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REVIEW ARTICLE

Management of Acute Rejection in Paediatric Liver Transplantation

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Abstract The success of paediatric liver transplantation is attributed to improved surgical techniques and the advent of calcineurin inhibitor-based immunosuppression. Acute rejection (AR) rarely results in graft loss with calcineurin inhibitor immunosuppressive regimens, and the advent of newer agents like interleukin (IL)-2 receptor antibodies. The latter have the benefit of reducing the incidence of AR further and may be of use in patients who are susceptible to recurrent AR, were retransplanted for graft rejection or are in a steroid-sparing regimen. A total of 60 % of all paediatric liver transplants result in AR; however, there is a 75 % response rate to initial steroid therapy. Steroid therapy remains the mainstay of initial AR management, coupled with an increase in baseline immunosuppression. Steroid-resistant rejection (SRR), previously an immediate indication for potent anti-lymphocyte preparations, is now effectively treated with chimeric or humanised IL-2 receptor monoclonal antibodies. Recurrent AR can be treated by adding adjuvant immunosuppressive agents such as mycophenolate mofetil (MMF) or sirolimus. Studies have also demonstrated the efficacy of MMF as rescue therapy for SRR. Anti-lymphocyte preparations such as anti-thymocyte globulin (ATG) and OKT3 are rarely used in SRR but may be of use as rescue therapy for severe SRR. The challenges of the management of AR remain in the management of recurrent AR and SRR. We discuss the pathogenesis, diagnosis and management of AR, including prevention, and specific management of AR and SRR based on current evidence and our own experience at the King's College Paediatric Liver, Gastroenterology and Nutrition Centre in London.

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1 Background

1.1 Introduction

Early human liver transplantation (LT) was complicated by frequent acute rejection (AR) and graft loss during the pre-calcineurin inhibitor (CNI) era.

The success of paediatric LT is the result of several key issues, including advances in surgical techniques and immunosuppressive therapy. These developments have resulted in improved patient and graft survival as well as outcomes in terms of quality of life when compared with pre-transplantation [2, 3]. The North American SPLIT (Studies of Pediatric Liver Transplantation) registry, published in 2008, reports the graft survival rate in children at 1, 3 and 5 years post-transplantation were 93, 90 and 88 %, respectively [3]. Recent data from the USA and Canada documenting outcomes of children alive 10 years after paediatric LT show survival rates after first allograft at 1, 5 and 10 years were 94, 91 and 88 %, respectively [4]. Consequently, paediatric LT is now the treatment of choice for children with end-stage liver disease. Despite the use of CNIs ciclosporin and tacrolimus, which has reduced not only the incidence and severity of AR but also AR-related graft loss compared with the pre-CNI era, the incidence of mild–severe AR after primary LT in children remains relatively high (60 % [2]). The transplanted liver is unique among solid organ allografts as it has certain immunological privileges that enable it to be relatively resistant to AR.

These privileges are explained by the liver allograft's ability to develop immune tolerance, a subject that is not discussed in detail in this review but is an important concept to mention in the context of AR. Mechanisms that facilitate this are thought to lie in the liver's unique microvascular architecture. It is thought to protect hepatocytes from immune attack by acting as a barrier and has a dampening effect on antigenic presentation to T cells. In addition, the liver has remarkable regenerative abilities even after an acute immune insult. It is recognised that early 'immune engagement' (i.e. AR) may lead to the development of tolerance and better graft survival [5].

The use of agents such as monoclonal antibodies in induction regimens, in combination with renal-sparing agents such as sirolimus (rapamycin) and mycophenolate mofetil (MMF), have demonstrated similar clinical outcomes to CNIs and may reduce long-term renal dysfunction. The caveats to such potent immunosuppressive agents are the consequences of their administration. Due to the drug toxicities, adverse effects and co-morbidities affect both patient and graft survival. References to product licensing signify the UK licensed status of the medicines only, as discussion of wider geographical use is outside the scope of this review. The aim of this article is to cover diagnosis and classification of AR, as well as current treatments, and to outline recent advances in immunosuppressive agents in the management of steroid-resistant rejection (SRR).

1.2 Diagnosis

The early recognition of acute cellular rejection is paramount, as it is easily reversed, with a 75 % response rate [6]. Those that fail to respond completely or have recurrent episodes have a higher risk of progression to chronic ductopenic rejection. The management of AR and SRR, recurrent AR and chronic rejection (CR) are still a challenge as they predispose patients to graft loss. In the North American cohort, 5 % of children went on to have CR, and 38 % of this group went on to have re-transplantation [2].

Due to baseline immunosuppression, clinical symptoms are preceded by liver serological abnormalities. Clinical symptoms, though uncommon, are the result of graft swelling (abdominal pain, hepatomegaly) due to inflammation, and systemic features related to cytokine release (fever, malaise). AR is clinically suspected by elevation of serum aminotransferases and alkaline phosphatase. Due to the subtle clinical features, or absence thereof, diagnosis of AR is made on liver biopsy and histological findings. AR occurs early within the first month of transplantation. It has a classic triad of histological features: portal inflammation, bile duct inflammation, and venous inflammation. At least two of these three features must be present for a diagnosis of AR. Perivenular inflammation is a poor prognostic factor in AR, and such patients are more likely to have recurrent AR episodes or progress to CR.

Table 1 Grading of acute liver allograft rejection, modified from Demetris et al. [8]

Severity	Criteria
Mild	Lymphocytic infiltrate in minority of triads, that is mild and confined within portal spaces
Moderate	Lymphocytic infiltrates expanding most or all of the triads
Severe	As for moderate but spill over into the periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis with bile duct damage

Overview of the immunology of acute rejection

In 1997, the Banff Schema for grading liver allograft rejection was introduced and resulted in a standard nomenclature for classifying AR. Severity of AR can further be defined according to histological findings (Table 1). The definition of AR I based on histological findings is as follows:

Definition of Acute Rejection

'*Inflammation of allograft elicited by antigenic disparity between donor and recipient primarily affecting interlobulary bile ducts and vascular endothelia, including portal vein and hepatic venules and occasionally the hepatic artery and its branches*', International Working Party. Terminology for hepatic allograft rejection [7]. The main differential in the diagnosis of AR is any cause of preservation injury to the graft. This includes primary graft dysfunction, and problems with the vascular and/or biliary tree. Thus, all patients should have concurrent imaging to exclude these complications. Often these may co-exist with AR at presentation; for example, histologically perivenular necrosis can occur in preservation injury and severe AR. It can be difficult to separate necro-inflammatory and ischaemic damage of rejection from non-rejection insults. Other possible differentials, such as cytomegalovirus (CMV) infection, should also be excluded.

1.3 Overview of the Immunology of Acute Rejection

Following an organ transplant, foreign antigens or alloantigens are shed into the circulation [9]. Transplanted organs express donor major histocompatibility complex (MHC) molecules, resulting in two pathways of recipient immune activation: directly via MHC-I molecules on donor cells and indirectly via host antigen recognition (allo-recognition) by T cells. In the latter, antigen-presenting cells (APCs) incorporate fragments of endocytosed donor antigens with surface MHC-II molecules. The primary targets of the immune response to allogenic tissues are MHC

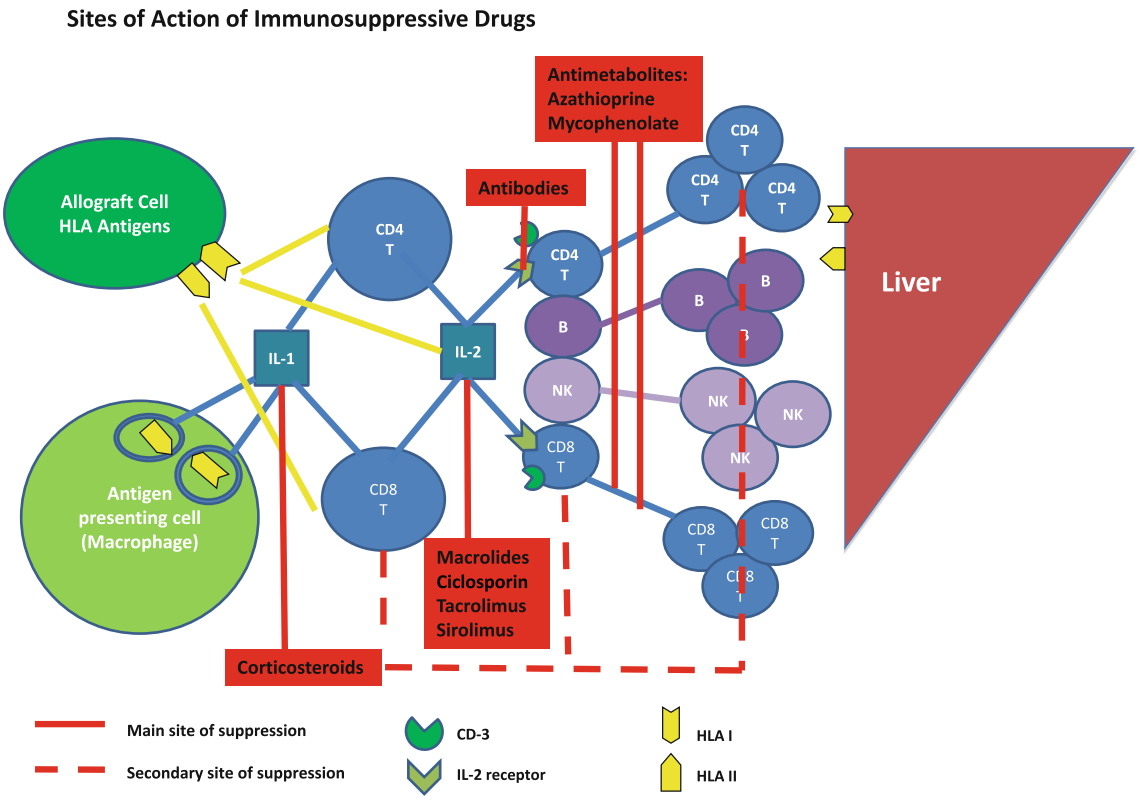

Fig. 1 Points of action of Immunosuppressive drugs, Corticosteroids inhibit production of IL-1. Macrolides (i.e., cyclosporine, tacrolimus, and sirolimus) inhibit production or use of IL-2, thus inhibiting stimulation of a clone of cytotoxic T lymphocytes directed against specific human lymphocyte antigen types. Antimetabolites (i.e., Mycophenolate Mofetil, azathioprine) inhibit purine production, thus impairing cell proliferation. Antibodies impair the normal function of cell surface markers, thus inhibiting stimulation of T-lymphocyte clones directed against foreign antigens (modified from D.Hatch MD [1]). *HLA* human leukocyte antigen, *IL* interleukin, *NK* natural killer

molecules on donor cells or APCs. MHC molecules on the surface of donor cells and APCs are recognised by the T-cell receptor (TCR/CD3). This leads to T-cell activation. This first signal, which results in TCR activation, is the first signal in T-cell activation in AR. The second signal (co-stimulatory signal) is not antigen specific. Instead, many T-cell molecules may serve as receptors for co-stimulation. The second signal is a calcium-independent pathway involving an interaction between CD28 on the CD4+ T cell and the proteins CD80 or CD86 on the APC. Both these proteins activate the CD28 receptor and are also known as co-stimulatory molecules. Once the naïve T cell has both the pathways activated, the biochemical changes induced by signal 1 are modified so that the cell undergoes immunological activation. The second signal then becomes obsolete and only signal 1 is needed for future activation [9–11].

After this, the T cells undergo clonal proliferation. It achieves this by releasing a potent T-cell growth factor called interleukin (IL)-2, which acts upon itself in an autocrine fashion. The IL-2 binds to the gamma chain of the IL-2 receptor and activates the Janus kinases (JAK) 1 and 3. This in turn triggers a cascade of intracellular signalling pathways, resulting in cell proliferation, DNA synthesis, and cell division as demonstrated by transition of the cell cycle from G1 to the S phase. After this initial T-cell activation and proliferation, release of cytokines and CD8 T cells cause donor cells to necrose or lyse. If this immune cascade is allowed to continue, it will eventually result in graft loss [9, 11, 12]. Since classical cellular AR is T-cell driven, most therapies are directed against these cells. This important pathway can be interrupted by azathioprine, MMF, sirolimus and everolimus (Fig. 1) [9, 13].

2 Prevention of Acute Rejection

The management of AR begins immediately post-transplant with maintenance therapy; in some centres, this initial management will include induction therapy. The level of immunosuppression during these phases is important as they give the allograft the best hope for long-term graft survival in that they prevent AR. The transplanted organ represents a continuous source of human leukocyte antigen (HLA) allo-antigens capable of inducing a rejection response at any time post-transplantation. Because it cannot be eliminated, the allograft continuously activates the

immune system. This results in lifelong overproduction of cytokines, constant cytotoxic activity, and sustained alteration in the graft vasculature. Therefore, lifelong immunosuppression is required to ensure allograft survival [10, 11]. Maintenance immunosuppression is the key to prevention of AR and CR throughout the life of the graft [11].

Due to differences in pharmacokinetics and pharmacodynamics in the paediatric population, pharmacists and clinicians are presented with challenges in prescribing safe and effective doses of therapeutic agents. This is owing to differences in body composition, liver enzyme maturity and protein binding variability that alter with age, weight and organ function [14]. This is further compounded in the paediatric transplant, as changes in the liver's ability to metabolise agents alter due to changes in liver perfusion. There are three distinct phases: at the point of transplantation (clamping of the hepatic blood supply), to removal of the recipient's liver (anhepatic), through to reperfusion [15]. In general, children who are younger than 5 years have a rate of clearance that is higher irrespective of the organ transplanted or the drug used. In younger patients, there is thus a need for higher dosages during the early post-transplant period as a result of these differences [16, 17]. Thus, the importance of effective drug monitoring is paramount in the immediate post-transplant phase to maintain appropriate immunosuppression levels and prevent AR [2]. Not all children need the same intensity of immunosuppression immediately post-transplant; therefore, target levels of immunosuppression should be on an individual basis, e.g. first liver transplantation vs. second transplantation. It should be noted that there is no difference in the target immunosuppressive levels with respect to recipients of cadaveric versus living related liver transplants.

2.1 Induction Agents

Some centres use induction agents as an initial hard immune hit. High-dose corticosteroids and high levels of CNIs remain the mainstay of immunosuppression immediately post-LT in the paediatric population. Induction with antibodies is used to decrease the incidence of AR in the immediate post-transplantation period, when the risk is highest. The rationale of induction is to tame the initial recipient immune response and T-cell activation during the early influx of donor cells and tissue injury from preservation and reperfusion immediately after transplant. Monoclonal or polyclonal antibodies are increasingly being used as induction agents, because they have fewer renal side effects, and are efficacious in preventing AR in susceptible recipients, thus preventing early AR and lowering maintenance immunosuppression. The benefits of induction therapy are that it enables the dosage of CNIs immediately post-transplant to be reduced to minimize their adverse effects. This is of particular use in recipients who have renal dysfunction. Induction is also of benefit in patients who have a higher risk of resistant or recurrent rejection (e.g. those who are retransplanted due to rejection, or transplanted for autoimmune liver disease). Their use also enables a reduction in the use of steroids, which is of particular interest in the paediatric population due to an association between steroid use and lower height centiles [4]. This section focuses on these newer agents. Some agents are used as induction agents and as rescue therapy in AR management, thus, use of these drugs is discussed for both indications in each section.

2.2 Introduction to Biological Therapies and Monoclonal Antibodies

These agents target specific immune components that are involved in the process of AR [13]. IL-2 receptor antibodies allow for the selective inhibition of IL-2-induced T-cell proliferation [10]. Animal studies were the first to show the promise of anti-IL-2-receptor antibodies in the prevention of cellular rejection. These antibodies are non-depleting and specific for the alpha chain of the IL-2 receptor. Blockade of the alpha chain results in inhibition of production of interferon (IFN)-γ in CD4+ and CD8+ T cells through suppression of IL-12 expression. It was not until the development of the chimeric or humanized forms that antibody therapy in transplantation was used to the full. The role of IL-receptor monoclonal antibodies in paediatric LT continues to evolve.

Use in induction regimens and as rescue therapy for SRR has been effective in children [18, 19]. They have fewer side effects, and rarely cause the typical first-dose infusion reaction associated with OKT3 [20]. They are also associated with a lower risk of opportunistic infections and post-transplant lymphoproliferative disease (PTLD). These agents are not part of standard immunosuppressive regimens. However, there has been an increase in their use, primarily to reduce or avoid the use of CNIs (particularly in patients with renal dysfunction) or to eliminate the use of corticosteroids, the latter of value in children. Some paediatric non-randomised studies have shown a significantly lower incidence of AR episodes with these agents (Table 2); thus, they may be of use in induction regimens for patients in whom the risk of rejection is highest.

2.2.1 Basiliximab

Several paediatric studies have demonstrated that basiliximab induction consistently significantly lowers the incidence of AR [21]. Studies comparing steroid-containing regimens with steroid-free basiliximab regimens have shown a greater growth catch-up post-LT in the basiliximab group [15, 22]. These data are summarized in Table 3. What is interesting from these studies is that

Table 2 Basiliximab dose guideline, (From 'Paediatric Liver Transplantation guidelines', Paediatric Liver, GI and Nutrition centre, King's College Hospital protocol September 2010)

Recommended Basiliximab dosing regimen in Paediatric Liver transplantation

The patient should receive two doses, on day 0 and day 4:

>35kg: 20mg per dose for two doses

< 35kg: 10mg per dose for two doses

Age <1 year old: 12mg/m^2 per dose for two doses

Table 3 Paediatric basiliximab efficacy studies

Reference	Study type	*n*	Immunosuppressive group	OLT type	AR rate (%)	Graft survival (1 year, %)	Patient survival (1 year, %)
Gras et al. 2008 [23]	PR	50	TAC, BAS	DD	28	95 (3 years)	96 (3 years)
		34	TAC, Ste		59	88 (3 years)	91 (3 years)
Spada et al. 2006 [15]	PR	36	TAC, BAS	DD	11	80	89
		36	TAC, Ste		31	86	91
Ganschow et al. 2005 [18]		54	CsA, Ste, BAS	DD, LD	17	98	98
		54	CsA, Ste (historical)		54	94	94
Gibelli et al. 2004 [20]	R	32	BAS, CsA, Ste	DD, LD	57.1		
		28	CsA, Ste (historical)		67.8		
Reding et al. 2003 [22]	PR	20	TAC, BAS	DD	94	75	75
		20	TAC, Ste		74	50	50
Strassburg et al. 2002 [24]	R	12	CsA, Ste		42		100
		9	CsA, AZA, Ste		66		100
		21	CsA, Ste, BAS		33		100
Asensio et al. 2002 [25]	R	13	TAC, Ste, BAS		30	80	80
		21	TAC, Ste		63	80	80
Kovarik et al. 2002 [26]	R	37	CsA, Ste, BAS		55		

AR acute rejection, *AZA* azathioprine, *BAS* basiliximab, *CsA* corticosteroids, *DD* deceased donor, *LD* living donor, *OLT* orthoptic liver transplantation, *PR* prospective, *R* retrospective, *Ste* steroids, *TAC* tacrolimus

although the AR rate lowered with the use of basiliximab, the overall graft and patient survival rates did not significantly lower. Basiliximab is considered, in our centre, for induction in children with renal dysfunction, or transplantation after recurrent graft loss due to recurrent AR or CR and sometimes for autoimmune liver disease.

Limited data exist on the use of IL-2 receptor antibodies in SRR. We reported our experience in a prospective study of seven children from King's College London with biopsy-proven SRR [19]. Of the seven, five received two doses of basiliximab, and two received one dose. Five children were rejection free at a median of 22 months' follow-up. There were no immediate side effects reported. In our unit, the use of basiliximab is considered in children where allograft rejection continues despite good levels of tacrolimus, and MMF or sirolimus and steroids have failed. In the past, potent anti-lymphocyte preparations such as ATG and OKT3 would have been used in our centre but, with the development of these newer IL-2 antibody agents, their use has been superseded.

Basiliximab is licensed for the prophylaxis of acute organ rejection in de novo allogeneic renal transplantation in adult and paediatric patients (aged 1–17 years). It is to be used concomitantly with ciclosporin for microemulsion- and corticosteroid-based immunosuppression, in patients with panel reactive antibodies less than 80 %, or in a triple maintenance immunosuppressive regimen containing ciclosporin for microemulsion, corticosteroids and either azathioprine or MMF. It is used outside the product licence for prophylaxis and treatment of acute organ rejection in paediatric LT. The licensed dose regimen for induction is two doses on day 0 and day 4. The same regimen is used locally for treatment of rejection (Table 2). CD25 counts are usually monitored before and after treatment.

2.2.2 Alemtuzumab Anti-CD52

Alemtuzumab (Campath®-1H) is a humanized antibody directed toward CD52 determinants on the surface of human B and T cells and monocytes. CD52 is the most prevalent cell surface antigen on lymphocytes. Alemtuzumab results in a profound and prolonged depletion of T and B cells in the peripheral circulation. Natural killer (NK) cells and monocytes are depleted to a lesser extent. There are few studies of its use in LT; much of its use is in renal transplantation. Alemtuzumab has been in use as an induction agent in adult LT since 2001. Alemtuzumab is licensed for the treatment of patients with B-cell chronic lymphocytic leukaemia (B-CLL) for whom fludarabine combination chemotherapy is not appropriate. Its use in both adult and paediatric LT falls outside the product licence. Use in the paediatric population is limited to a few studies. The dosing regimen used at King's College Hospital for treatment of rejection is similar to the induction regimen quoted in the literature for paediatric use: 0.3 mg per kg on days 0, 4 and 7 [27, 28]. Due to the profound immunosuppressive affects, patients commenced on alemtuzumab should be given prophylaxis therapy against opportunistic infections. Full blood counts and platelet counts should be obtained at regular intervals during alemtuzumab therapy.

The use of alemtuzumab in paediatric LT is limited. The University of Miami have reported their experience in children with autoimmune hepatitis (AIH) [28]. This group of patients are more susceptible to AR and graft loss. In their cohort of ten children, six had AIH and eight children were re-transplants. Alemtuzumab was administered as induction therapy on day 0, 4 and 7 post-transplant. Tacrolimus was maintained between 5 and 10 ng/ml. No patient received steroids. No patient received steroids, no opportunistic infection was observed, and lymphopenia lasted 4–6 months. They compared the incidence of AR in the AIH study group and with historical AIH controls who received tacrolimus steroid-based immunosuppression (no induction). Alemtuzumab significantly prolonged the time to the first episode of AR and significantly prolonged rejection-free graft survival. From this study we can conclude that alemtuzumab may be of use in paediatric LT for those who are susceptible to AR or those who have lost a previous graft due to rejection. Our centre's experience is limited to a few patients who developed persistent rejection under a setting where IL-2 antibodies and ATG were ineffective.

2.3 Maintenance Therapy

Adjuvant agents are sometimes combined with CNIs and include steroids, purine analogues (MMF and azathioprine), and mammalian target of rapamycin (mTOR) inhibitors (sirolimus). The rationale behind the addition of these agents is that the level of immunosuppression can be maintained but it enables the dose and toxicity of individual agents to be decreased.

2.3.1 Calcineurin Inhibitors

CNIs (tacrolimus and ciclosporin) remain the major players in maintenance immunosuppression in paediatric LT. Tacrolimus is a macrolide antibiotic with a potent immunosuppressive profile. Tacrolimus binds to cytoplasmic receptors (FK binding protein 12) and inhibits calcineurin. This is an important enzyme required in T-cell receptor signalling and activation. It results in the inhibition of the production of cytokines, especially IL-2, which is paramount in amplification of T-cell response. Tacrolimus has a tenfold greater in vivo potency than ciclosporin in inhibiting T-cell activation [29].

In the recent 20-year prospective study by Jain and colleagues, in which 1,000 consecutive orthotopic LTs (OLTs), performed between 1989 and 1992, were maintained under tacrolimus-based immunosuppression, significantly better survival rates were observed in children [30]. Graft loss due to rejection was rare (1.2 %). Adjunctive immunosuppression was observed in 26.2 % (20.5 % prednisolone, 4.75 % azathioprine and 8.8 % MMF). Earlier work by Reyes and colleagues reports a comparative long-term evaluation of tacrolimus- versus ciclosporin-based immunosuppression. Post-primary OLT children (n = 233) enrolled in the study [31]; 42.9 % remained rejection free in the tacrolimus group versus 43.3 % in the ciclosporin group. However, fewer children in the tacrolimus group had more than four episodes of AR (2.9 vs. 18.3 % in the ciclosporin group). A meta-analysis of 16 randomized trials comparing ciclosporin with tacrolimus for LT showed that tacrolimus is superior to ciclosporin in preventing AR. Tacrolimus reduced the AR and SRR rates by 18 and 43 %, respectively [3].

Tacrolimus is licensed for prophylaxis of transplant rejection in adult or child recipients of liver, kidney or heart allografts. It is also used for treatment of allograft rejection resistant to treatment with other immunosuppressive medicinal products. Our unit policy for dosing and drug levels is as below in Table 4. Dosing is as per the product licence [32].

2.3.2 Adjuvant Agents

Adjuvant agents are sometimes combined with CNIs and include steroids, purine analogues (MMF and azathioprine) and mTOR inhibitors (sirolimus). The rationale behind the addition of these agents is that the level of immunosuppression

Table 4 Unit protocol for Tacrolimus immunosuppression in paediatric transplantation, (From 'Paediatric Liver Transplantation guidelines', Paediatric Liver, GI and Nutrition centre, King's College Hospital protocol April 2010)

Recommended Tacrolimus dosing regimen in Paediatric Liver transplantation

Tacrolimus starts the night before transplantation if possible

Starting dose: 150μg/kg/dose orally twice a day
If > 20kg maximum dose of 3mg twice a day as initial dose

Target Trough guidelines post Liver transplantation

- Up to the first 3 months: 8-12μg/l
  Higher levels up to 15μg/l early on in patient who have rejection resistant to treatment
- After 3months: 6-8μg/l
- After 1 year: 3-5μg/l
  (or occasionally lower when graft function reliably normal)

can be maintained but they enable the dose and toxicity of individual agents to be decreased. Sirolimus and steroids have been discussed earlier, thus we discuss the use of azathioprine, MMF and sirolimus.

2.3.2.1 Mycophenolate Mofetil MMF is an ester pro-drug that is hydrolysed to the active immune suppressor mycophenolic acid (MPA). In 1969, MMF was discovered to block de novo purine nucleotide synthesis by inhibiting type 2 inosine monophosphate dehydrogenase (IMPDH) and the production of guanosine nucleotides such as guanosine monophosphate (GMP). Depletion of de novo guanosine causes a lack of deoxyguanosine triphosphate, suppressing DNA synthesis. This blockade impairs B- and T-cell proliferation. MMF is a teratogen, thus, use in adolescents should be considered carefully. MMF has also been used in CNI- and corticosteroid-sparing immunosuppressive protocols, without increasing the risk of rejection [33, 34]. In conjunction with tacrolimus in AR, it allows for tapering of steroid and limits tacrolimus toxicity.

MMF is licensed in combination with ciclosporin and corticosteroids for the prophylaxis of acute transplant rejection in adult patients only receiving allogenic renal, cardiac or hepatic transplants. In LT, MMF is used as an adjunctive agent in patients with CR or severe CNI toxicity. There are no suitable randomized controlled studies (RCTs) to support a role for MMF in LT.

MMF has been used to rescue grafts with SRR [34]. In another study from King's College London, 26 children who received LTs were given MMF for SRR [35]. Primary immunosuppression was ciclosporin based in 22 of the 26, with the remainder receiving tacrolimus. Of the 28 episodes of SRR, 21 responded to MMF therapy. In the seven non-responders, three went on to develop CR and subsequent graft loss.

2.3.2.2 Azathioprine Azathioprine is a derivative of 6-mercaptopurine. It acts as an antimetabolite of DNA and RNA synthesis and is used in maintenance immunosuppression and rescue therapy for AR in LT. Azathioprine is licensed in combination with other immunosuppressive agents for the prophylaxis of transplant rejection in adult patients receiving allogenic kidney, liver, heart, lung or pancreas transplants. CNIs combined with steroids, with or without azathioprine or MMF, have been standard immunosuppression after LT in adults and children. Azathioprine is more myleotoxic and hepatotoxic than MMF. Since 2000, many centres have substituted azathioprine with MMF. There are little data on its use in the paediatric population. Generally, its use is considered in light of MMF intolerance or where teratogenicity should be considered, i.e. in the adolescent patient. Adult studies have examined its efficacy compared with MMF. Two RCTs in adult patients compared MMF with azathioprine for AR. Fewer instances of AR occurred with MMF in one RCT (38.5 vs. 47.7 %), with no difference in patient and graft survival [36]. Two adult studies have evaluated the substitution of azathioprine with MMF. One study was stopped due to severe AR [37]. A recent 5-year prospective RCT of 100 adult LTs compared triple therapy (ciclosporin microemulsion, steroids and azathioprine) with double therapy (tacrolimus and steroids) [37] for induction. Of the triple therapy group, 62 % switched their immunosuppression to the tacrolimus-based regimen. Subjects who remained on triple therapy were shown to have an association with increased severity of AR. In this study, it is not clear whether this was attributed to the difference in CNI or the lack of azathioprine. However, from this study, the conversion to tacrolimus from the ciclosporin/azathioprine regimen was accomplished safely, with a good long-term outcome. Other studies demonstrate that azathioprine may be advantageous over MMF due to the reduced reoccurrence of hepatitis C virus (HCV); however, this is not as common in the paediatric population. In the paediatric population, azathioprine may be of benefit in adolescents,

as, unlike MMF, it has not been shown to be a teratogen in humans. In our unit, for immunosuppression after LT, we use a dose of 1.5 mg/kg orally daily; often we start at 0.5–1 mg/kg and increase if tolerated [38].

2.3.2.3 Sirolimus (Rapamycin) Sirolimus is a macrolide antibiotic that binds the FK-binding protein; its mechanism of action is via the target of rapamycin (TOR). It inhibits G1 to S-phase cell division and therefore cell proliferation. Sirolimus also inhibits B-cell proliferation and growth factor-mediated proliferation of non-immune cells. Other actions include inhibition of platelet-derived growth factor stimulation of smooth muscle. These factors may contribute to the efficacy of sirolimus in preventing and treating CR [39]. Since sirolimus is thought to have anti-proliferative properties against neoplastic cells, its use is advocated in patients who undergo LT for hepatocellular carcinoma [40]. Sirolimus does not have the nephrotoxicity of CNIs and does not reduce the glomerular filtration rate [41].

Sirolimus is licensed for the prophylaxis of organ rejection in adult patients at low to moderate immunological risk receiving a renal transplant. Use in paediatric LT recipients is outside the product licence. There are few paediatric studies on the efficacy of sirolimus-based immunosuppression. The use of sirolimus early post-LT is limited due to it being associated with adverse effects, including hepatic artery thrombosis and poor wound healing. Pittsburgh describes their experience of sirolimus as primary immunosuppression in combination with tacrolimus [42]. Fifteen children were given a single dose of anti-thymocyte globulin in combination with delayed sirolimus due to concerns of thrombogenicity immediately post-transplant. Six children received conventional steroid induction and maintenance, and nine received no steroids. In this study, there was a higher rate of steroid-sensitive AR in the steroid-free group (33 vs. 17 %). However, 11 of the 15 were steroid free. The authors of this study suggest that sirolimus may facilitate early elimination of steroids [42, 43].

Our centre recently published our paediatric experience with sirolimus. Its use as a rescue for allograft rejection, CNI-sparing agent for nephrotoxicity, and side effect profile with a follow-up period over 6 months was reported. Sirolimus was given to 37 patients, median age 10.4 years [44]. The indications for sirolimus therapy included resistant-proven rejection ($n = 12$), early CR ($n = 12$), CNI-induced nephropathy and MMF intolerance ($n = 11$), and reoccurrence of bile salt export pump (BSEP) disease in the allograft. We reported that 10 of 12 patients with resistant AR, and 6 of 12 patients in the early CR group responded to sirolimus therapy with normalization of aminotransferases. In addition, we demonstrated that sirolimus significantly improved renal function in the nephropathy group by improving the serum creatinine and cystatin C levels. We concluded that sirolimus is effective as rescue therapy in resistant AR and early CR. Sirolimus is not licensed for use in LT. Further studies are needed to warrant the efficacy and side effect profile of the use of sirolimus in LT. In our unit, we may convert a child from CNI-based immunosuppression if there is CNI nephrotoxicity or SRR or CR. Below is our unit policy for dosing and monitoring (Table 5).

3 Anti-Rejection Therapy

3.1 Acute Rejection

This section focuses on the management of histological proven AR.

3.1.1 Corticosteroids

The first-line treatment of acute (moderate or severe) rejection is pulsed high-dose corticosteroids in combination with an increase in baseline immunosuppression. Our standard regimen is as Table 6. Steroids prevent the production of IL-1 and IL-6 by macrophages and inhibit all stages of T-cell activation; the mechanism of action is through interaction with gene transcription. In the era of tacrolimus-based immunosuppression, the response rate to steroids is good. A meta-analysis of 16 randomized trials showed that steroids reverse 60–75 % of rejection episodes [45].

3.2 Steroid-Resistant Acute Rejection

Steroid-resistant AR is defined as AR that is unresponsive after three doses of high-dose pulsed steroids despite adequate immunosuppression levels, as defined by biochemical features and/or persistent histological features of AR. The incidence of SRR in paediatric liver recipients is between 6 and 30 % with tacrolimus-based immunosuppression [46]. SRR, previously an immediate indication for the use of anti-lymphocyte preparations, is effectively treated with chimeric or humanised IL-2 receptor monoclonal antibodies. SRR is a risk factor for CR, which often results in graft loss. Patients at risk of developing SRR and subsequent CR include younger age recipients, SRR, CMV infection, transplantation for autoimmune disease, occurrence of PTLD, HLA match/mismatch and positive lymphocytotoxic cross matching [47]. Use of sirolimus and IL-2 receptor antibodies for SRR has been discussed earlier, see Sects. 2.2 and 2.3.2.1.

3.2.1 OKT3 (CD3 Receptor, Muromab-CD3)

OKT3 is a murine monoclonal IgG2a antibody that specifically reacts with the T-cell receptor–CD3 complex on

Table 5 Sirolimus dose guideline post Orthoptic Liver transplantation, (From 'Paediatric Liver Transplantation guidelines', Paediatric Liver, GI and Nutrition centre, King's College Hospital protocol April 2010)

Recommended Sirolimus dosing regimen in Paediatric Liver transplantation

1. Without CNI -Stop ciclosporin or tacrolimus. Give 2mg/m2 loading dose on day one and 1mg/m^2 mg daily thereafter
2. In Combination with CNI -Start to taper ciclosporin or tacrolimus dose whilst at the same time starting sirolimus 1mg/m2 daily.

Monitor whole blood trough levels of Sirolimus and Cholesterol and triglycerides

	In combination with ciclosporin/tacrolimus	Without ciclosporin/tacrolimus
Sirolimus trough level	6-8ng/ml	8-10ng/ml

Table 6 Management of Acute rejection in Paediatric liver transplantation, Steroid use guideline in paediatric liver transplantation, (From 'Paediatric Liver Transplantation guidelines', Paediatric Liver, GI and Nutrition centre, King's College Hospital protocol April 2010)

Recommended steroid dosing regimen Paediatric Liver transplantation for acute rejection

Methylprednisolone: 10mg/kg as a single dose intravenously
Give daily for 3 days. Max 1gram daily

Restart at 2mg/kg/day oral prednisolone on the fourth day and reduce every 2-3 days back to the maintenance dose (0.1mg/kg/day).

the surface of circulating human T cells. It blocks T-cell function, and binds specifically to the CD3 receptor, reacting with more than 95 % of the peripheral mature T lymphocytes without affecting immature thymocytes. Nearly all functional T cells are transiently eliminated from the peripheral circulation. OKT3 has been removed from the market and is no longer available. It was used historically as rescue therapy for AR. It has been superseded by IL-2 antibodies, and is not discussed further in the context of AR management. It is not currently manufactured for therapeutic use. In our centre, we have not used it since it has been removed from the market. However, as historical adult and emerging paediatric data indicate, it may need to be revisited in the management of severe AR with cholestatic features. This is discussed in Sect. 3.2.2.

3.2.2 Polyclonal Antibodies: Anti-Thymocyte Globulin

These agents are derived from injecting animals with human lymphoid cells, and then the antiserum is collected. Preparations include horse anti-thymocyte globulin (Atgam), and rabbit anti-thymocyte globulin (thymoglobulin). A purified gamma globulin fraction (ATG) is used to prevent serum sickness. The efficacy of ATG in treating SRR is largely attributed to its ability to deplete T cells. Thymoglobulin also exhibits other effects on surface lymphocyte antigens that result in the interference of other immune effector cells: NK cells, B cells, regulatory T cells and dendritic cells [48]. ATG is a potent immunosuppressant; as a result it has significant adverse effects, including cytokine release syndrome, thrombocytopenia and lymphopenia. The risk of opportunistic infection is thus magnified, as is the risk of CMV disease. Patients receiving ATG should receive opportunistic infection prophylaxis. At King's College Hospital we use thymoglobuline, which is licensed in the UK but for prevention of graft rejection in renal transplantation. It is also licensed for treatment of steroid-resistant graft rejection in renal transplantation and prevention of graft rejection in heart transplantation (see Table 7 for our dosing regimen).

Few paediatric data exist on the efficacy of polyclonal antibodies in the treatment of SRR. Recent paediatric data from the USA report the successful use of anti-lymphocyte therapy (ATG/OKT3) in children who had biopsy-proven late 'cholestatic' rejection, i.e. severe AR [49]. In this study, 14 children who presented either with SRR ($n = 8$) or features of cholestatic AR ($n = 6$) were treated with ATG or OKT3. The latter received OKT3 or ATG as first-

Table 7 Use of Anti-human Thymocyte Globulin (Thymoglobulin®) in paediatric transplant patients, (From 'Paediatric Liver Transplantation guidelines', Paediatric Liver, GI and Nutrition centre, King's College Hospital protocol April 2010)

Recommended ATG dosing regimen in children for steroid resistant rejection.
1.5mg/kg/dose once a day for a minimum of 5 days (Maximum cumulative dose: 21mg/kg/course).

line therapy. All 14 patients were successfully treated and survived without re-transplantation at median follow-up of 2.9 years. No subject developed PTLD. The authors suggest that anti-lymphocyte therapies should be considered as first-line therapy for severe cholestatic rejection due to the high incidence of graft loss (49 %) associated with this type of AR. Thus, ATG is effective at treating SRR; however, sepsis remains a significant complication. Consequently, we rarely use ATG as first-line treatment for SRR in our unit; however, it should be considered in cases where IL-2 antibodies have failed or there are features of cholestasis.

3.3 Late Acute Rejection

Late AR occurring >3 months after transplantation has less typical histological features of AR. It has features that are associated with early CR, such as bile duct atrophy, early duct loss and or centrilobular fibrosis [11]. Due to these overlapping features, it can be difficult to diagnose. It can be asymptomatic in the early phase, hence an insidious course, but can present like AR. Serological markers are predominantly hepatitic (rising ALT and AST). Late AR tends to occur when there may be other issues with the graft, such as reoccurrence of disease. In a series in paediatric patients from our institution, the main factor appeared to be inadequate immunosuppression due to non-compliance [50] and this could explain the late presentation and insidious course. This differs from the adult population, where reoccurrence of underlying disease may arise, e.g. HCV. Unlike AR, late AR has a significant risk of progressing to ductopenic CR and graft loss in approximately 49 % [51]. The use of anti-lymphocyte preparations may be considered in this scenario and has been described in children in Sect. 3.2.2.

3.4 Recurrent Acute Rejection

Patients who develop recurrent episodes of AR are at increased risk of graft loss. Patients are more likely to develop recurrent AR if central perivenulitis is present on histology [11, 50]. Central perivenulitis is centrolobular necro-inflammatory changes with hepatic venous inflammation and perivenular hepatocyte loss. This is required to make a diagnosis of severe AR using Banff criteria. Difficulties arise when central perivenulitis exists in isolation; it represents an indolent form of subclinical rejection. Management of these findings may include increasing the levels of baseline immunosuppression with or without the addition of adjuvant immunosuppression.

3.5 Antibody-Mediated Rejection

In some recipients, although very rare in LT recipients, high titres of preformed antibodies exist that are sensitized to HLA molecules on the allograft. Accelerated AR can occur when the recipient has been previously exposed to low levels of donor tissue antigens and makes a rapid memory response when the donor organ is transplanted. Accelerated AR manifests within a few days to a few weeks following transplantation, and leads to allograft loss. Such antibodies are thought to arise as the result of HLA mismatch. In recipients with primary autoimmune liver disease or multiple transplants, antibody-mediated AR may have a role to play in reduced graft survival. HLA-sensitized cases often exhibit more intraoperative bleeding and a longer operative time. This is the result of antibody-mediated complement activation and endothelial damage. A cascade of events results in hepatic sinusoidal infiltrates of neutrophils, fibrin and erythrocytes as well as portal oedema leading to haemorrhagic infarction. In such cases, there is focal IgM, fibrin and C1q and C4d deposition [12]. Portal haemorrhage occurs in more severe cases, which is associated with poor graft outcome. In failed allografts there is evidence of large bile duct necrosis and hepatic artery thrombosis [50]. Antibody-mediated rejection (AMR) is thought to be driven mainly by B cells and complement activation. The future of AMR management is targeting B cells and complement activation with new monoclonal antibodies. Rituximab, a chimeric murine/human monoclonal antibody, approved in the USA only for the treatment of refractory or relapsed B-cell lymphomas, reacts with the CD20 antigen. Rituximab has been used off label in the prevention of rejection, in ABO incompatible renal transplantation and for desensitization in HLA-sensitized patients [13, 47]. None of the reports are in LT.

Treatment of PTLD has probably seen the greatest use of rituximab with great success, but there is no formal indication for this use. Rituximab may have a role to play in the treatment of AMR, and may improve the outcome in mixed cellular and humoral rejection. Eculizumab is a humanized monoclonal antibody that binds to C5 protein, thus inhibiting cleavage to C5a. It may have a role in the management of AMR as complement is thought to play a major role in the initiation of severe AR [48].

4 Conclusion

In the era of tacrolimus-based immunosuppression, the prevalence of AR is declining; however, it still can occur in up to half of all children in the 5 years following LT. It occurs most frequently in the 3–6 months following transplantation, and the mainstay of treatment of biopsy-proven moderate to severe AR remains high-dose intravenous corticosteroids while maintaining high levels of CNIs, and possibly the addition of adjuvant agents. Management of SRR is still an area that is challenging to the clinician, as it can result in recurrent AR and CR, ultimately leading to allograft loss. Newer agents, such as IL-2 receptor antibodies, sirolimus and MMF, have been shown to be efficacious in the treatment of steroid-resistant AR and have fewer adverse effects owing to their specificity compared with the potent anti-lymphocyte preparations OKT3 and ATG. In our experience, we would consider IL-2 antibodies with the addition of adjuvant therapy to increase baseline immunosuppression. These agents have been superseded by the newer agents; however, they may have a place as rescue therapy in recipients with features of cholestatic rejection, features of perivenular inflammation and steroid resistance. Further studies are needed in children to ascertain efficacy versus adverse effects.

Our understanding of the pathogenesis of recurrent AR and CR continues to develop. Antibody-mediated mechanisms may contribute to allograft damage in these scenarios and thus induction/AR therapy should be targeted at B cells, T cells and complement in this group. Such agents are under development, and use in the paediatric population is limited.

Induction therapy continues to evolve. Induction is not standard in paediatric LT immunosuppressive protocols. Some centres have demonstrated a reduction in AR rates with the addition of IL-2 receptor antibodies as induction agents. In addition, these agents offer reduced toxicity with results comparable to those of conventional immunosuppressive therapy. It may be of benefit in children who are susceptible to recurrent AR or severe rejection, e.g. AIH, re-transplantation and those with renal dysfunction.

The focus of AR management should be on those recipients at risk of recurrent AR and those with features of severe rejection, due to a higher prevalence of graft loss in this group. Further studies are needed to evaluate the efficacies and adverse effects of newer agents as well as potent emerging anti-lymphocyte preparations in the management of SRR and recurrent AR.

Conflicts of interest D. Thangarajah and M. O'Meara declare they have no conflicts of interest. A. Dhawan has served on the speakers' bureau for Astellas Pharma and has provided expert testimony for Boehringer Ingelheim and The Cytonet Group.

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