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Options for Induction Immunosuppression in Liver Transplant Recipients

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Contents

Abstract

Immunosuppression administered in the early postoperative period following liver transplantation plays a crucial role in the survival of the graft and the patient. The introduction of cyclosporin was an important landmark in transplantation, and to this day, calcineurin inhibitors form the basis of most induction immunosuppression regimens. New drugs are being developed which are more specific-

ally targeted to prevention of rejection, and multiple drug combinations have been proposed as a means of reducing the adverse effects of individual drugs.

Azathioprine and the newer antimetabolite mycophenolate mofetil have been added to calcineurin inhibitor-based regimens with varying amounts of success. Antibody induction has evolved as a potent form of immunosuppression as well as a means of avoiding certain adverse effects, particularly nephrotoxicity. The numerous adverse effects encountered with polyclonal preparations have been reduced with the development of more specific monoclonal antibodies such as muromonab CD3 (OKT3) or interleukin (IL)-2 receptor (IL-2R) antagonists. The anti–IL-2R antibody preparations basiliximab and daclizumab have shown excellent early results due to their potent yet highly targeted immunosuppressive effect and minimal adverse effects. Further study is needed to determine the most appropriate dosage, timing and patient population for these new drugs in the setting of liver transplantation.

Although a number of different induction regimens have been described, no single protocol is suitable for all liver transplant recipients. Rather, certain regimens have advantages that could favour their use in a specific subgroup of patients. A number of clinical trials are underway to identify new, more specific drugs and combinations which could be useful in induction immunosuppression.

1. Induction Immunosuppression

Induction therapy refers to the initial immunosuppression used in the days to weeks after organ transplantation. This early period is critical to the outcome of both the patient and the graft, and represents a time when the recipient is usually the most unstable, open to infections and vulnerable to drug adverse effects, such as nephrotoxicity. During this time, the graft is also highly susceptible to acute rejection episodes and to damage as the graft recovers from preservation. Induction immunosuppression thus requires a careful balance between too much and too little immunosuppression.

1.1 Characteristics of an Ideal Induction Regimen

An ideal induction regimen would be one that prevents acute rejection while minimising infection and toxicity. Whereas early acute rejection episodes have a negative impact on the future long term survival of the renal allograft, early mild-to-moderate acute rejection has not been shown to have a similar effect on the liver allograft.[1] Rather, the main concern lies in the heavy immunosuppression required to treat an established acute rejection episode. High doses of corticosteroids or muromonab CD3 (OKT3 antibody) have been shown to predispose to opportunistic infections, hepatitis C reactivation and malignancies.[2,3]

In the early postoperative period, the recipient is often in poor general condition and particularly susceptible to the toxic effects of drugs. Nephrotoxicity, for example, has been a major problem that has plagued the use of calcineurin inhibitors. Liver transplant patients are especially prone to renal failure secondary to preoperative hepatorenal syndrome, renal congestion secondary to caval clamping and volume fluctuations during the transplant.[4] Another example is bone marrow toxicity, which is associated with the use of the antiproliferative agents azathioprine or mycophenolate mofetil.

1.2 Multi-Drug Combinations

One approach to reducing toxicity is through the use of multiple drugs, each acting on a different portion of the rejection cascade. This allows the use of reduced doses of each drug thereby limiting the toxicity of that drug, all the while achieving an adequate amount of immunosuppression from the

Table I. Dual drug induction immunosuppression

Cyclosporin 2.5 mg/kg constant IV infusion[a]
or
Cyclosporin 5 mg/kg PO q12h[a]
or
Tacrolimus 0.05-0.1 mg/kg PO q12h[b]
Plus
Corticosteroid taper (table II)

a During the first 4 weeks post-transplant, the dosage of cyclosporin should be adjusted to maintain whole blood trough concentrations of 250 to 350 μg/L or 2-hour post-dose concentrations of 800 to 1200 μg/L.

b During the first 4 weeks post-transplant, the dosage of tacrolimus should be adjusted to maintain whole blood trough concentrations of 10 to 5 μg/L.

IV = intravenous; **PO** = oral; **q12h** = every 12 hours.

sum of the action of several drugs. Many triple and quadruple drug regimens have been formulated with this in mind.

1.3 Increased Drug Specificity

Another approach involves the development of drugs that are more specific to the prevention of organ rejection and less likely to cause global immunosuppression. This strategy has evolved over the last two decades from total body irradiation to antiproliferative agents to calcineurin inhibitors, each in turn affecting a more focused part of the immune system. Monoclonal antibodies have shown great potential as drugs with high specificity for a single receptor or molecule involved in the rejection cascade, while having negligible effects anywhere else.

The drugs and combinations used for induction immunosuppression continue to improve. While a number of induction regimens exist for liver transplantation, none currently exists which is clearly superior to the others in all situations. On the other hand, certain combinations appear to be better suited to specific patient groups. The purpose of this review is to compare the various regimens in current use and discuss which may be most beneficial in certain situations.

2. Calcineurin Inhibitors

The introduction of cyclosporin is a milestone in solid organ transplantation.[5] To this day, the calcineurin inhibitors cyclosporin and tacrolimus (table I), in combination with tapered corticosteroids (table II), remain the most widely used drugs in induction immunosuppression. While the merits of each drug continue to be debated, cyclosporin and tacrolimus have similar mechanisms of action and similar adverse effect profiles. Nephrotoxicity remains a major drawback of both drugs. One study suggested that tacrolimus may be less nephrotoxic than cyclosporin[6] but this has not been borne out in further studies.[7,8]

Large multicentre US[9] and European trials[7] comparing tacrolimus and cyclosporin induction regimens have shown similar 1-year patient and graft survival (table III). Tacrolimus may have an advantage in terms of reduced incidence of acute rejection as well as steroid-resistant rejection.[9] However, neurological and diabetogenic adverse effects were increased with tacrolimus use.[9] It is important to note that when these studies were conducted, tacrolimus blood concentration monitoring was not yet widely available and often could not be done on a daily basis. The initial tacrolimus doses were given intravenously and may have led to excessively high tacrolimus blood concentrations, which contributed to the high rates of nephrotoxicity and neurotoxicity.[10] In addition, the cyclosporin groups in both of these trials included

Table II. Examples of corticosteroid taper regimens

Prednisone 60mg PO od on first postoperative day
50mg PO od on second postoperative day
Taper by 10 mg/day until down to 20mg
20mg PO od on days 5 to 30
or
Methylprednisolone sodium succinate 100mg IV first postoperative day
80mg IV second postoperative day
Taper by 20 mg/day until down to IV 20mg, then start PO prednisone 20mg od

IV = intravenously; **od** = every day; **PO** = oral.

Table III. Published series of induction immunosuppression regimens in liver transplant patients using calcineurin inhibitors and antiproliferative agents (results at 1 year unless otherwise specified)

Reference	Study type	Treatment groups	No. pts	Acute rejection (%)	Steroid-resistant rejection (%)	Graft survival (%)	Patient survival (%)	Comments
US Multicenter[9]	r, mc	CSA, STE (AZA)	266	76	36	79	88	Various CSA regimens used
		TAC, STE	263	68 ($p < 0.002$)	19 ($p < 0.01$)	82	89	
European Multicenter[7]	r, mc	CSA, STE (AZA)	265	50	5	73	78	Various CSA regimens used
		TAC, STE	264	41 ($p = 0.04$)	1 ($p = 0.005$)	78	83	
Neuhaus et al.[11]	r, mc	TAC, STE	68	35	4	77	79	Different doses of tacrolimus and corticosteroids in the two groups
		TAC, STE, AZA	62	44	5	81	89	
Boillot et al.[12]	r, mc	TAC, STE	100	38	7	82	86	AZA added to full TAC doses
		TAC, STE, AZA	95	38	7	85	88	
Fischer et al.[13]	r	THY, CSA, STE, AZA	32	41	6	81	88	
		THY, CSA, STE, MMF	31	19 ($p = 0.06$)	3	84	90	
Wiesner et al.[14]	r, mc	CSA, STE, AZA	287	48	8	85	87	
		CSA, STE, MMF	278	39 ($p = 0.025$)	4	85	89	
Fisher et al.[15]	r	CSA, STE, MMF	48	22		92	98	6-month endpoints
		TAC, STE, MMF	48	16		94	98	
Glanemann et al.[16]	r	CSA, STE, MMF	40	75		88	98	
		TAC, STE, MMF	40	45 ($p < 0.05$)		95	95	
Eckhoff et al.[17]	re	TAC, STE	80	45	4	93	93	Lower dose TAC possible with MMF allowed better renal function
		TAC, STE, MMF	50	26 ($p = 0.03$)	0	91	91	
Jain et al.[18]	r	TAC, STE	99	41	4	80	85	High rate of MMF discontinuation
		TAC, STE, MMF	101	32 ($p = 0.15$)	3	79	83	
Klupp et al.[19]	r	CSA, MMF, STE	40	83	23	72	82	
		TAC, MMF, STE	40	50 ($p < 0.01$)	13	95	95	
		TAC, STE	40	52	13	85	90	

AZA = azathioprine; **CSA** = cyclosporin; **mc** = multicentre; **MMF** = mycophenolate mofetil; **STE** = corticosteroids; **r** = randomised; **re** = retrospective; **TAC** = tacrolimus; **THY** = thymoglobulin.

a variety of triple and quadruple induction regimens.

A few years after the appearance of these large studies, it was shown that tacrolimus could be given orally without the need for intravenous administration, with only a 35% acute rejection rate.[11] As a result, intravenous tacrolimus is no longer used except in very rare circumstances.

The debate over which drug is more effective will continue; meanwhile, a calcineurin inhibitor, whether tacrolimus or cyclosporin, continues to form the backbone of most induction regimens.

3. Antiproliferative Agents

3.1 Azathioprine

The use of the antiproliferative agent azathioprine predates the appearance of cyclosporin. Azathioprine is metabolised by the liver to the active drug 6-mercaptopurine, which inhibits DNA and RNA synthesis in rapidly proliferating cells. While azathioprine is effective in inhibiting T-lymphocyte proliferation, other haematopoetic, mucosal and endothelial cells are also affected. Bone marrow suppression can be a serious and limiting adverse effect of azathioprine.

Azathioprine was traditionally used alone (with corticosteroids) or in combination with cyclosporin and corticosteroids, and it is only recently that studies have examined the use of tacrolimus in combination with azathioprine (table IV).[11] 130 patients were randomised to receive either dual therapy of corticosteroids and tacrolimus 0.1 mg/kg or triple therapy of corticosteroids, tacrolimus 0.06 mg/kg and azathioprine 1 to 2 mg/kg. Comparisons between the two groups were difficult because different tacrolimus and corticosteroid dosages were used in the two groups. The study showed similar rates of acute rejection, and graft and patient survival. Approximately half of the patients randomised to azathioprine discontinued use of the drug because of adverse effects.

Another group obtained similar results when full dose tacrolimus and identical corticosteroid regimens were used in the two groups.[12] The authors concluded that azathioprine provides no additional benefit when added to tacrolimus and that azathioprine may in fact increase morbidity.

Table IV. Triple drug induction immunosuppression

Drug	Full dose	Reduced dose
Cyclosporin IV	2.5 mg/kg	1.25 mg/kg[a]
or		
Cyclosporin PO	5 mg/kg q12h	2.5 mg/kg q12h[a]
or		
Tacrolimus PO	0.05-0.1 mg/kg q12h	0.03-0.05 mg/kg q12h[b]
Plus		
Corticosteroid taper (see table II)		
Plus		
Azathioprine 1-2 mg/kg/day IV or PO		
or		
Mycophenolate mofetil 1-1.5g PO bid		

a During the first 4 weeks post-transplant, the dosage of cyclosporin should be adjusted to maintain whole blood trough concentrations of 150 to 250 μg/L.

b During the first 4 weeks post-transplant, the dosage of tacrolimus should be adjusted to maintain whole blood trough concentrations of 5 to 10 μg/L.

bid = twice daily; **IV** = intravenous; **PO** = orally; **q12h** = every 12 hours.

3.2 Mycophenolate Mofetil

A newer antiproliferative agent mycophenolate mofetil works by a mechanism similar to that of azathioprine but in a more selective manner. Mycophenolic acid, the active metabolite of mycophenolate mofetil, inhibits the synthesis of nucleotides, but only B and T lymphocytes are vulnerable to this effect.[20] Other cell types have a salvage pathway that allows nucleotide synthesis to continue.

Compared with azathioprine, mycophenolate mofetil appears to have fewer myelotoxic and hepatotoxic adverse effects.[21] A number of groups have sought to include mycophenolate mofetil as one of the drugs in triple or quadruple drug induction combinations as an alternative to azathioprine.[22]

Two studies have compared the efficacy of mycophenolate mofetil to that of azathioprine in the setting of a cyclosporin-based induction regi-

men. One group compared two quadruple drug regimens, each including thymoglobulin, cyclosporin, corticosteroids, and the study drug (mycophenolate mofetil or azathioprine).[13] Patients were randomised to receive either azathioprine 1 to 2 mg/kg/day or mycophenolate mofetil 1g twice daily. Acute rejection in the azathioprine group was numerically double that in the mycophenolate mofetil group; however, this did not achieve statistical significance ($p = 0.06$) because of small numbers. Azathioprine was discontinued in 44% and mycophenolate mofetil was discontinued in 29% of the patients.

A larger multicentre trial compared cyclosporin, corticosteroids and azathioprine with cyclosporin, corticosteroids and mycophenolate mofetil.[14] This study showed a significant difference between the azathioprine group and the mycophenolate mofetil group in terms of acute rejection (48 *vs* 39%, $p = 0.025$) and steroid-resistant rejection (8.2 *vs*. 3.8%, $p < 0.002$). These studies suggest that mycophenolate mofetil used as part of a combination regimen with cyclosporin is more effective than azathioprine in the prevention of acute rejection and is better tolerated.

Two studies have compared cyclosporin to tacrolimus, each in combination with mycophenolate mofetil. Fisher et al.[15] documented low 6-month rejection rates of 22% for the cyclosporin group compared with 16% for the tacrolimus group. The study by Glanemann et al.[16] used reduced dosages of cyclosporin and tacrolimus, and achieved 1-year acute rejection rates of 75% for the cyclosporin group versus 45% for the tacrolimus group ($p < 0.05$). Part of the reason for the advantage of tacrolimus over cyclosporin may be that the maximum concentration (C_{max}) and the area under the concentration/time curve (AUC) of the active metabolite mycophenolic acid was higher when mycophenolate mofetil was used in combination with tacrolimus. This is in keeping with studies in renal transplantation,[23] where it was also shown that equivalent mycophenolic acid concentrations could be obtained by increasing the mycophenolate mofetil dose by 50% in the cyclosporin group.

The combination of tacrolimus with mycophenolate mofetil would be advantageous if the addition of mycophenolate mofetil permitted the safe reduction of tacrolimus dosage without leading to an increase in the acute rejection episodes or infections. A retrospective study included patients who had received oral mycophenolate mofetil 1g twice daily, oral tacrolimus 0.2 mg/kg/day and corticosteroids, compared with historical controls who received oral tacrolimus 0.3 mg/kg/day and the same corticosteroid taper.[17] Lower daily doses of tacrolimus were indeed observed in the study group at 1 month (11.4 ± 0.5 mg/day *vs* 18.7 ± 0.8 mg/day, $p = 0.01$), and this resulted in lower tacrolimus whole blood trough concentrations and lower serum creatinine levels at 1 week and 1 month. Furthermore, the rate of acute rejection was significantly lower in the mycophenolate mofetil group, even in the presence of reduced concentrations of tacrolimus.

While it remains to be shown in a prospective study that mycophenolate mofetil is helpful in reducing tacrolimus dose administration, the addition of mycophenolate mofetil to full dose tacrolimus does not appear to be beneficial on the basis of two prospective studies.[18,19] The randomised trials compared the outcome of liver transplant patients receiving tacrolimus and corticosteroids to patients receiving tacrolimus, mycophenolate mofetil and corticosteroids. Patients in the mycophenolate mofetil group received mycophenolate mofetil 1g twice daily and both groups received full dose tacrolimus. There was no significant difference in patient or graft survival, or incidence of rejection; however, approximately half of the patients withdrew from the mycophenolate mofetil groups because of adverse effects of bone marrow toxicity or gastrointestinal disturbances.

While studies suggest that mycophenolate mofetil may be more effective if used with tacrolimus, compared with cyclosporin, simply increasing mycophenolate mofetil dosages may circumvent this problem. As suggested by a retrospective study, mycophenolate mofetil may help reduce the dose of tacrolimus and decrease nephrotoxicity,

but a prospective trial of mycophenolate mofetil in this situation is needed to clarify the matter, particularly given the high rate of mycophenolate mofetil discontinuation.

4. Antibody Induction

4.1 Polyclonal Antibodies

Antilymphocyte antibodies were among the first immunosuppressive agents used in clinical liver transplantation. Antilymphocyte globulin was derived from horse or rabbit serum following inoculation with human lymphocytes. Although such preparations were potent immunosuppressives, severe allergic reactions and serum sickness were common as a result of the large volume and variety of animal proteins in the preparations.[24] The production of this serum also resulted in variable potency and effectiveness, which meant consistent dosing produced unpredictable effects. Furthermore, the use of such antibody preparations is associated with an increased incidence of lymphoproliferative disease.[2] Today, more purified forms of antilymphocyte and antithymocyte globulin are available but they are rarely used in liver transplant patients except in the treatment of severe rejection in instances where the patient may have been sensitised to muromonab CD3.

4.2 Monoclonal Antibodies

Muromonab CD3,[25] a more specific, monoclonal form of antilymphocyte antibody was developed using hybridoma technology in an attempt to provide a more targeted form of antibody induction.[26] Muromonab CD3 is a murine preparation of monoclonal antibodies directed against CD3, which is found on the cell surface of all T lymphocytes. With this specific drug, problems with cross-reaction, allergies and serum sickness are uncommon. Administration of muromonab CD3 can, however, be associated with a variety of adverse effects, known as the cytokine release syndrome,[27] that results when muromonab CD3 binds to the CD3 receptor. By far the commonest symptom observed is fever, but most patients also experience diarrhoea, nausea and vomiting, severe headaches, myalgias and shortness of breath. Such adverse effects are generally worst with the first doses and can be reduced with the use of premedication. Information on the administration and dosage of muromonab CD3 is shown in table V.

Table V. Muromonab CD3 administration

Before the first and second doses, premedicate with
Diphenhydramine 50mg IV
Paracetamol (acetaminophen) 650mg PO
Methylprednisolone sodium succinate 250mg IV
then
Muromonab CD3 5mg IV od x 7 to 10 days[a]

a Increase to 10mg IV od if the CD3+ count does not decrease to less than 5%.

IV = intravenous; **od** = every day; **PO** = oral.

Three randomised, controlled trials have evaluated the use of muromonab CD3 given during the first 14 days in order to delay the start of cyclosporin therapy (table VI).[28-30] The control group in each of these studies consisted of patients who were randomised to receive cyclosporin, corticosteroids and azathioprine. Patients in the muromonab CD3 groups received a similar regimen of azathioprine and corticosteroids while muromonab CD3 was being administered, and cyclosporin was delayed until day 11. There was no advantage shown in terms of graft or patient survival. Acute rejection was significantly less in those patients receiving muromonab CD3, but only in the first 2 weeks postoperatively. Follow-up of the groups for 1 to 6 months, however, revealed that eventually a similar incidence of acute rejection was attained between the two groups in all three studies.[28-30] The studies showed a significant improvement in renal function by day 14 in the muromonab CD3 group; longer-term follow-up revealed that renal function equalised in the two groups.

Although at one time some programmes utilised muromonab CD3 routinely in all liver transplant recipients, the concern over an increased risk of lymphoproliferative disease[41] and the availability of more specific immunosuppressive drugs has led to a marked decrease in the use of muromonab CD3 in liver transplantation. Nonetheless, the use of

Table VI. Published series of induction immunosuppression regimens in liver transplant patients using antibodies (results at 1 year unless otherwise specified)

Reference	Study type	Treatment groups	No. pts	Acute rejection (%)	Steroid-resistant rejection (%)	Graft survival (%)	Patient survival (%)	Comments
Millis et al.[28]	r	CSA, STE, AZA	39	74	31	74	84	CSA started on day 11
		CSA, STE, AZA, OKT3	46	48	0 ($p < 0.05$)	61 (2y)	67 (2y)	
Cosimi et al.[29]	r	CSA, STE, AZA	41	78	54	61	73	
		CSA, STE, AZA OKT3	38	68	19 ($p < 0.05$)	76	84	
Farges et al.[30]	r	CSA, STE, AZA	50	75	22	77	78	
		CSA, STE, AZA, OKT3	44	67	5	74	82	
Reding[31]	r	CSA, STE, AZA	42	96		75	79	Murine anti-IL–2R antibody (Lo-Tact-1) used
		OKT3, STE, AZA	44	81		86	86	
		IL-2, STE, AZA	43	91		97	100	
Langrehr et al.[32]	r	CSA, STE, AZA, ATG	41	27	15	83	90	Murine anti-IL–2R antibody (inolimomab; BT-563) used
		CSA, STE, AZA, IL-2	39	8 ($p < 0.025$)	8	80	85	
Langrehr et al.[33]	r	CSA, STE, AZA	21	43	0	95	95	Murine anti-IL–2R antibody (inolimomab; BT-563) used
		CSA, STE, AZA, IL-2	19	11 ($p < 0.034$)	24	68	79	
Hirose et al.[34]	re	CNI, MMF, STE		60		93	93	
		CNI, MMF, STE, DAC[a]	32	50		81	88	CNI delayed by 1 week
Eckhoff et al.[35]	re	TAC, STE, (MMF)	58	40		91	93	Values at 6 months
		TAC, STE, (MMF), DAC[a]	38	18 ($p = 0.01$)		97	97	TAC started median 3.2 days postop
Heffron et al.[36]	pr	TAC, MMF, STE	47	54		'similar'	'similar'	
		TAC, MMF, STE, DAC[a]	54	26 ($p = 0.03$)		'similar'	'similar'	TAC delayed 7 days
Emre et al.[37]	re	DAC, MMF, STE	25	28		96	96	TAC delayed until Cr improved by more than 25%
		OKT3, MMF, STE	56	45		91	95	
		TAC, MMF, STE	48	25		91	94	
Calmus et al.[38]	ps	CSA, STE, AZA, BAS	102	23	10	89	92	6 month data
Grannas et al.[39]	ps	CSA, STE, BAS	109	27	0	93	94	
Heffron et al.[36]	pr	TAC, MMF, STE	87	41	4			Single dose of DAC
		TAC, MMF, STE, DAC[a]	93	26 ($p = 0.026$)	1			Follow-up time not stated TAC delayed 7 days
Neuhaus et al.[40]	r	CSA, STE	193	46	26	80	84	
		CSA, STE, BAS	188	39	18	84	87	TAC delayed 7 days

a Calcineurin inhibitor (CSA, TAC) administration delayed (see comments column for details in individual trials).

ATG = antithymocyte globulin; **AZA** = azathioprine; **BAS** = basiliximab; **CNI** = calcineurin inhibitor; **CSA** = cyclosporin; **DAC** = daclizumab; **IL** = interleukin; **IL-2R** = IL-2 receptor; **MMF** = mycophenolate mofetil; **OKT3** = muromonab CD3; **pr** = prospective; **ps** = pilot study; **r** = randomised; **re** = retrospective; **STE** = corticosteroids; **TAC** = tacrolimus.

muromonab CD3 to avoid the early use of calcineurin inhibitors in patients with poor renal function seems justified by these studies.

4.3 Antireceptor Antibodies

One approach to increasing the specificity of induction immunosuppression is the development of monoclonal antibodies with very specific targets.

4.3.1 Mechanism of Action

T lymphocytes play a central role in the initiation and progression of the rejection response. When foreign antigen is presented to it, the T lymphocyte becomes activated, secretes interleukin (IL)-2 and produces more IL-2 receptors. IL-2 then acts in an autocrine and paracrine fashion to drive the response forward. Because only activated T lymphocytes express IL-2 receptors, it was suggested that blocking this receptor with a monoclonal antibody could allow for an even more highly selective approach to preventing rejection than with muromonab CD3.[42]

4.3.2 Murine Antibodies

One of the first published experiences documenting the use of anti-IL-2 receptor monoclonal antibodies was the pilot study by Otto and associates.[43] The authors showed that the murine monoclonal antibody inolimomab (BT-563) was tolerated remarkably well and appeared to have no adverse effects in their series of nine patients. Encouraged by the apparent safety of the antibodies, a number of centres pursued this approach.

Langrehr and colleagues compared a 10-day course of inolimomab with antithymocyte globulin in a phase III trial.[32,44] Each drug was used in combination with cyclosporin, azathioprine and corticosteroids. Rejection was decreased in the inolimomab group (20.5%), compared with the antithymocyte globulin group (46.9%), while infectious complications were similar. The patients receiving inolimomab tolerated the drug well and reported fewer adverse effects than those receiving antithymocyte globulin.

Another study by Langrehr examined the effect of inolimomab versus placebo each in combination with cyclosporin, azathioprine and corticosteroids.[33] Again, less rejection was seen with inolimomab (10.5 compared with 28.6%), while similar rates of adverse effects and infections were seen in both groups.

These initial studies with murine anti-IL-2 receptor monoclonal antibodies suggest that the antibodies are well tolerated without leading to an increase in the incidence of infections.

4.3.3 Humanised and Chimeric Antibodies

Initial results with murine IL-2 receptor antibodies appeared promising but a significant limitation was the short half-life because of the use of a foreign protein. Inevitably, anti-murine antibodies were formed and the drugs were rendered ineffective within 2 weeks post-transplant. To circumvent these problems, chimeric and 'humanised' forms of these antibodies were developed. Using hybridisation techniques, monoclonal antibodies were made that retained the majority of human immunoglobulin (Ig)G sequences but incorporated murine sequences in the hypervariable regions specific to the α chain of the IL-2 receptor.[45] The theoretical advantages include prolonged action, lack of development of anti-drug antibodies and more potent effector activity as a result of the presence of human Fc.[42]

Two such antibodies are now commercially available; both contain the hypervariable regions of the murine anti-IL-2 receptor antibody Tac involved in binding the α subunit. Basiliximab is a chimeric antibody that contains less than 10% murine sequences and has a terminal half-life of approximately 6.5 days in renal transplant patients.[46] Daclizumab is a humanised antibody containing a greater proportion of human sequences, making it theoretically less immunogenic, with a terminal half-life of approximately 11 days.[47]

Although the two preparations are quite similar, the suggested dosage regimen for each drug has been different. Basiliximab is usually administered in two 20mg doses, given by slow intravenous in-

jection within 8 hours of graft reperfusion and then again on postoperative day 4.[46] Daclizumab is given in five doses of 1 mg/kg, given within 24 hours of graft reperfusion, then at 2, 4, 6, and 8 weeks postoperatively.[47] Other dosage regimens are shown in table VII. Receptor suppression has been documented for about 3 to 4 weeks following basiliximab and up to 10 weeks for daclizumab when the suggested dosage regimen is followed,[48,49] but the optimal length of receptor suppression and whether suppression beyond 4 weeks is of significant benefit remains unknown.

A number of phase III trials have demonstrated that daclizumab is effective and well tolerated in the setting of renal transplantation.[50,51] The drugs are only recently being used in the area of liver transplantation.

4.3.4 Considerations in Liver Transplant Patients

The properties of basiliximab were studied in this group of patients to determine how the characteristics of liver transplant recipients would affect the pharmacokinetics of the drug. The half-life was decreased in liver compared with renal transplant recipients.[48] The increased blood loss seen in liver transplantation did not account for significant drug elimination. Losses via ascitic fluid drainage, on the other hand, accounted for on average about 20% of drug elimination, and patients with extreme amounts of ascitic drainage lost significant amounts of drug.[52] Basiliximab administered either as 4 x 10mg doses or 2 x 20mg doses (as for kidney transplantation) produced similar AUCs and IL-2 receptor suppression of 23 ± 5 days or 2 weeks following the last dose [52] and up to 38 ± 16 days in another study.[53] While drug elimination is enhanced in liver transplant recipients compared with kidney transplant recipients, IL-2 receptor suppression with the 2-dose regimen seems to be long lasting. It has been suggested that dose adjustments should be considered for patients with massive ascitic fluid drainage.

Table VII. Administration of anti–interleukin-2 receptor monoclonal antibody preparations

Basiliximab
20mg IV on day 0 and day 4[46]
Daclizumab
1 mg/kg IV on day 0, 2 wk, 4 wk, 6 wk, 8 wk[47]
or
2 mg/kg IV on day 0 as a single dose[36] a
or
2 mg/kg IV on day 0 and day 5[35] a

a Calcineurin inhibitor should be started within 1 week post-transplant.

IV = intravenous.

4.4 Daclizumab - Early Clinical Results

Most of the early clinical studies have focused on using these drugs in order to reduce or delay the use of calcineurin inhibitors in the early postoperative period. In kidney transplant recipients, the combination of daclizumab, mycophenolate mofetil and corticosteroids was shown to be effective in preventing acute rejection while allowing the sparing of calcineurin inhibitors.[54]

The possibility of using such a regimen in liver transplantation was explored in a pilot study.[34] Daclizumab 1 mg/kg was given on day 0 then at 2-week intervals for a total of five doses, along with mycophenolate mofetil 1g twice daily and corticosteroids. The study was halted after all seven patients developed acute rejection. The authors concluded that 'renal' dose administration without calcineurin inhibitors is insufficient to prevent rejection in liver transplant patients. This is somewhat surprising, given that the liver has always been considered to be somewhat immunoprotected compared with the more immunogenic kidney. One reason for the ineffectiveness of the drug in liver recipients may be that daclizumab drug concentrations were noted to fall to subtherapeutic (<5 mg/L) by postoperative day 4 to 6, probably as a result of a shorter half-life in liver transplant recipients and drug elimination via fluid losses.

The same study retrospectively examined patients who had received daclizumab for induction in order to delay the use of calcineurin inhibitors for various reasons, the most common of which was nephrotoxicity.[34] The 25 patients all received daclizumab 1 mg/kg immediately pre- and postop-

eratively, followed by four more doses at week 2, 4, 6 and 8, in addition to mycophenolate mofetil 1.5mg twice daily and prednisone. Calcineurin inhibitors were started on average 7 days postoperatively. All patients tolerated daclizumab extremely well and no adverse effects could be directly attributed to the use of the drug. Compared with historical controls who received a calcineurin inhibitor, mycophenolate mofetil and prednisone, a similar rate of rejection was seen. The most important factor influencing acute rejection appeared to be the delay in instituting calcineurin inhibitors. Ten of 11 patients (91%) who received their first dose of calcineurin inhibitors after day 8 experienced acute rejection versus six of 21 patients (29%) if calcineurin inhibitors were started before day 8.

Another study looked at a two-dose schedule for daclizumab administration,[35] on the basis of the observation that concentrations fell to subtherapeutic by days 4 to 6 in an earlier study.[34] In this retrospective study, 38 patients with preexisting renal failure or thought to be at risk for renal dysfunction received intravenous daclizumab 2 mg/kg on day 0 and 1 mg/kg on day 5. Tacrolimus was started at an average of 3.2 days, once renal function showed evidence of returning (serum creatinine <2 mg/dl or urine output >1000 ml/day). These patients were compared with another 58 patients transplanted during the same time who were not thought to be at risk of renal dysfunction and thus received conventional immunosuppression.

While the daclizumab group had markedly increased creatinine preoperatively, serum creatinine for the two groups equalised by 1 week posttransplant. Rejection was decreased in the induction group at one month (8 *vs* 29%) and at 6 months (18 *vs* 40%). In spite of the fact that the patients in the daclizumab group were in worse medical condition preoperatively, there was no difference in graft or patient survival nor opportunistic infection between the two groups. It is important to recall that uraemia may have an immunosuppressive effect[1] and that this is a potential confounder in all nonrandomised studies involving patients with renal dysfunction.

The use of a single dose of daclizumab was suggested to provide coverage for the first postoperative week. On the basis of earlier studies that show IL-2 receptor suppression for over 2 weeks following the last dose, this seems to be a reasonable assumption. A prospective trial compared 54 patients who received a single dose of daclizumab postoperatively and with tacrolimus delayed until day 7, with 47 patients who received tacrolimus immediately postoperatively.[36] Both groups also received mycophenolate mofetil 1g twice daily and corticosteroids. The incidence of acute rejection was lower in the daclizumab group compared with the control group (26 *vs* 49%). Furthermore, serum creatinine was lower in the daclizumab group at 7 days (1.16 ± 0.5 mg/dl *vs* 1.54 ± 0.93 mg/dl). These results were similar to those that have been previously shown with muromonab CD3, however daclizumab had the advantage of having very few adverse effects recorded.

Three different approaches to induction in patients with poor renal function were studied retrospectively in 129 patients with preoperative serum creatinine >2 mg/dl.[37] The first group included those patients who received a single dose of daclizumab and had the initiation of tacrolimus delayed until serum creatinine improved by greater than 25%. The second group included patients who received muromonab CD3 for the first 10 to 14 days postoperatively, while the third group received low-dose tacrolimus, titrated to achieve a trough blood concentration of 10 μg/L. The incidence of acute rejection did not differ significantly between the groups. Renal function improved more quickly in the patients receiving daclizumab (3.6 days) or muromonab CD3 (4.6 days) than those receiving tacrolimus (12.2 days). Whereas only one patient in the daclizumab group required dialysis, 13 patients in each of the other two groups required dialysis.

4.5 Basiliximab - Early Clinical Results

A pilot study was performed examining 102 liver transplant recipients receiving basiliximab 20mg on day 0 and day 4 in combination with cyclosporin, azathioprine and corticosteroids.[38] A 6-month acute rejection rate of 24% was seen; no episodes of severe rejection were seen; however, a 10% incidence of steroid-resistant rejection was recorded. Both patient and graft survival were excellent. Similar results were shown in another pilot study in which azathioprine was not used.[39]

The largest trial yet to study the use of anti–IL-2 receptor monoclonal antibodies included 381 patients randomised to receive placebo or basiliximab 20mg on day 0 and 4 in addition to cyclosporin and corticosteroids.[40] The results of this multicentre, randomised, controlled trial show decreased acute rejection in patients receiving basiliximab, particularly in the hepatitis C virus (HCV)-negative subgroup (33.1 *vs* 47.6%, p = 0.034). The rate of severe acute rejection was also decreased. Patients receiving basiliximab had the same incidence of complications as the patients in the placebo group and the drug was well tolerated.

4.6 Comments

Available data on the use of anti–IL-2 receptor monoclonal antibodies in liver transplant patients suggest that the drugs have good safety and are well tolerated in this setting. Because of the lack of nephrotoxic adverse effects, their most promising use may be in patients with elevated creatinine levels or at risk of renal failure. Certainly, the drugs could be used to avoid neurotoxic adverse effects as well. Delaying the initiation of calcineurin inhibitors until the end of the first postoperative week seems to allow renal function to recover without risking an increased incidence of acute rejection.

The most effective dosage regimens of basiliximab and daclizumab have yet to be decided. Two 20mg doses of basiliximab have been shown to provide IL-2 receptor saturation for weeks after the last dose and individualised dose administration based on bodyweight does not appear to be necessary.[35] It is not clear that the administration of five doses of daclizumab at 2-week intervals is beneficial, particularly in light of the added costs and the fact that the maximal risk of acute rejection occurs in the first 4 weeks postoperatively.

At the current time, the addition of monoclonal antibodies whether muromonab CD3, basiliximab or daclizumab adds approximately $US3000 to $US5000 to the drug acquisition costs alone. An economic analysis has yet to be conducted examining the acquisition and administration fees for these drugs versus the savings from decreased renal dysfunction and rejection treatments. More randomised trials are needed to better elucidate the ideal dose administration and indications for these promising drugs.

5. Induction in Specific Situations

While there is no clear 'best' induction regimen for all liver transplant recipients, certain combinations may be beneficial to certain subgroups of patients (table VIII).

5.1 Renal Insufficiency

The avoidance of nephrotoxicity caused by calcineurin inhibitors motivated much of the research on induction immunosuppression and, as a result, most information is available in this subgroup of patients. A number of approaches have been suggested in order to minimise nephrotoxicity during the induction phase (table VIII). One approach is to decrease the dosage of calcineurin inhibitors and add another agent such as mycophenolate mofetil, as suggested in a retrospective study.[17]

Because of their virtual absence of nephrotoxic effects, monoclonal antibodies have long been used in patients with renal insufficiency undergoing liver transplantation. Three studies have shown a protective effect on renal function when muromonab CD3 is used during the first 14 days post-transplant.[28-30] One prospective trial has suggested that daclizumab can be used in a similar manner.[36]

Table VIII. Induction immunosuppression in specific situations

Patient subgroup	Possible induction regimens	Evidence (study type)	References
Renal insufficiency	Low-dose tacrolimus and MMF	Historical	17
	Muromonab CD3	Randomised	28-30
	Anti–IL-2R antibodies	Randomised	36
	Low-dose tacrolimus and sirolimus	Case series	55
Hepatitis C	Avoid muromonab CD3	Retrospective	56
	TAC + MMF	Retrospective	14
	Corticosteroid-free protocol	Retrospective	57-60
Fulminant hepatic failure	TAC may be preferable to CSA	Prospective	60

CSA = cyclosporin; **IL-2R** = interleukin-2 receptor; **MMF** = mycophenolate mofetil; **TAC** = tacrolimus

Finally, one of the newer anti-rejection agents, sirolimus (see section 6.1), is notable for its lack of nephrotoxic adverse effects. This promising agent has been used experimentally with great success in patients unable to tolerate calcineurin inhibitors because of nephrotoxicity. The combination of low-dose tacrolimus and sirolimus also shows much potential in this situation. The results of a multicentre trial comparing low-dose tacrolimus and sirolimus to full dose tacrolimus immunosuppression in liver transplant patients are eagerly awaited.

5.2 Hepatitis C

While it is true in general that early acute rejection does not affect the long term prognosis of liver grafts, the subgroup of patients transplanted for hepatitis C cirrhosis has been shown to be affected by acute rejection episodes and the extra immunosuppression required to treat them.[61] Whether used in the setting of induction or in order to treat acute rejection, corticosteroids and muromonab CD3 have been implicated in hepatitis C reactivation and progression.[56,57]

A number of studies have compared the use of cyclosporin versus tacrolimus in these patients, however, there does not appear to be an advantage to using one drug over the other.[62,63]

Wiesner et al.[14] documented a decreased recurrence of hepatitis C in a group treated with mycophenolate mofetil rather than azathioprine, and it was suggested that mycophenolate mofetil might have a protective 'antiviral' effect on hepatitis C. Other studies, however, suggested a worse outcome for hepatitis C liver transplant patients treated with mycophenolate mofetil.[64]

A recent prospective trial sought to resolve this debate and randomised 106 patients to receive tacrolimus and corticosteroids with or without mycophenolate mofetil.[65] In this compelling study, which included follow-up for over 4 years, no difference was seen between the two groups in terms of hepatitis activity, fibrosis scores, and graft and patient survival. On the basis of this recent study there is no evidence to support or contraindicate the use of mycophenolate mofetil in liver transplant recipients with hepatitis C.

It has been proposed that a corticosteroid-free protocol may be beneficial in patients transplanted for hepatitis C. In early studies, the use of azathioprine[58] or daclizumab,[59] or daclizumab and sirolimus[66] to substitute for corticosteroids has been associated with an acceptably low rate of rejection and may result in decreased viral replication.

5.3 Fulminant Hepatic Failure

Infection remains a major cause of death in the early post-transplant period in this group of patients. Because of the varied population and the critical presentation of this subgroup of patients, only a few trials specifically addressing the question of induction immunotherapy in these patients have been performed. It has been suggested that tacrolimus may have an advantage over cyclo-

sporin in terms of the reduced rate of infections and decreased corticosteroid requirements.[60]

6. Future Directions

With increased experience in monoclonal antibody production and use, the numbers of drugs and antibodies directed to specific targets will continue to grow, providing safer induction regimens.

6.1 Sirolimus

Sirolimus is a potent immunosuppressive drug, which was discovered over three decades ago.[67] The structure of sirolimus was elucidated and found to be similar to that of tacrolimus, however, the mechanism and adverse effect profiles of the two drugs are quite different.[68] In particular, sirolimus has no nephrotoxic or neurotoxic effects.

Much of the experience with this drug in liver transplantation comes from early studies where the drug was used because of calcineurin inhibitor toxicities. One of the first reports documented the results in 21 liver transplant recipients who received sirolimus 5 mg/day because of calcineurin inhibitor toxicity, mainly nephrotoxicity.[69] No rejection episodes were reported with a follow-up of 31 to 367 days and the drug was well tolerated.

Other studies have suggested that the best use of sirolimus may be in combination with low dose tacrolimus, such that adverse effects from either drug are minimised.[55,70,71] Although a number of studies have yielded excellent results with the use of sirolimus in renal transplantation, the drug has not yet received approval for use in liver transplant patients. Sirolimus, with its unique mechanism of action, potent immunosuppression and lack of nephrotoxicity, has the potential to become an important part of induction regimens in the future.

6.2 Antibodies

On the basis of the success of monoclonal antibodies targeted to CD3 and CD25 (IL-2 receptor), efforts have been made to find other specific targets to use for immunosuppression.[72] One promising target is the CD54 molecule [also known as intercellular adhesion molecule (ICAM-1)], an adhesion molecule involved in lymphocyte adhesion to targets or to antigen presenting cells. Results seen in a group of renal transplant patients suggest that the preparation can safely achieve a satisfactory level of immunosuppression.[73] The results of an animal study in which mice were treated with anti-CD54 are intriguing; graft survival appears to be very long, suggesting 'tolerance'. Other monoclonal antibody targets that may prove to be clinically useful include CD28[74], CD40[75] and CD154.[76]

7. Conclusions

In spite of the numbers of drugs and induction regimens available, no single combination can be advocated for all liver transplant recipients. More specific immunosuppressive drugs and safer combinations of these drugs are constantly being sought.

While mycophenolate mofetil may be used to decrease doses of calcineurin inhibitors, mycophenolate mofetil itself may have adverse effects that limit its use as well. Monoclonal antibodies are being produced to more specific and safer targets. Daclizumab and basiliximab are unique drugs in that minimal if any adverse effects have been reported thus far. Early results with these drugs suggest that they can be used safely even in patients with renal failure and that the incidence of acute rejection is low when these drugs are used in the proper combinations. Sirolimus appears promising in the setting of liver transplantation because it lacks nephrotoxic and neurotoxic effects.

As more is learned about the steps involved in rejection, an increasing number of monoclonal antibodies will be developed with more and more specific targets. In the future, improvements in the drugs and combinations in the critical early postoperative period should lead to a decrease in the early morbidity and mortality following liver transplantation.

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