Oral immunosuppressive drugs

Sujoy Khan MRCP, Immunology Specialist Registrar

WA Carrock Sewell PhD FRCP MRCPath, Consultant Immunologist and Professor of Immunology

Path Links Immunology, Scunthorpe General Hospital; University of Lincoln, Lincoln

Clin Med 2006;6:352–5

Understanding the immune network

The last few decades have witnessed remarkable achievements in the development of immunosuppressive drugs, made possible by significant advances in molecular immunology and improved understanding of the mechanisms by which cells communicate. The identification of the relevant cells involved in a pathological process is the key to understanding effective immunosuppressive strategies. For example, understanding the role of the activated $CD4^+$ T cell in cellular rejection of grafts has revolutionised transplant therapies.

Many immunosuppressive drugs are used for unlicensed indications. It is difficult to achieve a balance between adequate control of the disease process and overimmunosuppression leading to infections and malignancy. This review examines the commoner agents in use, highlighting which part of the immune system they modify, their most important side effects and monitoring strategies (Tables 1–3).

The adaptive immune system of both T and B cells relies on the non-specific uptake of antigen by antigen presenting cells (APCs) (eg monocytes and dendritic cells). Specific inhibitors of this process are not commercially available. Drugs such as minocycline[4] and chloroquine[4,5] can inhibit the processing of antigens by APCs, which is the reason for their anti-inflammatory effect.

Non-specific immunosuppressive drugs

Corticosteroids influence numerous gene transcription and post-transcriptional events and inhibit cytokine production and leukocyte migration, and thus exert anti-inflammatory and systemic effects. They are used extensively in many fields

Key Points

- **Many drugs are immunosuppressive; knowledge of how they work, combined with understanding the immunopathology of the disease, allows better selection of an effective agent**
- **Some immunosuppressive agents require blood level monitoring to reduce toxicity, but the drug level is not proportional to the degree of immunosuppression**
- **Combinations of agents are usually more effective than single agents; they reduce the incidence of toxicity by allowing the use of lower doses**
- **Excess immunosuppression may manifest as infections, but an increased incidence of malignancy should also be borne in mind**
- **Several new immunosuppressive agents are in advanced stages of development and may have novel and unexpected applications**

KEY WORDS: azathioprine, ciclosporin, mycophenolate, oral immunosuppressive drugs

Table 1. Side effect profile of common immunosuppressive agents.

Drug	Side effects/ *Special precautions*
Ciclosporin A	Very common (≥10%): nephrotoxicity, tremor, headache, ↑BP, ↑lipids Common (≥1% to <10%): fatigue, anorexia, nausea, vomiting, abdominal pain, diarrhoea, gingival hyperplasia Rare: confusion, haematuria, palpitations, paraesthesiae, myotoxicity *Creatinine >130 μmol/l: adjust dose; >180 μmol/l: withhold*
Tacrolimus	Frequent: anaemia, nephrotoxicity, neurotoxicity, hyperkalaemia, tremor, nausea, fever, diarrhoea, hypertension, hyperglycaemia Less frequent: vomiting, ascites, pancreatitis, alopecia, cardiomyopathy *ECG (or echo) for arrhythmias: dose reduction or discontinue*
Sirolimus	Common: oedema, hyperlipidaemia (worse in diabetics), hypertension Less common/rare: rash, venous thromboembolism, skin lymphoma *Monitoring of blood levels during hepatic impairment recommended*
Everolimus	Common: leukopenia, thrombocytopenia, hyperlipidaemia *Significantly increased risk of rejection at trough levels <3ng/ml* *Halve dosage in hepatic impairment*
Mycophenolate	Common: haematological (anaemia, leukopenia, thrombocytopenia), GI (nausea, vomiting, diarrhoea, haemorrhagic gastritis, erosive enterocolitis) *Fewer GI side effects with enteric-coated mycophenolate sodium* *Reduce dose in patients with decreased GFR*
Azathioprine	Dose-related bone marrow suppression, hepatic impairment, nausea, changes in hair colour/texture, pancreatitis, hepatic veno-occlusive disease (*rare*) *Prior testing with TPMT assay (phenotype or genotype) recommended*
Methotrexate	Common: GI, haematological, mucocutaneous toxicity pneumonitis (5%): stop methotrexate *Obtain baseline chest radiograph*
Cyclophosphamide	Common: nausea, vomiting, alopecia, bone marrow suppression, bladder toxicity, infertility, carcinogenicity (most are dose-dependent) *Adequate hydration (for oral and iv), mesna administration during iv bolus therapy reduces bladder toxicity*

BP = blood pressure; GFR = glomerular filtration rate; GI = gastrointestinal; iv = intravenous; TPMT = thiopurine methyltransferase.

of medicine and have been reviewed widely elsewhere.[6] Since all nucleated cells express glucocorticoid receptor, adverse effects associated with long-term use are inevitable and adequate prophylaxis to protect bone must be considered.

Metabolites of cyclophosphamide alkylate DNA bases and affect both T and B cells. Low doses of cyclophosphamide affect cell-mediated immunity more than bolus doses which tend to impair antibody responses. Cyclophosphamide bolus doses drop the B cell counts and significantly more $CD8^+$ T cells than $CD4^+$T cells.[7] Cyclophosphamide works synergistically with steroids and other immunosuppressive agents like azathioprine (AZA).

Methotrexate is a folic acid antagonist, given once weekly, which inhibits the enzyme dihydrofolate reductase. Its main effect is inhibition of DNA synthesis but it also interferes with RNA and protein synthesis and causes clonal deletion of activated T cells.[8] Methotrexate suppresses inflammatory responses through release of adenosine; this induces apoptosis of activated lymphocytes and inhibits the synthesis of purines and pyrimidines. Methotrexate is well absorbed orally, but subcutaneous, intramuscular or intravenous bioavailability is higher.

Specific immunosuppressive drugs

The advent of HIV and AIDS forcibly demonstrated the importance of the T helper cell in controlling the immune response. T cells recognise antigen presented to them by APCs using the T cell receptor (TCR). Following TCR engagement, a complex cascade of signals results in activation and proliferation of the T cell. This process is critically dependent upon the concentration of intracellular calcium.

Calcineurin inhibitors

The calcium-dependent phosphatase calcineurin is critical for diverse T cell signalling activities such as interleukin (IL)-2 production, apoptosis, cytoskeletal deployment and control of ion channels. Calcineurin inhibitors cause apoptosis of activated T cells as well as inhibiting the production of the key T cell cytokine IL-2.

Calcineurin inhibitors such as ciclosporin (CSA) and tacrolimus revolutionised transplantation surgery but are associated with significant dose-limiting side effects. Blood calcineurin inhibitor concentrations correlate with toxicity but do not directly translate into degree of immunosuppression. T cell counts and T cell proliferation assays do not correlate with immunosuppression either, although tests such as ImmuKnow[9] are being assessed and may be commercially available in the near future.

Several forms of CSA are available including microemulsions and capsules. CSA has a narrow therapeutic window, with wide inter- and intrapatient pharmacokinetics. It is essential always to prescribe CSA by brand name as there are wide differences in bioavailability between products.[10]

Mammalian target of rapamycin

T cell function is also controlled by another enzyme, mammalian target of rapamycin (mTOR), which is essential for regulating intracellular signalling through the IL-2 receptor. Inhibition of mTOR results in arrest of cell-cycle

Table 2. Therapeutic drug levels and useful laboratory tests in monitoring of immunosuppressive agents.*

Drug	Therapeutic levels	Useful laboratory tests**
Ciclosporin A	100–300 ng/l (12-hour pre-dose trough whole blood) Mark separate lumens for administration and level testing in iv regimens	U&Es (↑creat, ↑K^+, ↓Mg^{2+}, ↑uric acid), LFTs (cholestasis)
Tacrolimus	5–15ng/ml (whole blood trough levels for adult and paediatric liver and renal transplant); >30 ng/ml: toxic	U&Es (↑creat, ↑K^+, ↓Mg^{2+}), LFTs (hepatic dysfunction), ↑glucose
Sirolimus	Pre-dose (trough) blood-levels with CSA: 4–12 µg/l; pre-dose levels of CSA: 12–20 µg/l	FBC (all lineages affected), lipid profile (↑cholesterol, ↑triglycerides), U&Es (↑creat)
Everolimus	Trough: 3–8 ng/l >8 ng/l: increased toxicity[1]	FBC (anaemia, ↓platelets), lipid profile (↑cholesterol, ↑triglycerides)
Mycophenolate	Trough ≥2 mg/l: less rejection early post-transplant (cardiac allograft);[2] levels >10 mg/l: risk of side effects	FBC weekly first 4 weeks, then twice monthly for 2 months, then monthly for a year
Azathioprine	Drug levels not routinely available TPMT metabolises azathioprine; ↑risk of myelosuppression in low activity and homozygous-deficient patients	FBC weekly first 4 weeks, then 3-monthly, LFTs (cholestasis), U&Es
Methotrexate	Drug levels not routinely available	FBC, LFTs, creat every 4–8 weeks Rising procollagen III aminopeptide levels may predict hepatic fibrosis[23]
Cyclophosphamide	Drug levels not routinely available	FBC, U&Es, urinalysis every 2–4 weeks

*Drug levels may be monitored using a variety of approaches; local laboratories should be consulted regarding the timing of samples in relation to the dose and acceptable blood levels.
**Brackets denote common abnormalities.
creat = creatinine; CSA = ciclosporin; FBC = full blood count; iv = intravenous; LFT = liver function test; TPMT = thiopurine methyltransferase; U&E = urea and electrolyte.

Table 3. Effect of immunosuppressive drugs on T cells and immunoglobulin (Ig) levels.

Drug	T cells	Ig
Ciclosporin A	↓CD4 T cells	Total Igs not significantly altered, but specific antibody levels may fall
Tacrolimus	↓CD4 T cells	Total Igs not significantly altered, but specific antibody levels may fall, usually less than with ciclosporin
Sirolimus	↓CD4 T cells	Not established hypogammaglobulinaemia seen when used with mycophenolate
Everolimus	↓	Not established
Mycophenolate	↓	May cause hypogammaglobulinaemia
Azathioprine	↓	May cause severe hypogammaglobulinaemia
Methotrexate	↓(variable counts)	May cause hypogammaglobulinaemia (rare)
Cyclophosphamide	↓(CD8 more than CD4)	May cause hypogammaglobulinaemia
Glucocorticoids	↓(CD4 more than CD8)	↓IgG and IgA levels with long-term use

progression and inhibition of proliferation of T cells, B cells and vascular smooth muscle in response to cytokine signals. Two mTOR inhibitors, sirolimus (previously known as rapamycin) and everolimus, are used in various centres for transplant immunosuppression. Their side effect profile is different to that of calcineurin inhibitors, causing less nephro- and neurotoxoicity but a greater incidence of hyperlipidaemia and bone marrow suppressive effects. Both calcineurin and mTOR inhibitors can be combined, thereby reducing the dose-limiting calcineurin inhibitor side effects.[11]

A subset of T cells, $CD4^+CD25^+$ regulatory T cells (T_{REG}), play an important role in allograft tolerance; their functional activity depends on signalling via the IL-2 receptor. Rapamycin promotes apoptosis of alloreactive T cells and strongly preserves T_{REG}, in contrast to CSA, which may favour the use of rapamycin in the induction of tolerance.[12]

De novo nucleotide synthesis inhibitors

AZA is enzymatically converted and incorporated into DNA, inhibiting purine biosynthesis and proliferation of T and B lymphocytes. The major side effect of AZA is therefore myelosuppression, including leukopenia, anaemia and thrombocytopenia. These side effects are generally dose-dependent and resolve in 7–10 days with dose reduction.

Individuals with a polymorphism of the thiopurine methyl transferase enzyme (TPMT) cannot metabolise AZA effectively, leading to severe side effects. Screening for this is now possible in advance of AZA therapy by measuring TPMT activity (phenotype) or genotype. Patients with intermediate TPMT activity or who are heterozygotes need a 50% reduction in the standard dose; those with homozygous TPMT deficiency should not receive AZA or only extremely low doses.[13,14]

Mycophenolic acid inhibits inositol monophosphate dehydrogenase, preventing *de novo* purine biosynthesis in a similar way to AZA. Its function is unique in that it blocks IL-2-mediated proliferation but does not inhibit cell survival and Fas-mediated apoptosis of activated T lymphocytes.[15] Mycophenolic acid-based drugs include mycophenolate mofetil (MMF) and a newer agent, mycophenolate sodium, an enteric-coated prodrug of mycophenolic acid associated with fewer gastrointestinal side effects than MMF.[16]

Dihydroorotate dehydrogenase (DHOD) is a key enzyme in *de novo* pyrimidine synthesis. Leflunomide, FK778 (a leflunomide derivative), brequinar sodium and atovaquone are chemically distinct but all block DHOD activity.[17] Leflunomide is used as an immunomodulatory agent and is licensed as a disease-modifying anti-rheumatic drug in rheumatoid arthritis.[18] The malononitrilamide FK778 is a new class of low molecular weight immunosuppressants that block both T cell and humoral immune responses.[19]

Newer immunomodulatory agents

Thalidomide and its analogues, lenalidomide and CC-4047 (Actimid) provide immunomodulation through inhibition of tumour necrosis factor-α production in monocytes and costimulatory effects on human $CD8^+$ T cells.[20]

Mizoribine, like mycophenolate, inhibits inosine monophosphate dehydrogenase; long-term use seems to prevent renal relapses and reduce prednisolone requirement among severe proliferative lupus nephritis patients. A few anecdotal reports show mizoribine is effective in steroid-resistant nephrotic syndrome.[21]

15-Deoxyspergualin has properties similar to CSA and tacrolimus. It inhibits the activation of APCs and monocytes without affecting IL-2 synthesis.[22]

FTY 720 is a synthetic molecule structurally related to myriocin, a fungal metabolite. FTY 720 reduces the number of circulating lymphocytes, especially T helper cells, by downregulation of sphingosine receptors and redirecting them to the lymph nodes. FTY 720 has shown considerable promise in prolonging renal allograft transplant survival[23] and is undergoing trials in pancreatic islet cell transplantation for type 1 diabetes.

Practicalities of immunosuppression

Treatment with immunosuppressive therapies requires an understanding of the speed of onset of the drugs and effective monitoring systems (Table 2). Corticosteroids, acting within hours, enable rapid symptom control. Bolus dose cyclophosphamide begins to work within days. Other drugs need to be started early because their onset of action

is considerably slower. Effective blood levels of calcineurin inhibitors are reached in days, but sustained effects may be seen only after several weeks, whereas AZA may take two or three months to peak effect. As non-steroid agents begin to take effect, the steroid dose can gradually be tapered.

A good general principle is that powerful combinations of immunosuppressants (eg methyl prednisolone with cyclophosphamide) are used initially, with milder agents (eg AZA or methotrexate) replacing them once disease control is established. However, this approach may not be required in all clinical settings. Disease-specific, evidence-based guidelines should be consulted frequently as new studies emerge.

The future

Oral immunosuppressive drugs are widely used for both licensed and unlicensed indications. All physicians need to be aware of the possible uses, interactions and side effects of these drugs as patients may present acutely with infectious, malignant or other complications related to their immunosuppression.

References

1 Mabasa VH, Ensom MH. The role of therapeutic monitoring of everolimus in solid organ transplantation. *Ther Drug Monit* 2005;27:666–76.

2 Yamani MH, Starling RC, Goormastic M *et al.* The impact of routine mycophenolate mofetil drug monitoring on the treatment of cardiac allograft rejection. *Transplantation* 2000;69:2326–30.

3 Chalmers RJ, Kirby B, Smith A *et al.* Replacement of routine liver biopsy by procollagen III aminopeptide for monitoring patients with psoriasis receiving long-term methotrexate: a multicentre audit and health economic analysis. *Br J Dermatol* 2005;152:444–50.

4 Kalish RS, Koujak S. Minocycline inhibits antigen processing for presentation to human T cells: additive inhibition with chloroquine at therapeutic concentrations. *Clin Immunol.* 2004;113:270-7.

5 Accapezzato D, Visco V, Francavilla V *et al.* Chloroquine enhances human $CD8^+$ T cell responses against soluble antigens in vivo. *J Exp Med* 2005;202:817–28.

6 Boumpas DT, Chrousos GP, Wilder RL, Cupps TR, Balow JE. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. Review. *Ann Intern Med* 1993;119:1198–208.

7 Lacki JK, Mackiewicz SH, Leszczynski P, Muller W. The effect of intravenous cyclophosphamide pulse on peripheral blood lymphocytes in lupus erythematosus patients. *Rheumatol Int* 1997;17:55–60.

8 Genestier L, Paillot R, Fournel S *et al.* Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *J Clin Invest* 1998;102:322–8.

9 Hooper E, Hawkins DM, Kowalski RJ *et al.* Establishing pediatric immune response zones using the Cylex ImmuKnow assay. *Clin Transplant* 2005;19:834–9.

10 Johnston A, Belitsky P, Frei U *et al.* Potential clinical implications of substitution of generic cyclosporine formulations for cyclosporine microemulsion (Neoral) in transplant recipients. Review. *Eur J Clin Pharmacol* 2004;60:389–95.

11 Nashan B. Maximizing the clinical outcome with mTOR inhibitors in the renal transplant recipient: defining the role of calcineurin inhibitors. Review. *Transpl Int* 2004;17:279–85.

12 Coenen JJ, Koenen HJ, van Rijssen E, Hilbrands LB, Joosten I. Rapamycin, and not cyclosporin A, preserves the highly suppressive CD27+ subset of human CD4+CD25+ regulatory T cells. *Blood* 2006;107:1018–23.

13 Clunie GP, Lennard L. Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine. *Rheumatology (Oxford)* 2004;43:13–8.

14 Cara CJ, Pena AS, Sans M *et al.* Reviewing the mechanism of action of thiopurine drugs: towards a new paradigm in clinical practice. *Med Sci Monit* 2004;10: RA247–54.

15 Nakamura M, Ogawa N, Shalabi A *et al.* Positive effect on T-cell regulatory apoptosis by mycophenolate mofetil. *Clin Transplant* 2001;15(Suppl 6):36–40.

16 Behrend M, Braun F. Enteric-coated mycophenolate sodium: tolerability profile compared with mycophenolate mofetil. *Drugs* 2005;65:1037–50.

17 Hansen M, Le Nours J, Johansson E *et al.* Inhibitor binding in a class 2 dihydroorotate dehydrogenase causes variations in the membrane-associated N-terminal domain. Review. *Protein Sci* 2004;13:1031–42.

18 Li EK, Tam LS, Tomlinson B. Leflunomide in the treatment of rheumatoid arthritis. Review. *Clin Ther* 2004;26:447–59.

19 Fitzsimmons WE, First MR. FK778, a synthetic malononitrilamide. *Yonsei Med J* 2004;45:1132–5.

20 Crane E, List A. Immunomodulatory drugs. Review. *Cancer Invest* 2005;23:625–34.

21 Yokota S. Mizoribine: mode of action and effects in clinical use. Review. *Pediatr Int* 2002;44:196–8.

22 Holcombe H, Mellman I, Janeway CA Jr, Bottomly K, Dittel BN. The immunosuppressive agent 15-deoxyspergualin functions by inhibiting cell cycle progression and cytokine production following naive T cell activation. *J Immunol* 2002;169:4982–9.

23 Budde K, Schmouder RL, Brunkhorst R *et al.* First human trial of FTY720, a novel immunomodulator, in stable renal transplant patients. *J Am Soc Nephrol* 2002;13: 1073–83.