reports (Abdel-Raheem *et al*, 2001; Seipelt *et al*, 2001; Galor & O'Brien, 2003; Knecht *et al*, 2004; Mantadakis *et al*, 2004; Quinn *et al*, 2004; Urban *et al*, 2004; Jubinsky & Rashid, 2005), seven achieved a CR, as did the patient in Quartier's series with AIHA and ITP (Quartier *et al*, 2001). Of these eight patients, five were adults and three were children; the only non-responder was a child who developed fatal secondary Evans syndrome refractory to all treatment 10 months after an unrelated donor SCT (Urban *et al*, 2004). In each case the dose of rituximab given was 375 mg/m^2 with a variety of dosing schedules (most commonly once a week for 4 doses). Complications associated with rituximab in these studies have been minimal with minor infusion-related toxicities (fever, facial erythema, upper airway oedema) described. The use of prophylactic IVIG infusion following rituximab was not recorded in most of the reports and no serious adverse effects attributable to rituximab were described. This is in contrast to studies of rituximab given for other conditions (e.g. hepatitis B

Table III. Rituximab for treatment of Evans syndrome.

Study	No of patients	Age (years)/ sex	Co-existing illness	Dose of rituximab	Type and duration of response
Abdel-Raheem *et al* (2001)	1	60 M	Renal ca rIL-2 Rx	375 mg/m^2 every 3 weeks 5 cycles	CR 11+ weeks
Galor and O'Brien (2003)	1	43 M	–	375 mg/m^2 weekly 4 cycles	CR 9+ months
Jubinsky and Rashid (2005)	1	16 M	Diabetes cold and warm AIHA	375 mg/m^2 1–2 weekly 6 cycles	CR 12+ months
Knecht *et al* (2004)	1	50 M	CIDP	375 mg/m^2 weekly 4 cycles + 5 cycles 3 monthly	CR 17+ months
Mantadakis *et al* (2004)	1	21 M	–	375 mg/m^2 weekly 4 cycles 375 mg/m^2 weekly 2 cycles	CR 5 months Relapsed 2nd CR 7 months
Quartier *et al* (2001)	1	13 M	Hashimoto's thyroiditis	375 mg/m^2 weekly 4 cycles	CR 15+ months
Quinn *et al* (2004)	1	27 F	Multicentric Castleman's HIV-positive	375 mg/m^2 weekly 4 cycles	CR 7+ months
Seipelt *et al* (2001)	1	65 M	CLL	375 mg/m^2 weekly 4 cycles	CR 11+ months even when CLL progressed
Shanafelt *et al* (2003)	4	25, 39, 42 M; 79 F	Hepatic ca	375 mg/m^2 weekly 3–8 cycles	2 PR, 1 NR NR
Urban *et al* (2004)	1	3 M	UD SCT	375 mg/m^2 weekly 4 cycles	NR
Zecca *et al* (2003)	5	0.3–12.5 2M, 3F	Rh Arth (2) Vitiligo (1)	375 mg/m^2 weekly 3–4 cycles	3 sustained CR 2 CR->rel at 7, 8 months 2nd CR with rituximab

AIHA, auto-immune haemolytic anaemia; Ca, carcinoma; CIDP, chronic inflammatory demyelinating polyneuropathy; CLL, chronic lymphocytic leukaemia; CR, complete remission; F, female; M, male; NR, no response; PR, partial remission; Rh arth, rheumatoid arthritis; Rel, relapse; Rx, treatment; UD-SCT, unrelated donor stem cell transplant.

reactivation, pure red cell aplasia secondary to parvovirus B19 infection and fatal visceral varicella; Bermudez *et al*, 2000; Dervite *et al*, 2001; Song *et al*, 2002) and may reflect the small numbers or younger age of patients studied.

Despite the relative lack of experience of rituximab use, especially in children, and uncertainty about possible long-term effects, the recent promising results described above suggest that rituximab is at least as effective as, and arguably safer than, splenectomy. It is now our practice to offer rituximab as therapy for Evans syndrome resistant to first-line therapy and ciclosporin/MMF as an alternative to splenectomy.

Splenectomy Although traditionally used as initial second-line therapy in patients with autoimmune cytopenias (ITP or AIHA) who have failed to respond or relapsed following standard therapy with steroids +/− IVIG, the role of splenectomy in the management of Evans syndrome is not clearly established. In general, the response rate to splenectomy in Evans syndrome is poorer than the 70–75% response rates reported in chronic ITP although there are so few data that accurate response rates cannot be quoted for Evans syndrome (Blanchette & Price, 2004). While splenectomy often produces immediate improvement or even complete normalisation of

blood counts, this response is frequently transient and relapse occurs in most cases 1–2 months post-splenectomy (Pui *et al*, 1980; Wang, 1988; Mathew *et al*, 1997) irrespective of whether steroids are continued postoperatively (Wang, 1988). Nevertheless, splenectomy occasionally results in sustained remission (one of eight patients reviewed by Wang (1988), remained in CR at 6 years follow-up postsplenectomy) and there is some evidence that splenectomy may be ultimately beneficial by reducing the frequency of relapses and lowering the maintenance dose of steroids (Pui *et al*, 1980; Wang, 1988).

The risks of splenectomy are not insubstantial and should be carefully considered prior to operation. In addition to the risks of a general anaesthetic or perioperative bleeding, the greatest risk is sepsis; in a retrospective review of five children undergoing splenectomy for Evans syndrome (Savasan *et al*, 1997), two children died of postoperative sepsis. This group of patients may be at particular risk because of the frequent occurrence of neutropenia and/or hypogammaglobulinaemia (Mathew *et al*, 1997) and standard advice on preoperative vaccinations and postoperative life-long penicillin should be emphasised.

In our view, splenectomy should be avoided in children <6 years of age but can be considered in older children and adults as an alternative to long-term immunosuppression where first-line therapy has failed. However, given increasing data about the effects of rituximab, many clinicians may feel that the high relapse rate and long-term risks may make rituximab a more attractive option than splenectomy for all age groups.

Third-line therapy

The majority of patients will respond to first or second-line therapy, at least for many years. However, for patients with severe, relapsing disease despite second-line therapy, other options will have to be considered. The main third-line options are cyclophosphamide, alemtuzumab or SCT. For older adults SCT is not an attractive option, because of the relatively high mortality and failure rate in Evans syndrome, as discussed below. However, for children with chronically relapsing and life-threatening Evans syndrome, SCT from a human leucocyte antigen (HLA)-identical donor is likely to offer the only realistic prospect of long-term remission and is therefore superior to other third-line therapies.

Cyclophosphamide There are few reports of cyclophosphamide specifically in Evans syndrome. It has been reported to induce remission of thrombocytopenia in patients with Evans syndrome refractory to other treatments in doses of 1–2 mg/kg/d orally for 2–3 months (Oda *et al*, 1985; Wang, 1988; Gombakis *et al*, 1999). Brodsky *et al* (1998) described the use of high-dose cyclophosphamide (200 mg/kg) in three patients with severe autoimmune conditions (Evans syndrome, ITP and AIHA). Stem-cell support was not given. In the patient with Evans syndrome, a partial response was achieved although thrombocytopenia (not severe) and a positive DAT persisted (the other two patients in this study died).

Alemtuzumab Alemtuzumab is a humanised IgG monoclonal antibody specific for the CD52 antigen present on T and B lymphocytes, monocytes and eosinophils. There are few data published about the use of Alemtuzumab in Evans syndrome. Willis *et al* (2001) treated 21 patients with autoimmune cytopenias, three of whom had Evans syndrome, and all of whom were refractory to previous therapies, which included prednisolone, IVIG, vincristine, azathioprine, ciclosporin and cyclophosphamide. Alemtuzumab was administered at a dose of 10 mg/d for 10 d by intravenous infusion. A response was seen in two of the three patients with Evans syndrome; however both relapsed at 3 months. Both patients responded to a second course although one subsequently relapsed again at 19 months and the other died of metastatic carcinoma 5 months after completing therapy. The third patient with Evans syndrome only had a transient response and died of a cerebral haemorrhage 80 days after treatment (Willis *et al*, 2001).

Other medical therapies Other immunosuppressive drugs and therapies have been tried in occasional patients in the literature and include azathioprine, ALG, 6-thioguanine, tacrolimus, anti-D and plasmapheresis with variable results. Often, the role of these therapies in achieving response has been difficult to ascertain because of concomitant use of other therapies (Mathew *et al*, 1997), reports of their use is limited and therefore will not be discussed in further detail in this review.

Haemopoietic stem cell transplantation

Autologous and allogeneic SCT, including cord blood, have been used in small numbers of patients with Evans syndrome, with mixed results (summarised in Table IV) and have been recently reviewed (Hough *et al*, 2004; Passweg, 2004). Overall around 50% of patients are alive in CR.

The use of high-dose cyclophosphamide plus autologous SCT for treatment of Evans syndrome has been reported by Huhn *et al* (2003) in five patients and Passweg *et al* (2004), on behalf of the Autoimmune Diseases Working Party of the European Group for Blood and Marrow transplantation (EBMT), in two patients. Of these seven patients, four achieved a CR, one had a transient response and two patients had no response (Huhn *et al*, 2003; Passweg *et al*, 2004). Martino *et al* (1997) also reported a 25-year-old woman with Evans syndrome who died from an intracranial haemorrhage following exacerbation of haemolysis and thrombocytopenia after cyclophosphamide/granulocyte colony-stimulating factor mobilisation of peripheral blood stem cells in preparation for autologous SCT (Martino *et al*, 1997).

Table IV. Haemopoietic stem cell transplantation for Evans syndrome.

Reference	Number of patients	Stem cell source	Age/sex	Conditioning regimen	Outcome
Raetz *et al* (1997)	1	Allogeneic cord blood	5 M	Cy/TBI	CR; *died 9/12 post-SCT
Oyama *et al* (2001)	1	Allo-BM + PBSC	28 M	Cy/ATG	CR 30+/12
Huhn *et al* (2003)	5	Autologous PB $CD34^+$	33–52 3M,2F	Cy 200 mg/kg	3CR; 2 NR 1 pt died 14/12 post-SCT†
Passweg *et al* (2004)	5	Allo-BM	NA	NA	1 CR; 2 NE 2 deaths (1 TRM, 1 Evans syndrome)
	2	Autologous	NA	NA	1 CR, 1 PR

Allo, allogeneic; BM, bone marrow CR complete remission; Cy, cyclophosphamide; F, female; M, male; NA, not available; NE, not evaluable; NR, no response; PR, partial remission; TRM, transplant-related mortality synd: syndrome.
*Patient died 9/12 post SCT from fulminant hepatic failure of unknown cause.
†Patient died 14/12 post SCT from myeloma (diagnosed 5/12 after SCT).

The first report of allogeneic SCT for Evans syndrome was from Raetz *et al* (1997) who described a 5-year-old boy with severe, refractory Evans syndrome who achieved CR following an HLA-matched sibling cord blood transplant after conditioning with total body irradiation and cyclophosphamide (120 mg/kg). Myeloid engraftment occurred by day +16 but platelet engraftment was delayed until day +170. Unfortunately, the patient died unexpectedly, still in CR from his Evans syndrome, 9 months following transplant from fulminant hepatic failure of unknown cause. Subsequently, Oyama *et al* (2001) described complete clinical and serological remission in a 28-year-old man following allogeneic SCT with cyclophosphamide/ATG conditioning, although complete donor chimaerism was only achieved after the patient developed grade IV acute graft-*versus*-host disease (GVHD) on withdrawal of immune suppression. At last reported follow-up 30 months post-SCT, the patient remained in remission and free from infections or GVHD.

The largest series of allogeneic transplants for Evans syndrome has been reported by the EBMT (Passweg *et al*, 2004). A total of five patients were transplanted, of which two died (one of progressive Evans syndrome and one, a haploidentical transplant, of transplant-related causes). Of the remaining three cases, two were not evaluable and one achieved CR after donor lymphocyte infusion (DLI). This interesting case, which was also separately reported by Marmont *et al* (2003), suggested the possible role of allogeneic SCT in achieving a 'graft-*versus*-autoimmunity' effect. A 21-year-old man with severe, refractory Evans syndrome underwent a reduced intensity (thio-tepa 10 mg/kg plus cyclophosphamide 100 mg/kg) bone marrow transplant from his HLA-matched sister. Increasing mixed chimaerism with rapidly falling donor cells in blood and marrow led to the administration of a total of five courses of DLI, which achieved 100% donor chimaerism, blood count normalisation and autoantibody negativity. There was associated grade two acute GVHD; at the 2-year follow-up the patient remained in CR off corticosteroids and with a tapering ciclosporin dose (Marmont *et al*, 2003).

Prognosis

As previously discussed, Evans syndrome is characterised by recurrent episodes of relapse and remission of both ITP and AIHA. In some patients it seems likely that long-term cure may only be achievable with SCT. In long-term follow-up most authors described more frequent episodes of ITP compared with episodes of AIHA (median of 3–5 episodes of ITP, range 1–22 compared with medians of two and three episodes, range 1–22, of AIHA; Wang, 1988; Mathew *et al*, 1997). Pui *et al* (1980) and Scaradavou and Bussel (1995) also found that episodes of ITP were more frequent and harder to control than AIHA. Long-term survival data are limited. A total of 75 patients followed for a median of 3, 7, 8 and 8 years (range 4 months to 19 years) have shown mortality rates of 7%, 36%, 33% and 30% respectively (Wang, 1988; Ng, 1992; Mathew *et al*, 1997; Savasan *et al*, 1997). Causes of death were mainly related to haemorrhage or sepsis and reassuringly, given the degree of immune dysregulation seen in many patients, none of the patients described in these long-term studies (mainly of children) developed malignancy.

Conclusion

In this review we have discussed the clinical and laboratory features of Evans syndrome and its possible pathophysiology. We have described the treatment options available; however the paucity of large patient surveys and the lack of randomised-controlled trials make it difficult to make evidence-based recommendations about the optimal management of these patients. At best, the data suggest a role for corticosteroids +/− IVIG as first-line therapy in the acute setting, with blood product support as necessary.

Second-line therapy, in the form of single agent or, for more severe cases, multi-agent, immunosuppressants will usually be required. We recommend a therapeutic trial of ciclosporin as the best second-line option for most patients, with MMF and then multi-agent therapy or rituximab for those that fail to

respond. Splenectomy commonly achieves only short-term responses but may reduce the frequency of relapses and allow reduction of immunosuppressive agents. The choice of splenectomy versus rituximab may be a difficult one and will need to be made on a case-by-case basis.

Finally, high-dose therapy plus stem cell support provides hope for long-term cure in patients with a matched family donor; the success of a reduced-intensity conditioning regimen is an encouraging approach for this group of patients who have usually been extensively pretreated and are often in poor clinical condition prior to referral for SCT. However, as with many other rare disorders, progress may depend upon the acquisition of detailed information through national/international databases and international, multicentre randomised trials to accrue sufficient numbers of patients; long-term follow-up is also essential given the chronic relapsing nature of this condition.

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