INTRODUCTION

Recognition of the importance of the Th2 lymphocyte as an orchestrator of the immune and inflammatory response associated with asthma has focused attention on the use of immunomodulator therapy. Immunomodulators that have been successfully used in other immunologically mediated diseases (e.g., methotrexate in rheumatoid arthritis; cyclosporin in transplant rejection; intravenous immunoglobulin (IVIG) in idiopathic thrombocytopenic purpura) are not currently routinely used in the therapy of asthma or allergy, either because of the lack of studies showing a consistent beneficial effect and/or the potential side effects related to therapy. Second-generation DNA-based immunomodulators have shown significant promise in animal models of asthma, but whether this potential will be borne out in human studies is at present unknown. In this chapter we review the safety and efficacy of currently available (methotrexate, cyclosporin, IVIG) and investigational (DNA-based, anti-cytokine) immunomodulators. Traditional allergen protein immunotherapy (Chapter 95) and novel immunomodulation strategies targeting adhesion molecules (Chapter 9) and chemokines (Chapter 11) are covered in other chapters.
pathways as does the parent compound. Serum methotrexate concentrations do not correlate with clinical efficacy in non-malignant conditions, whereas levels of intracellular polyglutamated methotrexate do.

Methotrexate and folic acid are structurally similar (Fig. 94.1). Methotrexate and its polyglutamated metabolites avidly bind the active site of the enzyme dihydrofolate reductase, thereby blocking the conversion of dietary folic acid to its reduced form, tetrahydrofolate. Depletion of the reduced form of folate leads to reduced synthesis of thymidine, thereby impairing DNA synthesis. Methotrexate also inhibits other enzymes relevant to nucleotide synthesis, including thymidylate synthetase. Allelic variation in the genes encoding enzymes affecting methotrexate’s uptake and metabolism, and also in various potential targets affecting their sensitivity to methotrexate, has been described. There is substantial interest in the potential for pharmacogenetic profiling as a means to enhance methotrexate’s efficacy while minimizing its toxicity.

**IMMUNOLOGIC EFFECTS**

Inhibition of cell replication may be the key therapeutic mechanism of methotrexate in neoplastic conditions. Lymphocytes divide rapidly in the course of the immune response, and thus immunosuppression may be one mechanism of action of methotrexate in various immunologic diseases. However, several observations have suggested that other mechanisms may be relevant in non-malignant conditions. For example, in contrast to its use in the treatment of malignancies, the lower doses of methotrexate commonly used in rheumatoid arthritis (RA) are infrequently associated with myelosuppression or clinical signs of systemic immunosuppression. In addition, supplementation with low doses of folic acid may obviate some of the toxicity seen in RA patients receiving methotrexate without significantly attenuating its clinical efficacy. In vivo studies have shown that methotrexate can induce various immunomodulatory effects, including effects on cellular immunity, humoral immunity, and inflammation.

Two relevant mechanisms underlie the anti-inflammatory effect of methotrexate at the doses typically used in non-malignant conditions: (1) inhibition of the intermediate step in purine metabolism catalyzed by amino-imidazole-carboxy-amido-ribonucleotide (AICAR) transformylase, leading to the increased release of adenosine; and (2) interference with transmethylation reactions, such as the methylation of homocysteine to methionine. The precise mechanisms by which AICAR inhibition results in increased extracellular concentrations of adenosine remain to be defined, but through interactions with specific receptors, adenosine exerts potent and diverse anti-inflammatory actions.

**ADVERSE EVENTS**

Methotrexate can be associated with a number of adverse effects (Table 94.1). Because dosing regimens, indications, and relevant factors such as renal function vary significantly among published reports, it is difficult to assign exact figures to the expected prevalence of these adverse effects. Many side effects, including myelosuppression, are dose-dependent and occur infrequently at the doses typically used in non-malignant diseases. Some side effects, such as pulmonary reactions, are idiosyncratic. Many side effects are more commonly seen early in the treatment course, or with changes in dose regimen. Many side effects can be attenuated by decreasing methotrexate dosages or by the supplemental use of folic acid. Although the administration of folic or folinic acid, particularly in large doses, can reduce efficacy, there appears to be consensus that the use of folic acid in low doses (e.g., approximately 1 mg/day) is a cost-effective way to reduce adverse effects in patients taking methotrexate while at the same time not substantially reducing efficacy. Moreover, supplemental folic acid reverses the increase in serum homocysteine that is observed with methotrexate therapy – a potentially important consideration for patients with other risk factors for the development of atherosclerotic disease. The potential hepatic toxicity of methotrexate may vary with factors such as frequency of dosing (weekly versus daily), comorbid
Although it is widely used as an immunomodulatory therapy in autoimmunologic rheumatic conditions, the use of methotrexate as a therapeutic agent for patients with allergic disease may be most appropriate in patients with severe asthma that has been refractory to or dependent on oral corticosteroids. In ‘corticosteroid-resistant’ asthma, methotrexate has been shown to have a modest corticosteroid-sparing effect. The greatest guidance for the use of methotrexate in non-malignant conditions comes from its use in RA. Typical weekly doses for patients with normal renal function range from 15 to 25 mg. Concurrent use of folic acid (1 mg/day) may obviate some adverse effects. Regular monitoring of selected liver function tests, blood cell counts, and renal function is useful to prevent adverse effects.

**IMMUNOPHILIN-BINDING AGENTS AND CALCINEURIN INHIBITORS: CYCLOSPORIN, TACROLIMUS, AND PIMECROLIMUS**

**PHARMACOLOGY AND METABOLISM**

Cyclosporin A (CsA) is an 11 amino acid cyclic peptide initially isolated in the 1970s from a soil organism (*Tolypocladium inflatum*) (Fig. 94.2). It was found to have substantial immunosuppressive properties, particularly inhibition of helper T-cell function. CsA blocks T cells via inhibition of calcineurin, which subsequently downregulates cytokines such as IL-2 that are critical for T-cell activation. Tacrolimus (previously referred to as FK506; Fig. 94.2), also initially isolated from a soil organism (*Tolypocladium tsukubanesis*), is structurally distinct from CsA, but shares its mechanisms of action.

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**Table 94.2 Recommendations for monitoring for methotrexate hepatic safety in patients with rheumatoid arthritis (RA).**

<table>
<thead>
<tr>
<th>A. Baseline</th>
<th>1. Tests for all patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Liver blood tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, albumin, bilirubin, serologic tests for hepatitis B and C)</td>
<td></td>
</tr>
<tr>
<td>b. Other tests (serum creatinine, complete blood count)</td>
<td></td>
</tr>
</tbody>
</table>

| B. Monitor AST, ALT, albumin at 4–8-week intervals |
| C. Perform liver biopsy if: |
| 1. Five of nine determinations of AST within a given 12-month interval (6 of 12 if performed monthly) are abnormal |
| 2. Albumin decreases below normal |

| D. If results of liver biopsy are: |
| 1. Roenigk grade I, II, or IIIa, resume methotrexate and monitor |
| 2. Roenigk grade IIIb or IV, discontinue methotrexate |

| E. Discontinue methotrexate in patients with persistent liver test abnormalities who refuse liver biopsy |

Tacrolimus and pimecrolimus have been approved for use in patients with moderate to severe atopic dermatitis. Another structurally similar compound, sirolimus (previously referred to as rapamycin), has a distinct mechanism of action and has not yet been introduced for clinical use.\(^1\)\(^5\) CsA and tacrolimus have variable pharmacokinetics and narrow therapeutic windows, making close monitoring mandatory.\(^1\)\(^6\) Monitoring of trough whole blood concentrations is standard for the use of these drugs in solid organ transplantation. Monitoring of drug concentrations is not standard in autoimmune conditions such as rheumatoid arthritis and psoriasis, partly because of the lower doses used and because of the lack of correlation of drug concentrations with relevant clinical outcomes in non-transplant diseases.\(^1\)\(^6\) The microemulsion formulation of CsA has more consistent bioavailability than with older preparations. Tacrolimus has about 100 times more immunosuppressive activity in vitro than CsA; therapeutic whole blood trough concentrations of tacrolimus are about 20 times lower than those for CsA.

CsA and tacrolimus are both available as intravenous and oral preparations. Typical intravenous doses are about one-third of the oral dose. In transplantation, doses of CsA are typically in the range of 5–8 mg/kg/day, whereas for tacrolimus a typical dose would be 0.3 mg/kg/day. In transplantation, dosing is guided by whole blood drug concentrations.

Steady-state whole blood levels of 400 ng/mL are often associated with trough concentrations of 300 ng/mL — a reasonable therapeutic target. In non-transplant conditions the doses are typically lower. These agents are lipophilic, and are highly bound to lipoproteins in the circulation. Topical tacrolimus and pimecrolimus have minimal systemic absorption, with low serum levels being detectable in approximately 20–25% of treated patients.\(^1\)\(^7\)

CsA and tacrolimus are eliminated primarily via biotransformation in the cytochrome P450 system (CYP3A) in the gut wall and liver. Impairment of hepatic function reduces elimination of metabolites, whereas renal failure has an insignificant effect. A number of other medications are also metabolized by this same pathway, and therefore drug–drug interactions may significantly affect concentrations of CsA and tacrolimus.

**IMMUNOLOGIC EFFECTS**

CsA, tacrolimus, and pimecrolimus exert their more prominent immunomodulatory effects on T-cell function. After being taken up by the cell, these agents bind to cytosolic proteins called immunophilins: CsA binds to cyclophilin, and tacrolimus to FK-binding protein (FKBP). The combination of drug and immunophilin binds to and inhibits calcineurin, a calcium–calmodulin-dependent serine/threonine phosphatase that activates various transcriptional regulatory factors (Fig. 94.3).\(^1\)\(^5\),\(^1\)\(^8\) A key target of calcineurin is nuclear factor of activated T cells (NF-AT). Upon activation, NF-AT activates the transcription of genes encoding...
ADVERSE EVENTS

Potential adverse effects occurring with oral and parenteral CsA and tacrolimus are similar (Table 94.3). One of the most common and significant adverse effects is nephrotoxicity. Whereas nephrotoxicity is seen similarly with both agents, hypertension, which can be a contributing factor in renal dysfunction, may occur less frequently with tacrolimus. Both an acute, reversible nephrotoxicity, related to alterations in intrarenal hemodynamics, as well as a chronic irreversible renal dysfunction can be seen. The arteriolar vasoconstriction relevant to the acute decrease in renal function seen with these agents may be mitigated by calcium channel blocking agents. Hypertension can be an insidious problem, even at lower doses, and may limit long-term use of these drugs.

A variety of adverse effects may occur with CsA and tacrolimus that, although not life-threatening, can be bothersome enough to preclude long-term use of these drugs. Neurologic symptoms, including headache, tremor, and dysesthesias, have been reported in up to 20% of transplant patients. Hypertrichosis, with hair growth typically on the face, arms, and shoulders, has been reported in up to 50% of transplant patients, as has gingival hyperplasia. These effects are observed less commonly among non-transplant patients receiving lower doses. Glucose intolerance, which is often compounded by the concurrent use of corticosteroids, may relate to a direct effect of these drugs on pancreatic islet cell function, and seems to be more of a problem with tacrolimus than with CsA. Cholestasis and increases in hepatic transaminase concentrations have also been observed.

As with any agents that suppress the immune response, potential side effects related to the interference with normal immunosurveillance, namely increased susceptibility to infection and malignancy, are a concern with CsA and tacrolimus. In the setting of allograft transplantation, where the situation is made more difficult because of the concurrent use of other immunosuppressants, cases of opportunistic infections have been observed. In addition, cases of lymphoproliferative disease and other malignancies have been reported among transplant patients. In non-transplant recipients, such as patients with autoimmune diseases, there does not appear to be an increased incidence of infection or malignancy related to the use of these agents.

Table 94.3 Adverse effects potentially associated with cyclosporin and tacrolimus

<table>
<thead>
<tr>
<th>Common</th>
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<tbody>
<tr>
<td>Renal insufficiency (acute and chronic)</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Hypertrichosis</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Gingival hypertrophy</td>
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<tr>
<td>Hyperuricemia</td>
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</table>

<table>
<thead>
<tr>
<th>Less common</th>
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</thead>
<tbody>
<tr>
<td>Paresthesias</td>
</tr>
<tr>
<td>Tremor</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Glucose intolerance</td>
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</table>

<table>
<thead>
<tr>
<th>Uncommon</th>
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</thead>
<tbody>
<tr>
<td>Increased risk of infection</td>
</tr>
<tr>
<td>Increased risk of lymphoproliferative diseases</td>
</tr>
<tr>
<td>Cholestasis</td>
</tr>
</tbody>
</table>

CLINICAL STUDIES IN ASTHMA AND ALLERGY

To date, the calcineurin inhibitors have been studied mainly in two allergic conditions: asthma and atopic dermatitis. In a blinded, 12-week crossover study, treatment with CsA, at an initial dose of 5 mg/kg/day, resulted in an improvement in FEV₁ of 12% compared to placebo. No attempt at corticosteroid sparing was investigated, but fewer increases in corticosteroid dose were needed among the CsA-treated patients. In a follow-up study, 39 patients with corticosteroid-dependent asthma experienced a 25% reduction in corticosteroid dose while on CsA. The potential utility of CsA in asthma would appear to be supported by studies showing that it can attenuate the allergen-induced late-phase reaction. Inhibition of the late-phase asthmatic response is associated with attenuation of allergen-induced increases in IL-5, GM-CSF, eotaxin, and eosinophilia in the airway.

Not all studies of CsA have had a positive outcome. In one study, neither improvements in lung function nor any corticosteroid-sparing effect could be demonstrated; hence CsA remains an investigational treatment in asthma. Novel routes of administration, such as nebulization, are under investigation.

T cells appear to have an important role in the initiation and maintenance of atopic dermatitis (AD), providing a rationale for the use of CsA and tacrolimus in this condition. In one double-blind, placebo-controlled, 8-week crossover study CsA significantly improved the extent and severity of AD. Of note, despite the short duration of the study, at the dose of CsA used (5 mg/kg/day), 20 of the 33 patients developed adverse effects, compared to 8 of 33 receiving placebo. Other studies have suggested that CsA may have a beneficial effect in AD, but concerns about
Toxicity remain. Because much of the relevant immune reaction driving AD takes place locally in the dermis, topical calcineurin inhibitor administration could theoretically minimize adverse effects while maintaining its therapeutic effect. A topical preparation of CsA was not effective, presumably related to poor absorption. In contrast, tacrolimus and pimecrolimus have been successfully developed as local immunomodulatory agents for the treatment of AD. Trials with both agents have shown notable improvements in signs and symptoms, with minimal toxicity (see Chapter 62).

**CURRENT RECOMMENDATIONS FOR USE IN CLINICAL PRACTICE**

Recommendations for the use of calcineurin inhibitors in clinical practice come from evidence accumulated to date in allergic diseases and extrapolation from other diseases. CsA remains an investigational agent in the treatment of asthma, in which its use is based on the wider experience with this agent in autoimmune diseases such as RA and psoriasis. In those conditions, therapy with CsA is often started with a dose of the microemulsion formulation of about 2.5 mg/kg/day, given in divided doses. Doses are typically titrated upwards in increments of 0.5 mg/kg/day at about 8-week intervals. Long-term doses higher than 4 mg/kg/day are not typically used outside the transplant setting. The utility of monitoring trough concentrations of CsA in non-transplant patients remains undefined. Longitudinal monitoring of renal function and blood pressure is required for patients using CsA or tacrolimus chronically. If the baseline creatinine increases by 30% or more, the dose should be reduced. Similarly, significant increases in blood pressure necessitate CsA dose reduction or the introduction of antihypertensive agents (i.e. calcium channel blocking agents such as felodipine, amldipine), with attention given to potential drug interactions. Patients should be monitored for signs and symptoms of infections, neurologic side effects, and other adverse effects.

The use of topical tacrolimus and pimecrolimus for atopic dermatitis has increased, and the clinical indications are reviewed in Chapter 62. At present, the greatest experience with this agent has been in patients with severe or refractory AD.

### INTRAVENOUS IMMUNOGLOBULIN

#### CLINICAL PHARMACOLOGY

In healthy individuals, following IVIG infusion there is a biphasic plasma IgG disappearance curve, with the initial phase representing distribution between body compartments and early catabolism, and the second phase representing catabolism. The half-life of IVIG ranges from 14 to 24 days in healthy individuals and is more prolonged (26–35 days) in patients with humoral immunodeficiencies.

#### IMMUNOLOGIC EFFECTS

IVIG has a variety of immunomodulating properties which are of potential benefit in immunologically mediated diseases such as immune thrombocytopenic purpura and Kawasaki syndrome. These immunologic effects include blockade of Fc receptors, anti-cytokine effects, downregulation of T- and B-cell function, inhibition of complement activation, enhanced clearance of endogenous IgG, anti-idiotype suppression, and neutralization of super antigens. Which, if any, of these effects may be useful in the treatment of asthma or allergy is currently not known. In vitro studies have shown that IVIG inhibits cytokine-dependent lymphocyte proliferation, as well as cytokine (IL-2, IL-4) production by T lymphocytes.

### Immunoglobulin E

Inhibition of IgE production by B cells is one postulated mechanism by which IVIG may exert an immunomodulating effect in asthma. This effect of IVIG is postulated to occur through co-ligation of the B cell Fcγ RIIB receptor and the B-cell antigen receptor, with resultant negative signaling in B cells. IVIG could thus provide an off signal to the B cell to inhibit B-cell proliferation and immunoglobulin production. In vitro, IVIG inhibits IgE production by B cells. In open-label studies IVIG has been shown to reduce the immediate skin reactivity to allergen. Although there is some experimental evidence to suggest that IVIG may reduce IgE levels in vivo, a reduction in IgE is not noted in the majority of studies which have investigated this immunomodulatory property of IVIG.

### Sinopulmonary infections

IVIG might theoretically improve asthma by reducing the incidence of sinopulmonary infections. Evidence for this has not adequately been addressed in current studies with IVIG.

### ADVERSE EVENTS

IVIG therapy is generally safe and well tolerated. Side effects such as nausea, headache, backache, chills, and flushing occur in approximately 5–10% of IVIG infusions (Table 94.4) and usually resolve with interruption of the infusion and resumption at a slower rate. More serious adverse events (anaphylaxis, aseptic meningitis, renal failure) related to IVIG therapy are fortunately uncommon. IgA deficiency should be suspected in individuals who develop anaphylaxis to IVIG. An explanation for why subjects who have no IgA may develop anaphylaxis following IVIG infusion is

<table>
<thead>
<tr>
<th>Table 94.4 Adverse effects potentially associated with IVIG</th>
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<tbody>
<tr>
<td><strong>Common</strong></td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Chills</td>
</tr>
<tr>
<td>Backache</td>
</tr>
<tr>
<td>Flushing</td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
</tr>
<tr>
<td>Renal insufficiency</td>
</tr>
<tr>
<td>Aseptic meningitis</td>
</tr>
<tr>
<td>Anaphylaxis</td>
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</table>
suggested from the observation that IgA-deficient individuals may develop anti-IgA IgE antibodies, as their immune system does not recognize infused IgA as a self protein. In subjects who develop a severe headache related to IVIG therapy the development of aseptic meningitis should be considered. These subjects will often have associated photophobia and a stiff neck, and their CSF will show a neutrophilic or a mixed cell pleocytosis. The induction of renal insufficiency by IVIG has also been reported and attributed to hyperosmolar renal damage induced by the breakdown of sucrose, which is used as a stabilizer in IVIG preparations.

As IVIG is derived from the pooled plasma of 3000–60000 individuals, the theoretic transmission of infectious agents requires continued vigilance. All IVIG donors are screened for HIV, hepatitis B, and hepatitis C. To date there have been no reported cases of transmission of HIV or hepatitis B following IVIG infusion. However, in the USA in 1993–1994 there were outbreaks of hepatitis C associated with certain brands of IVIG, particularly those produced by chromatography. Following the introduction of specific viral inactivation steps, no new cases of hepatitis C related to IVIG have been reported in the USA.

The theoretic potential for the transmission of Creutzfeld–Jakob disease (CJD) has also been reviewed by the FDA, NIH, and CDC, who suggest that the risk of transmission of CJD by blood products, if it exists, is considerably lower than the risk for harm to public health from withdrawal of the use of blood products. Although CJD transmission has never been documented with transfusion of these products, the prolonged incubation period and the lack of screening tests for CJD warrant continued research and surveillance for the potential transmission of this rare condition.

**CLINICAL STUDIES IN ASTHMA**

**Open-label high-dose IVIG**

Three open-label studies in the USA of high-dose IVIG (2 g/kg) in subjects with moderately severe asthma requiring daily or alternate-day prednisone have been performed. Overall, these studies demonstrate that IVIG (2 g/kg administered monthly for 6–9 months) has an oral corticosteroid-sparing effect, as in two studies subjects were able to significantly reduce their daily oral corticosteroid dosage (from 21 mg to 10 mg and from 31 mg to 5 mg) or in one study their alternate-day oral corticosteroid dosage (from 32 mg to 11 mg). Symptom scores, glucocorticoid bursts for exacerbations of asthma, and hospitalizations for asthma were all significantly reduced in subjects who received IVIG.

No change in methacholine responsiveness or response to exercise challenge was noted in subjects who received IVIG in these studies. Although the open-label IVIG studies suggest that IVIG has a corticosteroid-sparing effect, the lack of a double-blind placebo-controlled study design, and the small number of subjects studied (8–11 subjects in each study received IVIG) necessitate that these studies be interpreted and validated in the context of additional double-blind placebo-controlled studies with IVIG which have been performed.

**Double-blind studies of IVIG**

Double-blind placebo-controlled studies of IVIG have been performed in the USA and Europe. In the USA multicenter study, subjects with corticosteroid-dependent asthma were randomized to receive monthly infusions of either IVIG (2 g/kg or 1 g/kg) or placebo for 7 months. At entry into the study the mean prednisone dose in the group receiving IVIG was 16 mg/day, their mean FEV1 87% of predicted, and their mean age 16, characteristics that were not significantly different from those of the placebo group. The primary outcome measured in the study was the mean daily prednisone dose requirements (adjusted for weight) determined during the observation month preceding the institution of IVIG therapy, compared to the mean daily prednisone dose requirements determined during month 7 of the study. The mean daily prednisone dose requirements declined by 33% in the placebo group, and this reduction was not significantly different from the reduction in prednisone in the 2 g/kg IVIG group (33% reduction in prednisone), or the 1 g/kg IVIG group (39% reduction in prednisone). There was also no difference between the placebo and IVIG groups in other asthma outcomes measured, including FEV1, emergency department visits, hospitalizations, or missing days from work or school. The mean levels of IgE did not change significantly from baseline after the institution of IVIG therapy. The study was terminated prematurely as 20% (3/15) of the subjects randomized to the 2 g/kg IVIG group were hospitalized with symptoms of headache, nausea, vomiting, photophobia, and meningismus, consistent with aseptic meningitis.

IVIG was also evaluated in a European double-blind placebo-controlled study in moderately severe asthmatics (mean FEV1 77% predicted) who had a mean age of 14 years. After a 2-month stabilization phase, during which inhalation and oral corticosteroids were tapered to a minimum dose allowing effective control of asthma symptoms (mean dose of inhaled corticosteroid of 1350 μg/day; 31% of subjects on oral corticosteroids), subjects were randomized to receive IVIG (1 g/kg) or placebo monthly for 3 months. The addition of IVIG to a stable dose of inhaled and/or oral corticosteroid did not result in a significant improvement in asthma symptoms, FEV1, or airway hyperreactivity to histamine at the end of the study period. IVIG had no significant effect on IgE levels.

In contrast to the open-label studies of IVIG, the double-blind placebo-controlled studies of IVIG do not demonstrate a significant improvement in asthma symptoms, emergency department visits, hospitalizations, FEV1, airway hyperreactivity measurements, or reductions in IgE levels. In addition, the high-dose IVIG in one study was associated with a significant risk for adverse events such as aseptic meningitis.

**CURRENT RECOMMENDATIONS FOR USE IN CLINICAL PRACTICE**

Although the results of the open-label studies with IVIG in asthma are encouraging in terms of their oral corticosteroid-sparing effect, the placebo-controlled studies do not confirm that IVIG has a corticosteroid-sparing effect. The number of subjects randomized to IVIG groups in the double-blind studies (9–16 subjects per IVIG group) are small, but similar numbers of subjects have been studied in the open-label studies (8–11 subjects per IVIG group). At present IVIG is an experimental therapy for asthma. It should be evaluated further in the context of randomized, placebo-controlled clinical trials.

■ DNA-BASED THERAPIES

Several types of DNA-based therapy are currently being evaluated in the treatment of allergy and asthma. Although these therapies are similar in that they all utilize oligodeoxynucleotides, they differ in structural DNA sequences and their mechanism of action. The DNA-based therapies...
There are several potential mechanisms by which the antigen encoded by DNA vaccines are circular, extrachromosomal pieces of plasmid DNA that can be modified to carry genes of interest in the antigen coding region (e.g. dust mite allergen for allergen immunotherapy). The transcription unit includes a promoter which initiates the transcription of the antigen coding sequence. The plasmid backbone contains CpG (cytosine guanosine) motifs which bias the immune response to a Th1 response. Antibiotic resistance sequences allow for selection of the plasmid during preparation of the DNA vaccine.

**DNA VACCINES**

DNA vaccines are circular, extrachromosomal pieces of plasmid DNA that can be modified to carry genes of interest (i.e. dust mite allergen for allergen immunotherapy) (Fig. 94.4).

**Immune response**

There are several potential mechanisms by which the antigen encoded by the injected plasmid DNA vaccine is processed and presented to elicit an immune response. The subcutaneously injected plasmid DNA could be taken up by dendritic cells or somatic cells (skin and/or muscle) that then transcribe and express the encoded antigen or allergen and elicit an immune response. Alternatively, a non–antigen–presenting cell (i.e. muscle cell) injected with plasmid DNA could express and transfer the protein (cross-priming) to a professional antigen–presenting cell. The preponderance of evidence suggests that the primary immune response after DNA vaccination is mediated by dendritic cells. During cross-priming, antigen or peptides (both MHC class I and II) generated by plasmid DNA injected into somatic cells can be taken up by professional antigen–presenting cells such as dendritic cells to prime T-cell responses. Thus, somatic cells (i.e. myocytes and keratinocytes) may serve as a significant reservoir of antigen available for cross-priming, as these cells are the predominant cells transfected after DNA inoculation via muscle or skin injection, respectively. Cross-priming may serve as a mechanism to augment or maintain the immune response. DNA vaccination has generally been associated with the induction of a Th1 as opposed to a Th2 immune response. The presence of immunostimulatory DNA sequences containing a CpG motif in the plasmid backbone of the DNA vaccine are considered to be important in generating the Th1 immune response.

**Mouse models of allergy and asthma**

The use of DNA vaccines encoding an allergen to modify the Th2 immune response to that allergen has been studied extensively in mice. Both humoral and cellular immune responses to encoded allergens can be generated following intramuscular or intradermal injection of plasmid DNA encoding a specific antigen. Furthermore, studies using a rat model of asthma have demonstrated that the IgE response, histamine release into bronchoalveolar lavage fluid, and bronchial hyperreactivity following challenge with aerosolized dust mite allergen Der p1 were inhibited in rats immunized with a plasmid DNA encoding the Der p 5 allergen. The response to the DNA vaccine was antigen specific, as the rats immunized with plasmid DNA encoding the Der p 5 allergen were protected against Der p 5 allergen challenge, but not against a different allergen, i.e., ovalbumin challenge.

DNA vaccines also prevent anaphylactic reactions to peanut allergen (Ara h1) in mouse models of anaphylaxis. In these studies the DNA vaccine could be administered orally using chitin particles containing the DNA plasmid encoding Ara h1. The orally administered DNA vaccine remained functional, as assessed by its ability to inhibit Th2 immune responses as well as anaphylaxis following challenge with Ara h1. Additional studies in mice have demonstrated that DNA vaccines can significantly reduce mortality associated with anaphylaxis. Moreover, these vaccines can prevent allergic responses to birch pollen and latex allergens.

**Potential use in human allergy and asthma**

DNA vaccines in humans have been evaluated in infectious disease and in oncology, in which no serious adverse events related to injection of plasmid DNA have been reported. Although DNA vaccines alone can elicit humoral and cellular immune responses to many antigens, the immune response may be suboptimal for protection against infectious disease. At present there are no published studies of the human immune response to DNA vaccines encoding allergens. Several potential safety concerns regarding DNA vaccines will need to be addressed in all studies, including the theoretical potential for integration of the injected plasmid DNA into the host genome (with the theoretical risk of increasing the risk for malignancy), as well as the potential for triggering autoimmune responses to injected DNA. To date there is no evidence that plasmids integrate into the host genome.

**IMMUNOSTIMULATORY DNA THERAPY**

**Immune response**

An alternative method of DNA-based immunization that has received significant attention recently is the use of CpG-rich (cytosine phosphorothioate-linked guanosine DNA) immunostimulatory DNA
sequences as inhibitors of Th2 responses to antigen. Interest in this approach began when studies demonstrated that the CpG DNA motifs in *Mycobacterium bovis* BCG DNA induced interferon-γ, a cytokine produced by Th1 cells. In contrast, the immunostimulatory effect of vertebrate DNA is significantly lower than that of bacterial DNA. The reduced immunostimulatory effect of vertebrate DNA is probably related to a combination of the lower frequency of CpG DNA motifs in vertebrate compared to bacterial DNA, as well as a high frequency of cytosine methylation in vertebrate compared to bacterial DNA, which abolishes the immunostimulatory effect of vertebrate CpG DNA sequences. The DNA hexamer sequences generating the optimal Th1 adjuvant effect in mice include the motif 5′-purine–purine–CpG–pyrimidine–pyrimidine–3′, e.g. AAGCTT or GACGTC. These sequences are non-coding. Cytosine (C) methylation (CH3) abolishes the Th1 adjuvant effect of CpG DNA sequences.

**Fig. 94.5.** Cytosine–guanosine (CpG) DNA. A CpG DNA hexamer sequences that have an optimal Th1 adjuvant effect in mice include the motif 5′-purine–purine–CpG–pyrimidine–pyrimidine–3′, e.g. AAGCTT or GACGTC. These sequences are non-coding. B Cytosine (C) methylation (CH3) abolishes the Th1 adjuvant effect of CpG DNA sequences.

**Mouse models of allergy and asthma**

Several studies have demonstrated that CpG DNA can inhibit Th2 cytokine responses, as well as eosinophilic inflammation and airway hyperreactivity to methacholine in mouse models of asthma. In addition to inhibiting eosinophilia of the airway and lung parenchyma, CpG DNA also significantly inhibits blood eosinophilia, suggesting that CpG DNA exerts a significant effect on the bone marrow production and/or the release of eosinophils. The inhibition of the bone marrow production of eosinophils is associated with a significant inhibition of Th2 cell-derived cytokine production (IL-4, IL-5).

The cellular mechanism of action of CpG DNA in vivo may be more complex than that initially hypothesized based on results from in vitro studies. Although CpG DNA induces a strong Th1 response, the anti-allergic effect of CpG DNA may not be mediated by this mechanism in vivo. In support of this hypothesis are studies demonstrating that adaptive transfer of antigen-specific Th1 cells does not protect against eosinophilic airway inflammation in a mouse model of asthma, and studies demonstrating that CpG DNA can mediate its anti-allergic effect in vivo independent of the Th1 cytokines interferon-γ and IL-12. Thus, the ability of CpG DNA to induce a Th1 response and interferon-γ expression may not be essential to its inhibitory effect on allergic inflammation in vivo.

**Potential use in human allergy and asthma**

CpG-based therapeutics are currently undergoing evaluation in human clinical trials in allergy and asthma, infectious disease, and cancer. For therapeutic use, CpG DNA oligodeoxynucleotides are typically synthesized with a phosphorothioate linkage (rather than using the native phosphodiester linkage) to prevent nuclease digestion and increase the half-life of CpG. Subcutaneous administration of CpG DNA in healthy human volunteers results in high concentrations in the draining lymph node and induces high levels of serum cytokines. In contrast, intravenous administration of the same CpG DNA results in a significant dilution in the blood, binding of CpG DNA to serum proteins, and failure to induce a measurable serum cytokine response. Thus, the regional lymph nodes play an important role in the immune response to subcutaneously administered CpG DNA. The most common side effect noted with subcutaneous injection of CpG DNA is a mild local injection site reaction. Some individuals may develop transient 'flu-like' symptoms of headache, fever, myalgia, and nausea. Although induction of autoimmunity has not been noted, the duration of therapy with CpG DNA in the majority of studies has been less than 6 months; hence further studies are needed to address this issue.

At present there are limited published results of CpG DNA in subjects with allergy and asthma. In a phase I study of CpG DNA administered weekly by inhalation for 4 weeks to humans with mild asthma (FEV₁ 87% of predicted), the nebulized CpG DNA induced expression of IFN-γ in sputum cells, but did not attenuate the physiologic response to allergen inhalation challenge (early- or late-phase FEV₁), sputum eosinophils, or Th2 cytokines in sputum. Nebulized CpG DNA was well tolerated and no significant adverse events were noted. Further studies are needed to determine whether different doses of CpG DNA, different routes of CpG administration, or administration of CpG DNA in clinical asthma (as opposed to a model of allergen-induced asthma), demonstrate similar or different results.
DNA-BASED THERAPIES

The immune response to Amb a 1, the major short ragweed allergen, Recently, interest has developed in the use of allergen protein conjugated to CpG DNA for the treatment of allergic diseases, as this approach has certain theoretical advantages compared to unconjugated CpG DNA. The primary advantage of allergen protein conjugated CpG DNA is that physically linking CpG DNA to antigens would increase the likelihood of their delivery to the same antigen-presenting cell (APC), resulting in an amplified, antigen-specific Th1 immune response, which is much less likely to occur when CpG DNA and antigen are administered separately. The same APC would therefore process the antigen and present the antigen to Th cells, whereas CpG DNA would induce the APC to release IL-12, thus biasing the well-targeted Th cell to differentiate towards a Th1 phenotype. Furthermore, theoretically, the CpG DNA allergen protein conjugate might be less allergenic than the CpG DNA allergen protein mixture, as steric hindrance or electrostatic blockade of IgE-binding epitopes could occur with CpG DNA coating the allergen.

**Immune response**

The immune response to Amb a 1, the major short ragweed allergen, conjugated to CpG DNA has been investigated in mice, rabbits, monkeys, and humans. Administration of subcutaneous injections of a ragweed protein CpG DNA conjugate to ragweed-allergic individuals induced their peripheral blood mononuclear cells to express a Th1 rather than a Th2 cytokine profile. Individuals with ragweed-induced allergic rhinitis who have received immunotherapy with a ragweed protein CpG DNA conjugate respond to ragweed nasal challenge by increasing nasal mucosa Th1 cytokine production, as well as by decreasing Th2 cytokine production and nasal mucosa eosinophilia.

**Mouse models of allergy and asthma**

In a mouse model of asthma, concomitant administration of antigen conjugated to CpG DNA intratracheally prior to allergen challenge reduced airway eosinophilia and airway hyperreactivity to methacholine, downregulated the Th2 immune response, and enhanced Th1 cytokine expression. The allergen protein conjugated CpG DNA was found to be 100 times more efficient than the unconjugated mixture in reducing airway eosinophilia and bronchial hyperreactivity, with this inhibitory effect observed for at least 2 months. CpG DNA allergen protein conjugates therefore offer promise as therapy for asthma and other allergic disorders, although investigation of its immune modulating properties in humans requires further study.

**Potential use in human allergy and asthma**

In human studies a ragweed protein CpG DNA conjugate induces significantly less basophil histamine release than does ragweed alone. In a phase II clinical study of 25 individuals with allergic rhinitis due to ragweed, a short-course of immunotherapy with six weekly injections of a ragweed protein CpG DNA conjugate significantly reduced rhinitis symptom scores during the ragweed season, and these symptom improvements persisted through the second ragweed season without additional immunotherapy. Other than mild local injection site reactions the ragweed protein CpG DNA conjugate has not been associated with any significant side effects. The ability to reach a target dose of 12 μg of Amb a 1, the major ragweed allergen, without inducing a systemic allergic reaction using only six escalating dose injections of the conjugate is a significant advantage of the conjugate compared to standard immunotherapy, which requires many more injections to safely reach the maintenance dose. As some, but not all, studies have shown a clinical benefit of the ragweed protein CpG DNA conjugate in subjects with ragweed-induced allergic rhinitis, further large-scale studies are needed to determine both its effectiveness and its long-term safety.

**ANTISENSE OLIGODEOXYNUCLEOTIDE THERAPY**

The goal of antisense oligodeoxynucleotide (ODN) therapy is to selectively inhibit the expression of a specific gene product by preventing the translation of mRNA into protein (Fig. 94.6). Antisense ODN therapy acts by sequence-specific hybridization to mRNA, inhibiting the expression of that specific gene product while not affecting the expression of other genes. Antisense ODN therapy prevents the translation of the RNA message into protein and promotes the degradation of the message by ribonucleases. In order for antisense ODNs to be synthesized the coding sequence of the gene to be inhibited must be known. Antisense compounds are generally about 20 base pairs of oligodeoxynucleotides that have a sequence complementary to a portion of the targeted mRNA. The

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**Fig. 94.6.** Antisense oligodeoxynucleotides. Transcription of genes follows the sequence of DNA, mRNA, and translation into protein. Antisense oligonucleotides are designed to have complementary sequences to target and bind to specific known mRNA sequences. This binding blocks access of the mRNA/antisense hybrid to the ribosome and translation of the mRNA to protein. An enzyme Rnase H degrades the RNA strand of the mRNA/antisense hybrid.
poor stability of oligonucleotides in vivo has been improved by modifying the phosphodiester backbone to a sulfur-containing phosphorothioate backbone, which enhances the stability of oligonucleotides to resist enzymatic degradation.

### Animal models of allergy and asthma

Several gene products important to asthma and allergic inflammation have been targeted with antisense ODN in animal models of asthma. These include cell surface receptors (adenosine A1, IL-5 receptor), cytokines (IL-4, stem cell factor), intracellular signaling molecules (Syk protein tyrosine kinases), and transcription factors (GATA-3).68–70

The feasibility of using antisense therapy in asthma was first suggested in studies using antisense targeted to the adenosine A1 receptor in a rabbit model of asthma.68 Pretreatment of rabbits with inhaled adenosine A1 receptor antisense resulted in an increase in the dose of aerosolized adenosine required to reduce the dynamic compliance of the lung (a measure of bronchoconstriction) by 50%.68 Airway smooth muscle derived from rabbits treated with inhaled adenosine A1 receptor antisense demonstrated an approximately 75% decrease in the adenosine A1 receptor density, but did not affect the adenosine A2 receptor density, indicating the specificity of the targeted antisense therapy.68

GATA-3 is a transcription factor which is preferentially expressed in Th2 cells and regulates the expression of Th2 cytokine genes important to allergic inflammation. Studies with antisense targeted to GATA-3 in mouse models of asthma have demonstrated that GATA-3 antisense inhibits eosinophilic airway inflammation, airway hyperactivity, and cytokine expression, including IL-4 and IL-5.70 Targeting a transcription factor that regulates the expression of several Th2 cytokine genes is theoretically advantageous over targeting a single cytokine, although this approach has the potential for increased side effects.

Interestingly, antisense therapy to ameliorate allergic inflammation has also been targeted at grass pollen allergens to reduce the generation of pollen as opposed to targeting the allergic patient. The potential of this approach has been demonstrated in studies of antisense-mediated silencing of a gene encoding Lol p 5, a major ryegrass pollen allergen.71 A pollen-specific promoter was used to drive the antisense expression of Lol p 5 in ryegrass plants. Approximately two-thirds of the IgE reactivity of ryegrass pollen has been attributed to Lol p 5. The transgenic ryegrass pollen showed significantly reduced allergenicity, as reflected by low IgE-binding capacity of the transgenic pollen compared to that of control pollen.71 The transgenic ryegrass plants in which Lol p 5 gene expression is perturbed showed normal fertile pollen development, indicating that genetic engineering of hypoallergenic grass plants is possible.

### ALTERNATIVES TO ANTISENSE: SMALL INTERFERING RNAs (siRNA)

In 2006 the Nobel Prize in Physiology or Medicine was awarded to two scientists, Andrew Fire and Craig Mello, for their far-reaching discovery in 1998 about how genes are controlled within living cells. They discovered an unexpected system of gene regulation in living cells that resulted in a subsequent explosive phase of research in a field known variously as RNA interference or gene silencing. RNA interference may potentially lead to a new class of drugs that switch off unwanted expression of individual genes in disease.72 Two gene-silencing drugs using the RNA interference strategy have been designed to treat macular degeneration and are already in clinical trials.72

Post-transcriptional inhibition of gene expression at the mRNA level can therefore be accomplished not only by antisense-based therapy but also by small interfering RNAs (siRNAs).72 siRNAs are small double-stranded RNAs (about 21 nucleotides) designed to have complementarity to a specific single-stranded mRNA, and mediate destruction of the specific target mRNA.72 A pivotal point in mRNA silencing is the selection of the active strand of the siRNA duplex. The translation of the ability of siRNA to silence genes in cell culture in vitro and in animal models in vivo to human application still requires significant further development. In particular, improving targeting siRNAs in vivo without their degradation, as well as assessing the risks and benefits of such an approach in humans, remains problematic. siRNAs have been delivered by inhalation to the airway in mice and shown to inhibit genes such as heme oxygenase-1 in the lung, but not in other organs.73 Phase I human studies have begun to evaluate siRNA in the treatment of ocular neovascularization,72 but no human studies have yet been reported in allergy or asthma.

### Potential use in human allergy and asthma

The potential for antisense to be utilized in human disease has best been applied to cytomegalovirus (CMV) infection in subjects with AIDS. Vitravene is an antisense therapy that inhibits CMV replication and is approved for local therapy of CMV retinitis in patients with AIDS, providing conceptual proof of principle that antisense therapy can be used to treat human disease.74 At present there is no published information on the use of antisense therapy in the treatment of human allergic disease or asthma.

### MYCOBACTERIAL VACCINES

Epidemiologic studies suggest an inverse correlation between rates of tuberculosis and the prevalence of asthma in children. In Japanese schoolchildren the rate of current symptoms of asthma was reduced by approximately one–third in positive tuberculin responders. Based on epidemiologic evidence that mycobacterial exposure inhibits the development of allergy and asthma, animal model and subsequent clinical studies with heat-killed *Mycobacterium vaccae* or heat-inactivated bacillus Calmette–Guérin (BCG) have been investigated as a potential immunomodulatory therapy for asthma and allergy.

### ANIMAL MODELS OF ALLERGY AND ASThma

Intranasal infection of mice with *Mycobacterium bovis*-BCG 1–3 months prior to allergen challenge significantly inhibits the development of airway eosinophilia in a mouse model of allergen-induced asthma.75 The mycobacterium-induced inhibition of airway eosinophilia is associated with inhibition of the Th2 cytokine IL-5, but not with inhibition of IgE. The mycobacterium-induced inhibition of airway eosinophilia was significantly reduced in interferon-γ receptor-deficient mice, suggesting an important role for the Th1 cytokine IFN-γ in mediating the anti-eosinophilic effect of the mycobacterial infection.
To date, the principle that one can target a single cytokine and have a stable symptoms. Outcomes measured included the effect on asthma severity (change in morning peak flow) and immunologic response 3 months later. This study demonstrated that there was no clinical benefit or immunologic difference (eosinophil, IgE, T-cell proliferation, cytokine response) in subjects who received two intradermal doses of either of the two Mycobacterium vaccae vaccines tested compared to those who received placebo. Additional ongoing studies with Mycobacterium vaccae in asthma and allergy will determine whether the lack of benefit noted in this particular study relates to a lack of effectiveness of Mycobacterium vaccae immunization, or alternatively, relates to study design issues (route of administration, study population, outcome measure, sample size, etc.).

The effect on asthma severity of administering four weekly injections of heat-inactivated BCG has been investigated in a placebo-controlled study of 3 months’ duration in moderately severe asthmatics who were Mantoux skin test negative. The heat-inactivated BCG did not improve any of the markers of asthma severity (FEV1, peak flow, asthma exacerbations, asthma symptom scores, β-agonist use), and also had no effect on blood eosinophil and serum IgE levels. Recruitment for the trial was halted early and the number of injections was reduced in some patients owing to excessive local injection site reactions to BCG. In addition to the lack of efficacy of repeated heat-inactivated BCG injections, the occurrence of severe local injection site reactions limits the therapeutic application of this approach in asthma.

Cytokines play a key role in regulating the initiation, perpetuation, and resolution of allergic inflammation (Chapter 10). Based on studies demonstrating that several cytokines are expressed in the airway in asthmatics, novel therapeutics have been developed to target individual cytokines in patients with asthma to determine whether any of these individual cytokines may play an important role in disease pathogenesis. Pre-clinical studies in animal models of asthma have demonstrated that targeting individual cytokines such as TNF, IL-4, or IL-5 reduces levels of eosinophilic airway inflammation and airway responsiveness.

### IL-4

IL-4 mediates several important proinflammatory functions in allergic inflammation, including induction of the IgE isotype switch, induction of vascular cell adhesion molecule-1, promotion of eosinophil transmigration across the endothelium, stimulation of mucus production, and promotion of Th2 lymphocyte differentiation. The therapeutic potential of a recombinant soluble IL-4 receptor (IL-4-R) as an IL-4 antagonist has been studied in asthmatics. In two small studies, treatment with the IL-4 receptor antagonist improved asthma symptom scores and pulmonary function, reduced β2-agonist rescue use, as well as lowering levels of exhaled nitric oxide. However, subsequently two large phase III studies in moderate-to-severe asthma failed to reveal efficacy, possibly due to dose limitations and the short duration of action of the IL-4 receptor antagonist.

### IL-5

As IL-5 is a key regulator of eosinophil proliferation, studies have investigated whether targeting IL-5 would reduce eosinophilic inflammation and improve asthma outcomes. In asthmatics, therapy with an anti-IL-5
antibody has a dramatic effect in reducing blood eosinophilia (~90%), but interestingly does not inhibit the late-phase lower airway response to inhaled allergen, nor improve measures of clinical asthma, thereby raising questions about the previously held notion of the pivotal roles of IL-5 and eosinophils in allergic asthma. Subsequent studies have demonstrated that anti-IL-5 is less effective at inhibiting eosinophils in the lower airways (~50%) than in the blood (~90%), indicating that partial depletion of tissue eosinophils might be insufficient for clinical efficacy in a complex disease such as asthma. Interestingly, anti-IL-5 reduces levels of airway remodeling in asthma through inhibition of eosinophil expression of TGF-β, and improves eosinophilia and clinical responses in patients with eosinophilic esophagitis.

CONCLUSIONS

Immunomodulatory therapy has made a significant therapeutic impact in immunologically mediated diseases such as rheumatoid arthritis, but the search for safe and effective immunomodulator therapy continues in immunologically mediated diseases such as asthma and allergy. At present, however, immunomodulator therapy (other than traditional corticosteroid-sparing agents) remains investigational in the field of asthma and allergic disorders.

References

Methotrexate


Intravenous immunoglobulin


Immunophilin-binding agents and calcineurin inhibitors: cyclosporin, tacrolimus, and pimecrolimus

DNA-based therapies


Myocobacterial vaccines


Cytokine-based therapy


