*Kirk-Othmer Encyclopedia of Chemical Technology.*


# IMMUNOTHERAPEUTIC AGENTS

Advances in immunology during the last part of the twentieth century have continued at a rapid rate and cytokines and immune cells having specific markers continue to be defined. A number of natural and synthetic immunotherapeutic agents have been discovered that can modulate components of the normal or aberrant immune system, through stimulation or suppression. However, most of these substances also have inherent adverse side effects.

The immune system (1) is the primary mechanism of defense against invasive disease for human beings. Microorganisms can penetrate into the innermost parts of the body and once there, if not combatted, can cause disease. A competent immune system can recognize the molecular components of these organisms or substances released by them. These highly specific molecules, called antigens (Ag), can provoke immune responses. In addition to microorganisms, chemicals such as immunoglobulins (Igs), nucleic acids (qv), and other biopolymers (qv); eg, blood cells or plasma proteins, introduced through transfusions (see Fractionation, blood); food; and blood substitutes may be important sources of antigens. Moreover, internal tissue injuries induced physically or chemically can release antigenic material. Cell turnover, which may be accelerated under various conditions, can also lead to an increased antigen load. Many of the biopolymers released during cell death may be denatured or partially metabolized to become more antigenic. As a body's anabolic and catabolic processes fluctuate, the availability of these antigens may rise and fall with the state of health and disease. During aging, active stages of diabetes, starvation, or even a crash-diet program, deterioration of tissue components may expose the immune system to many antigens. The ability of the immune system to respond determines the difference between normalcy, ie, health, and pathology, ie, disease.

The immune system is composed of two principal components, known as limbs: the humoral immune system, primarily the domain of the B-lymphocytes; and the cell-mediated immune system, primarily the domain of the T-lymphocytes. In a normal, healthy state, regulatory mechanisms affect control so that these two limbs function in proper balance. The humoral immune system produces antibodies that react specifically with antigens; the cell-mediated immune system mobilizes the phagocytic leucocytes to ingest and destroy invading organisms or molecular material that contain or release the antigens. In the healthy state, the two limbs are regulated by complex feedback mechanisms employing mediators called cytokines and by cell-to-cell cooperation. Sudden exposure to antigens can elicit responses by the immune system such as clonal proliferation, an expansion process by which a few antigen-responsive cells can generate a large number of specific immune competent cells.

Whereas the rate and extent of an immune response may depend on the specific cells available at the moment an antigenic challenge occurs, the capacity to mount an immune response is largely determined genetically. For example, T-cell recognition of antigens, the ability of cells to present antigen, and the potential of the B-cell to produce antibodies are regulated by immune response genes. The ability to control immune responses, and thereby avoid excessive production of antibodies to self-antigens, is critical to prevention of autoimmunity.

Cytokines and antagonists (2–4), intercellular proteins produced by immune cells, play an important role in the regulation of immune responses. Cytokines are present in a variety of tissues under normal conditions.

Through insufficient or excessive production, these macromolecules can mediate chronic inflammatory diseases. An inability to respond to cytokines, eg, interleukin 1 (IL-1) or interleukin 2 (IL-2), may lead to an immunosuppressive state, whereas over-production can result in severe shock, autoimmune disease, or immunopathological conditions, such as leukemia and rheumatoid arthritis (RA). Specific communications between immune cells are constantly modulated by naturally occurring inhibitors.

The number of known cytokines, as well as the diversity of biological functions, have led to a very complex and often confusing picture of the immunologic and nonimmunologic processes involved. The role of cytokines in local or systemic homeostatic mechanisms related to physiological functions may be utilized therapeutically for treatment of cancer and a variety of other diseases (2). Pharmaceutical research and development efforts surrounding IL-1 are typical examples of the cytokine inhibition approach to chronic inflammation research (2).

T-Lymphocytes (4, 5) and other cellular components of the immune system also have equally wide implications in regulation of the normal immune system. The T-lymphocytes play a central role in the body's response to harmful antigens and tumor–host interaction (4). Responses involve antigens derived from viruses, bacteria, parasites, and tumors. T-cells also participate in the immune surveillance response, where self-antigens are recognized, but usually sequestered within the cell and, when exposed, become markers of cellular damage.

Because T-lymphocytes are capable of recognizing and destroying pathologically altered self-tissues, it may be possible to utilize T-lymphocytes for treatment of malignancy (2) or chronic and debilitating autoimmune diseases such as rheumatoid arthritis, thyroiditis, and neuromuscular diseases (5). Clinical studies for the use of T-cell lines for vaccination and treatment of autoimmune diseases, contemplated since the early 1980s, were initiated in 1990 at the Brigham and Women's Hospital (Boston, Massachusetts) (see Vaccine technology).

## 1. Immunodeficiencies

Primary immunodeficiencies are uncommon, and may occur in 1 in 10,000 individuals (6). Many primary immunodeficiencies are hereditary and congenital, and first appear in infants and children. Primary immunodeficiencies are classified into four main groups (7) relating to the lymphocytes (B-cells, T-cells, or both), phagocytes, or the complement cascade (8). Primary deficiency diseases result from B-cell defects in 50% of cases, from T-cell defects in ca 10%, and from combined B- and T-cell defects in ca 20%. Phagocytic disorders account for 18% and complement defects occur in 2% of all cases.

In severe combined immunodeficiency disease (SCID), B- and T-cell immunity are either absent or depressed. Children having SCID are susceptible to infection from almost any microorganism and often die within their first year of life, unless placed in a sterile environment. Individuals with the same primary immunodeficiency may express different signs and symptoms. In general, chronic or recurrent infections and unusual or selective infections suggest primary immunodeficiency. Quantitation of serum antibody levels and isotyping of the serum immunogammaglobin (IgG) levels are required to confirm primary deficiency disease. A deficiency in one subclass can result in signs and symptoms of immunodeficiency. Treatment consists of a maintenance dose of immune globulin intravenous (IGiv), usually 150 – 300 mg/kg every 3–4 wk. Higher frequency of dosing may be needed for some patients to maintain serum antibody levels at a concentration of 200 mg/dL. For acute infections, 500 mg/kg may be infused along with antibiotics (qv).

Secondary immunodeficiencies (9) are much more common than primary ones and frequently occur as a result of immaturity of the immune system in premature infants, immunosuppressive therapy, or surgery and trauma. Illnesses, particularly when prolonged and serious, have been associated with secondary immunodeficiencies, some of which may be reversible. Acquired immune deficiency syndrome (AIDS) (10–12) may be considered a secondary immunodeficiency disease caused by the human immunodeficiency viruses HIV-1 or

HIV-2. Hitherto unknown, the disease began to spread in the United States during the latter part of the 1970s. The agent responsible for this infection has been isolated and identified as a retrovirus.

The pathogenesis of AIDS (10, 12, 13) following HIV infection may be separated into primary and secondary effects. The primary effects are (*1*) quantitative and qualitative decreases in infected cells, ie, the $CD4^+$ T-lymphocytes; (*2*) impaired cellular immunity; (*3*) impaired immune surveillance; and (*4*) direct pathology in the central nervous system (CNS), gut, and elsewhere. Secondarily there are devastating effects of opportunistic infections and appearance of virally mediated tumors.

Gradual diminution of $CD4^+$ T-lymphocytes from the peripheral blood is the most consistent feature observed in HIV infection. Because the majority of $CD4^+$ cells are T-helper lymphocytes, removal leads to deficiency of cellular immunity, which depends on T-helper cells to initiate cytotoxic T-cell killing of virus-infected cells of cancer. The loss of immune surveillance leads to the appearance of virally induced tumors from unopposed clonal expansion of virally transformed cells. Furthermore, depletion of cellular immunity leads to exaggerated viral, fungal, and protozoal infections.

*Pneumocystis carini* pneumonia (PCP), the most common of the opportunistic infections, occurs in more than 80% of AIDS patients (13). Toxoplasmosis, a protozoan infection of the central nervous system, is activated in AIDS patients when the $CD4^+$ count drops and severe impairment of cell-mediated immunity occurs. Typically, patients have a mass lesion(s) in the brain. These mass lesions usually respond well to therapy and can disappear completely. Fungal infections, such as *Cryptococcal meningitis*, are extremely common in AIDS patients, and *Histoplasma capsulatum* appears when cell-mediated immunity has been destroyed by the HIV virus, leading to widespread infection of the lungs, liver, spleen, lymph nodes, and bone marrow. AIDS patients are particularly susceptible to bacteremia caused by nontyphoidal strains of *Salmonella*. Bacteremia may be cleared by using antibiotic therapy.

## 2. Immunotherapy for Various Disease States

### 2.1. Immunodeficiencies

Whereas elimination and neutralization of the antigen by avoidance and isolation is feasible for minor cases of allergies (14), patients with immunodeficiency are at greater health risk and require increased amount of care to maintain optimal health. Antibiotic (qv) therapy is an important modality for the treatment of immunodeficient patients and continuous prophylactic antibiotic treatment is often beneficial, especially when there is a potential for overwhelming infection, other forms of immune therapy have proven insufficient, or there is a high risk for a specific infection. The objective is the elimination of the organism or antigen as well as physiologically active substances that are not immunologically related. Antibiotics are lifesaving in treatment of infections, particularly in immunosuppressed or immunodeficient patients, and the selection and dosages used are critical.

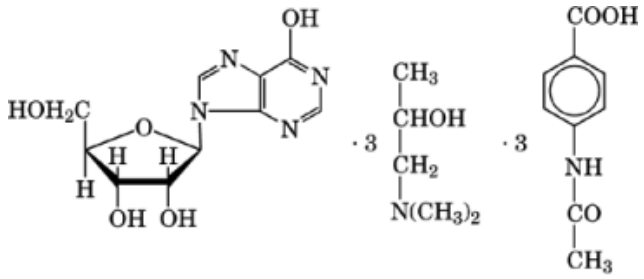
In passive immunotherapy immune globulin (Ig) is an effective replacement in most forms of antibody deficiency (14). In the past, plasma was used instead of immune globulin, but plasma is rarely indicated in the 1990s because of the risk of disease, particularly AIDS, transmission. Because plasma contains many factors in addition to immunoglobulins (Igs), plasma is, however, of particular value in patients with protein-losing enteropathy, complement deficiencies, and refractory diarrhea.

Problems associated with active immunotherapy for a faltering immune system involve identifying the requirements for appropriate immunotherapeutic agents so that both efficacy and safety can be ensured. Immunological disturbances in patients can lead to pathogenesis of many diverse problems, such as senescence, primary and acquired immunodeficiency syndromes, acute and chronic infections, autoimmune diseases, and cancer. Immunoenhancing agents may be useful where a certain degree of immune capacity is present, but are of limited value in treating cellular or phagocytic immunodeficiencies.

## 4 IMMUNOTHERAPEUTIC AGENTS

Antiviral agents (qv) (15–17) are used in attempts to combat the devastating effect of HIV on the immune system. As of this writing there are three principal approaches to the treatment of AIDS: (*1*) use of anti-HIV agents to destroy the virus or control its growth; the National Cancer Institute (NCI) encourages submission of synthetic and characterized natural products for anti-HIV screening (18); (*2*) immunotherapy to restore impaired immune functions; and (*3*) treatment of specific opportunistic infections or tumors.

The majority of antiviral agents, whether purines, pyrimidinones, amantadines, or others, were designed primarily to interfere with viral replication. Purine (adenosine, guanosine, and inosine) and pyrimidine (cytidine and thymidine) nucleosides having the dideoxy configurations of the ribose moiety nucleosides, were shown in cell cultures to control the rapid proliferation of viruses (11, 12). However, there are few virus-specific enzyme systems available as targets for appropriate chemotherapeutic intervention without concomitant adverse effects on host cellular processes. Usually antiviral chemotherapy results in a wide variety of side effects and a narrow separation between efficacy and toxicity. Some antiviral agents, eg, inosine pranohex [36703-88-5], $C_{52}H_{78}N_{10}O_{17}$ (**1**), are also immunomodulators.

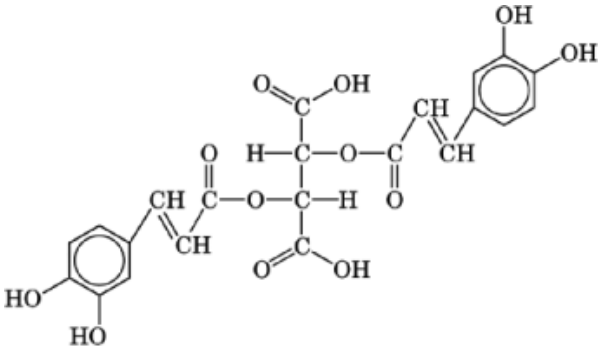


(**1**)

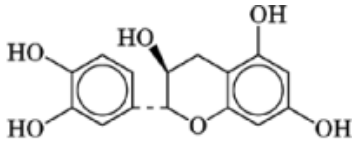
Plants and microorganisms produce unique and diverse chemical structures, some of which act as immunomodulators (18–28). Of specimens used in traditional medicine, approximately 450 plant species have shown antiviral activity out of 4000 plants screened (19). Several tannins (20) exhibit strong inhibition of tumor promotion experimentally. Pretreatment of mice with small amounts of tannins for several days strongly rejected transplanted tumors. This activity has been claimed to be effected through enhancement of host-mediated antitumor activity.

Whereas over 200 plant constituents are reported to have antiviral activity, as determined by *in vitro* methods, only 31 compounds have shown antiviral activity *in vivo* (19). Immunotherapeutic activity has not been determined.

A number of natural products exhibit immunological effects. Echinacea extracts contain a large number of constituents and the immunostimulatory activity of such extracts has been demonstrated (22). The biological activity of these extracts has warranted development of fermentation methods for large-scale production. Significant quantities of polysaccharide mixtures have been produced, leading to the isolation and availability of pure polysaccharides for biological testing. Lipophilic alkylamides and the polar caffeic acid [331-39-5] derivative, $C_9H_8O_4$, cichoric acid (**2**) (2,3-*O*-dicaffeoyltartaric acid), probably contribute to the immunostimulatory activity of the Echinacea extracts.



(**2**)

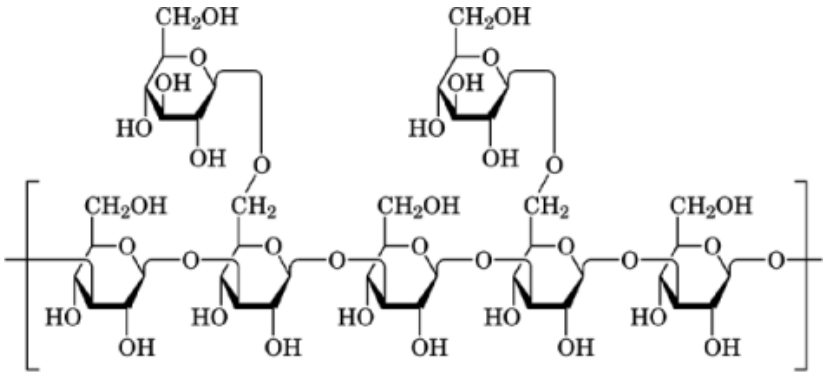


(**3**)

Cianidanol (Ci) or cathechin [154-23-4], $C_{15}H_{14}O_6$ (**3**), a flavonoid found primarily in higher woody plants (23), has been shown to have both some specific and nonspecific effects on the immune system (24). Cianidanol exerts an immunoenhancing effect on the function of various peripheral mononuclear blood cells (25). The T-cell activation by Ci at low doses stimulates the spontaneous and mitogen-induced proliferation and Ig secretion of human peripheral blood mononuclear cells. Ci is not able to stimulate B-cells directly, and T-cells are required for enhancement. The immunoenhancing properties of Ci were demonstrated (26) in a clinical study involving healthy volunteers and patients suffering from chronic liver diseases. After treatment for six months using $2-$ of Ci, the helper-to-suppressor T-lymphocyte ratio was significantly augmented. Some patients having high initial ratios showed a decrease with treatment, and T-suppressor cell concentrations were decreased at the same time. Significant clinical improvements were observed (27, 28) in a four-month-long, double-blind, multicenter trial involving 338 patients with chronic active hepatitis. The clinical improvements may be related to the immunoenhancing properties of this agent.

Ling Zhi-8 (LZ-8) is an immunomodulatory protein (29, 30) isolated from the mycelial extract of *Ganoderma lucidium*, that has been purified and shown to stimulate mouse spleen and human peripheral blood lymphocytes. LZ-8 is able to inhibit antibody production and prevent the development of autoimmune type I diabetes in NOD mice.

Polysaccharides (31–41) obtained from different sources have been shown to have immunostimulant and antitumor activity. One example is a glucan, isolated from yeast, a branched $\beta$-1,3-polyglucopyranose originally present in the yeast cell wall as a component of zymosan. It is known to be a broad-spectrum enhancer of host defense mechanisms (29–33). Immunopharmacological studies of this glucan demonstrated antitumor effects; prevention of carcinogenesis; increase in host resistance to bacterial, viral, fungal, and parasitic infections; and an increase in phagocytic and proliferative activity of the reticuloendothelial system. Lentinan [9051-97-21] (**4**), an adjuvant polysaccharide isolated from mushrooms and described as $\beta$-1,3-d-glucan having $\beta$-1,6-glucopyranoside branchings, has been shown to have immunomodulatory activity also.



(4)

Glucans have been shown to nonspecifically activate cells of the macrophage–monocyte series (34) and stimulate humoral (35) and cell-mediated immunity (36). Numerous studies have demonstrated that glucans profoundly enhance the production of colony-stimulatory factor (37) and the subsequent stimulation of diverse bone marrow progenitor cells (38, 39). In addition, treatment using glucan inhibited the growth of a variety of experimentally induced syngeneic neoplasms and enhanced survival in four syngeneic murine tumor models (40). Other studies have shown that therapeutic application of glucan increased long-term survival, inhibited metastases, decreased primary tumor weight, and maintained hepatic parenchymal cell functional integrity in animals with syngeneic reticulum cell sarcoma M5076 (41). The tumoricidal activity of splenic and peritoneal macrophage cytolytic activity against M5076 was increased after three iv doses of glucan. Clinically, glucan appears to be an immunomodulator with hematopoietic and radioprotective capabilities.

Synthetic immunomodulators (42) that have been developed are listed in Table 1. Structures are given in Figure 1. These compounds have been shown to modulate the immune system. Some also have antitumor activity. Inosiplex [36703-88-5] (**1**), also known as inosine pranobex, has been used to treat immunodeficiencies caused by cancer, radiotherapy, surgery, burns, aging, and AIDS (43). Treatment with inosiplex, for one week to several months, reduced the incidence of complications, infections, and mortality, ie, the immune status of these patients was enhanced. Natural killer cell cytotoxicity was improved; T-lymphocyte count, mitogen-induced proliferation, E rosettes, and skin test reactivity also improved. A study of immunosuppressed male homosexuals, most of whom presented clinical signs and symptoms of prodromal AIDS, indicated that the patients' depressed immune parameters returned to normal. Treatment with inosiplex was found to have a profound and lasting effect on a number of immunological parameters.

The immunorestorative potential of inosiplex has been evaluated in several clinical conditions, including post-surgical trauma, cancer patients with concurrent viral infections, and cancer patients receiving radiotherapy or chemotherapy. For example, most (84%) of the surgery patients remained immunologically depressed, but 56% of the inosiplex-treated surgery patients had complete restoration of normal skin test reactivity (probability level $< 0.0005$). The use of inosiplex as an adjuvant to chemotherapy or radiotherapy appears to be valuable in the prophylaxis against opportunistic infections.

Imuthiol 1, ditiocarb sodium, was first studied in the early 1980s (44–49) when animal data suggested that Imuthiol is a virtually nontoxic immunotherapeutic agent. It is active on the T-cell lineage and is devoid of immunosuppressive effects. This compound induces T-cells to generate enhanced levels of cytotoxic activities, lymphoproliferative responses, and IL-2 production. Imuthiol increases natural killer cell (NK) activity without effects on circulating interferon levels. Through its effects on T-cells, B-cells are induced to secrete primary antibodies of the IgG class and monocyte/macrophages to participate in delayed-type hypersensitivities, and to increase IL-1 production. Imuthiol can restore T-cell activities inhibited by cytotoxic agents. Imuthiol treatment in AKR mice, NZB mice, and virally induced diabetes or cancer has shown favorable results.

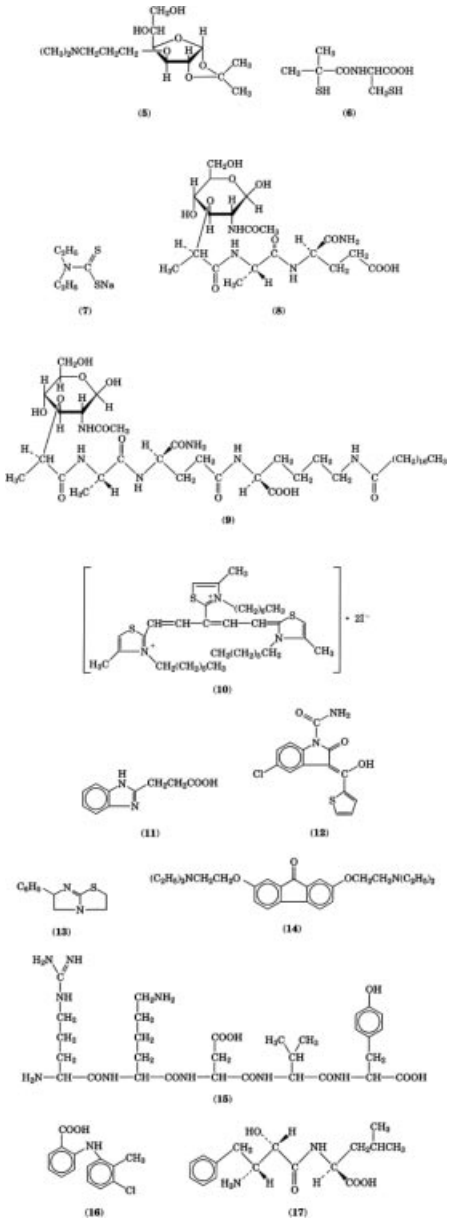


**Fig. 1.** Structures of synthetic immunomodulators. See Table 1.

Generally, clinical studies using Imuthiol have confirmed the animal results. The compound was found to be safe when administered orally or intravenously (2 – 20 mg/kg body weight) once a week for up to four years, without untoward side effects or incompatibility with other drugs. Imuthiol can restore abnormal T-helper:suppressor cell ratios, responses to T-cell mitogens, and delayed hypersensitivity. Immune restoration and beneficial effects were observed in chronic bronchitis, bronchiectasis, repeated upper respiratory infections, tuberculosis, post-surgery infections, infections in the elderly, and rheumatoid arthritis (49). Treatment

**Table 1. Synthetic Immunomodulators[a]**

| Compound | CAS Registry Number | Molecular formula | Structure number |
|---|---|---|---|
| amiprilose | [56824-20-5] | $C_{14}H_{27}NO_6$ | 1 |
| bucillamine | [65002-17-7] | $C_7H_{13}NO_3S_2$ | 1 |
| ditiocarb sodium | [148-18-5] | $C_5H_{11}NS_2{\cdot}Na$ | 1 |
| inosine pranobex | [36703-88-5] | [b] | |
| muramyl dipeptide | [53678-77-6] | $C_{19}H_{32}N_4O_{11}$ | 1 |
| muroctasin | [78113-36-7] | $C_{43}H_{78}N_6O_{13}$ | 1 |
| platonin | [3571-88-8] | $C_{38}H_{61}N_3S_3{\cdot}2I$ | 1 |
| procodazole | [23249-97-0] | $C_{10}H_{10}N_2O_2$ | 1 |
| tenidap | [120210-48-2] | $C_{14}H_9ClN_2O_3S$ | 1 |
| tetramisole | [5036-02-2] | $C_{11}H_{12}N_2S$ | 1 |
| tilorone | [27951-97-5] | $C_{25}H_{34}N_2O_3$ | 1 |
| thymopentin | [69558-55-0] | $C_{30}H_{49}N_9O_9$ | 1 |
| tolfenamic acid | [13710-19-5] | $C_{14}H_{12}ClNO_2$ | 1 |
| ubenimex | [58970-76-6] | $C_{16}H_{24}N_2O_4$ | 1 |

[a] See Figure 1.
[b] $C_{10}H_{12}N_4O_5{\cdot}3C_9H_9NO_3{\cdot}3C_5H_{13}NO$.

with Imuthiol in AIDS patients (46, 47) was followed by increased T-helper cell count and restoration of helper:suppressor cell ratios to normal. Three patients with lymphadenopathy associated syndrome were treated with Imuthiol with encouraging results. The use of Imuthiol as preventive therapy has been suggested for treatment of HIV seropositive subjects, particularly when they present a decrease in T-helper cell subset.

### 2.2. Rheumatoid Arthritis

Nonsteroidal antiinflammatory drugs (NSAIDs) are able to modulate many symptoms of chronic inflammation, but are unable to halt the underlying degenerative changes involved in rheumatoid arthritis (RA) (49–61), a common disease that affects $2 - 3 \times 10^6$ people in the United States alone (see Analgesics, antipyretics, and antiinflammatory agents). In general, there is no evidence of immunological effects for the group of classical NSAIDs; eg, NSAIDs show no significant effect on the synthesis of IL-1, and use of NSAIDs in an autoimmune disease, such as RA, has been questioned. As of this writing, however, the NSAIDs continue to be the first-line drugs for the traditional treatment of chronic inflammation, including RA. When NSAIDs are not sufficiently effective or beneficial, a number of second-line agents, known as disease-modifying agents, are available.

Steroids, also used for RA treatment, generally influence the synthesis and response to IL-1. Glucocorticoids, eg, prednisone [53-03-2] 2, can affect virtually every aspect, phase, and cell type involved in immunologic and inflammatory reactions (56). Some rheumatologists are now using immunosuppressive drugs such as methotrexate [59-05-2] 3 in early stages of RA with significant success. Antimalarials, gold compounds, penicillamine, and sulfasalazine are all used as antirheumatics. Traditional antirheumatic drugs having immunological activity are listed in Table 2 and structures are given in Figure 2. Immunosuppressive drugs that are increasingly used for treatment of severe and active RA are given in Table 3. Structures are shown in Figures 3 and 4.

Corticosteroids (60) are used to treat some patients with severe progressive RA. Low dose prednisone 2 may be a better alternative to second-line therapy for the elderly. In younger patients, disease control may be desired temporarily until second-line drugs, with a slower onset, can provide sufficient control. For patients who cannot tolerate NSAIDs or have severe systemic manifestations of RA, such a pericarditis or vasculitis, prednisone may be helpful. Intraarticular injections of corticosteroids are often helpful in treating acute inflammation of RA joints. There are many adverse side effects of corticosteroids. These include osteoporosis,

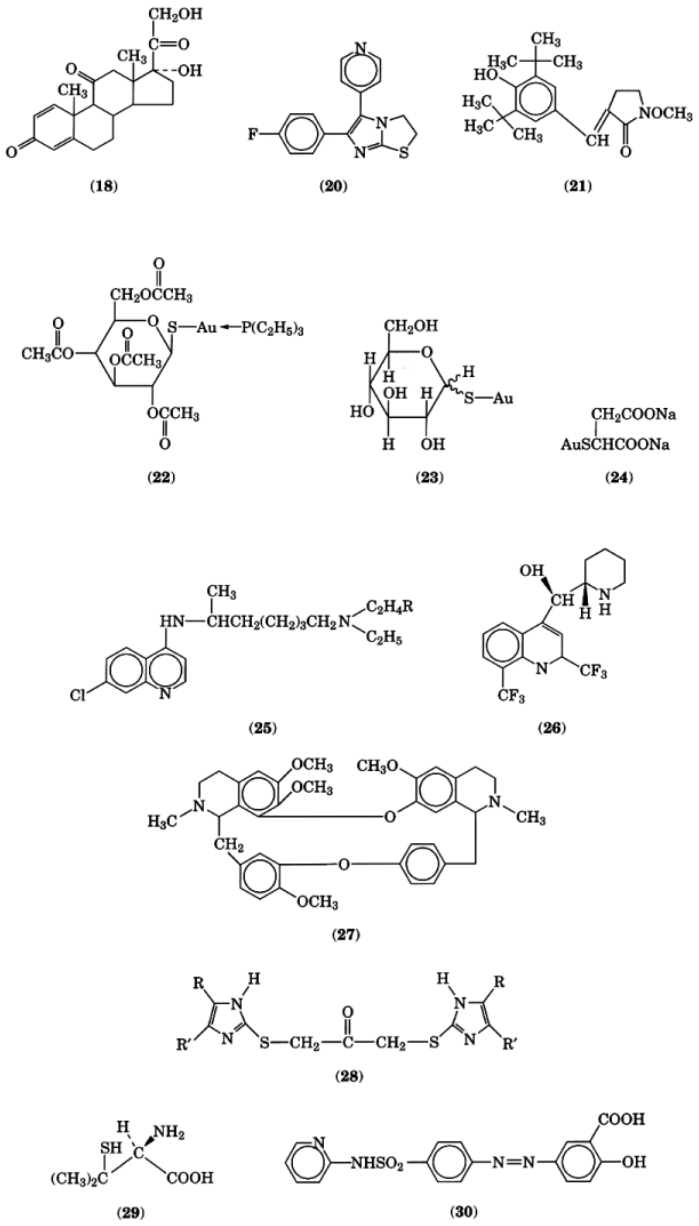


**Fig. 2.** Structures of traditional antirheumatic drugs having immunologic activity. See Table 2.

cataracts, poor healing, gastrointestinal bleeding, hyperglycemia, hypertension, and increased infection. Gastrointestinal bleeding and osteoporosis may be more severe in the elderly.

A new generation of antiinflammatory agents having immunosuppressive activity has been developed. The appearance of preclinical and clinical reports suggest that these are near entry to the pharmaceutical market. For example, tenidap (CP-66,248) 1 has been demonstrated to inhibit IL-1 production from human peripheral blood monocytes in culture (55). Clinically, IL-1 in synovial fluids of arthritic patients was reduced following

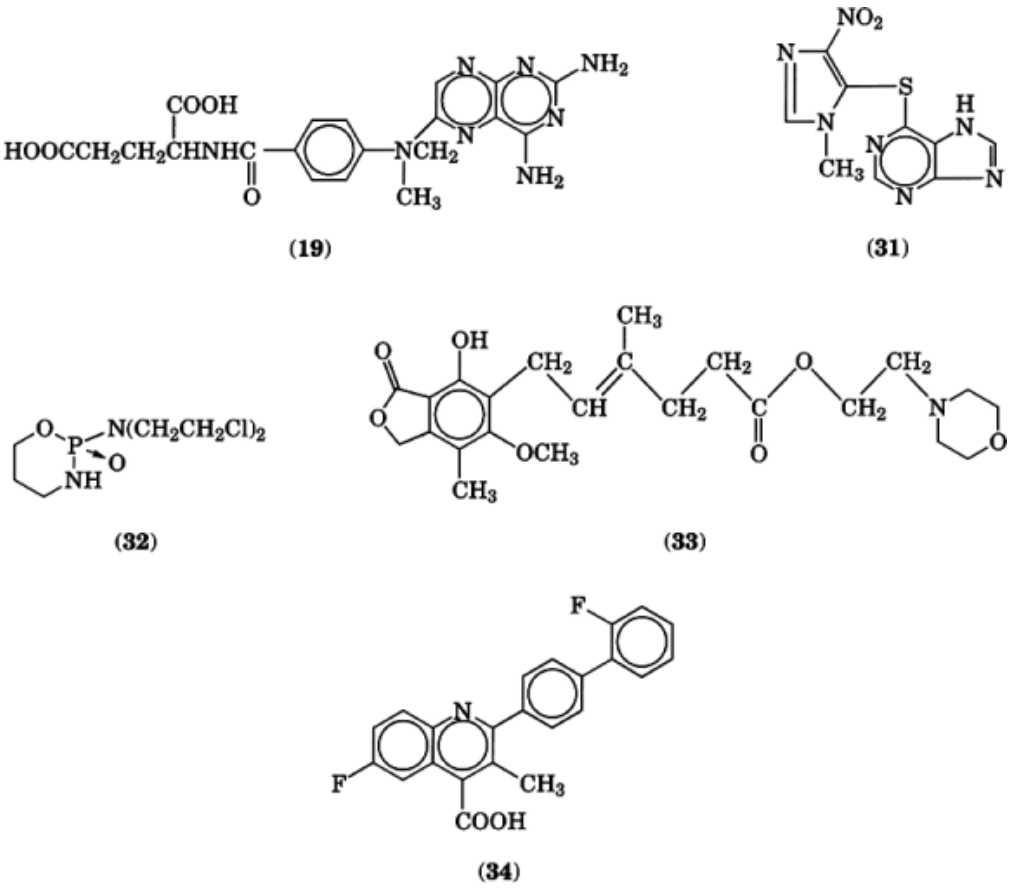



**Fig. 3.** Structures of synthetic immunosuppressive drugs. See Table 3.

treatment with tenidap. Patients with rheumatoid or osteoarthritis, when treated with tenidap, showed clinical improvement (57, 58). In addition to its immunological effects, tenidap also has an antiinflammatory profile similar to the classical NSAIDs (59). Other synthetic inhibitors of IL-1 production are SKF 86002 2 and E-5110 2 (55).

The hydroxy derivative of chloroquine (**25**, $R = H$), hydroxychloroquine [118-42-3] (**25**, $R = OH$), an antimalarial, has been shown to be effective for RA (60). Serious side effects are rare, but can include retinal damage and various skin, central nervous system, and bone marrow toxicities. Vision problems should be monitored at six-month intervals, and the drug discontinued at the first signs of renal toxicity. Higher doses cause greater risk. Mefloquine 2, another antimalarial compound of the quinoline series, and hydroxychloroquine have been shown to prevent the proteoglycan degradation in cartilage induced by IL-1 (55). Tetrandrine 2, a bisbenzylisoquinoline, can influence monocyte–macrophage functions, and has been shown to inhibit IL-1 production from human monocytes. A general generic structure 2 for IL-1 inhibitory activity has been postulated (61).

Gold compounds can be effective for active RA and may delay or prevent erosive progression of joints in some patients. Gold sodium thiomalate 2, and aurothioglucose 2 are available as injectable preparations. An oral preparation is also available, but is less effective and frequently causes diarrhea. Injectable gold is administered in 10-mg amounts as a test dose, followed by 25 mg once weekly for two weeks, then 50 mg

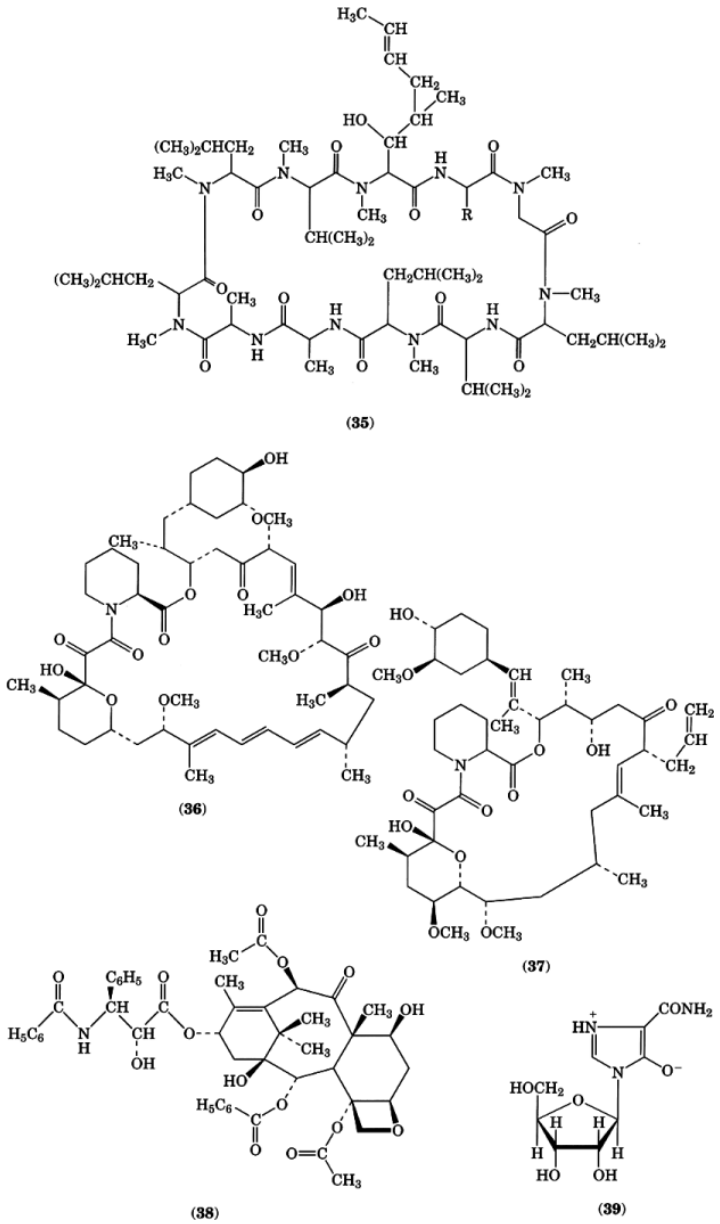



**Fig. 4.** Structures of natural immunosuppressive drugs. See Table 3.

weekly for 20 weeks. If a response occurs, treatment intervals may be lengthened to every two weeks, then three weeks, and then monthly. Patients who respond usually remain at least on monthly therapy. Discontinuation of gold therapy maintenance may result in recurrence of arthritic symptoms, which may not remit even with reinstitution of gold therapy. Auranofin 2, administered in 3-mg amounts twice daily or 6 mg once daily, should be continued for at least six months, assuming a favorable response.

**Table 2. Traditional Antirheumatic Drugs Having Immunological Activity[a]**

| Compound | CAS Registry Number | Molecular formula | Structure number |
|---|---|---|---|
| prednisone | [53-03-2] | $C_{21}H_{26}O_5$ | 2 |
| tenidap[b] | [120210-48-2] | $C_{14}H_9ClN_2O_3S$ | 2 |
| SKF 86002 | [72873-74-6] | $C_{16}H_{12}FN_3S$ | 2 |
| E-5110 | | $C_{20}H_{29}NO_3$ | 2 |
| auranofin | [34031-32-8] | $C_{20}H_{34}AuO_9PS$ | 2 |
| aurothioglucose | [12192-57-3] | $C_6H_{11}AuO_5S$ | 2 |
| gold sodium thiomalate | [24145-43-5] | $C_4H_5AuO_4S$ | 2 |
| chloroquine | [54-05-7] | $C_{18}H_{26}ClN_3$ | (**25**, $R = H$) |
| mefloquine | [53230-10-7] | $C_{17}H_{16}F_6N_2O$ | 2 |
| tetrandrine | [518-34-3] | $C_{38}H_{42}N_2O_6$ | 2 |
| general IL-1 inhibitor | | | 2 |
| D-penicillamine | [52-67-5] | $C_5H_{11}NO_2S$ | 2 |
| sulfasalazine | [599-79-1] | $C_{18}H_{14}N_4O_5S$ | 2 |

[a]See Figure 2.
[b]This drug is considered an immunomodulator. The structure appears in Figure 1.

**Table 3. Immunosuppressive Drugs[a]**

| Compound | CAS Registry Number | Molecular formula | Structure number |
|---|---|---|---|
| | *Synthetic products* | | |
| methotrexate | [59-05-2] | $C_{20}H_{22}N_8O_5$ | 3 |
| azathioprine | [446-86-6] | $C_9H_7N_7O_2S$ | 3 |
| cyclophosphamide | [50-18-0] | $C_7H_{15}Cl_2N_2O_2P$ | 3 |
| mycophenolate | [115007-34-6] | $C_{23}H_{31}NO_7$ | 3 |
| brequinar | [96187-53-0] | $C_{23}H_{15}F_2NO_2$ | 3 |
| deoxyspergualin | | | |
| | *Natural products* | | |
| cyclosporin A | [59865-13-3] | $C_{62}H_{111}N_{11}O_{12}$ | 4 |
| sirolimus | [53123-88-9] | $C_{51}H_{79}NO_{13}$ | 4 |
| FK 506 | [104987-11-3] | $C_{44}H_{69}NO_{12}$ | 4 |
| taxol | [33069-62-4] | $C_{47}H_{51}NO_{14}$ | 4 |
| mizoribine | [50924-49-7] | $C_9H_{13}N_3O_6$ | 4 |

[a]See Figures 3 and 4.

Adverse side effects of gold treatments include stomatitis, rash, and proteinuria. Complete blood counts and urinalysis should be performed before each or every other injection of gold compounds. Pruritic skin rash and stomatitis are more common adverse effects that may resolve, if therapy is withheld for a few weeks and then restarted cautiously at a lower dose. Oral gold causes less mucocutaneous, bone marrow, and renal toxicity than injectable gold, but more diarrhea and other gastrointestinal reactions appear.

Penicillamine 2 can be effective in patients with refractory RA and may delay progression of erosions, but adverse effects limit its usefulness. The most common adverse side effects for penicillamine are similar to those of parenteral gold therapy, ie, pruritic rash, protein uria, leukopenia, and thrombocytopenia. Decreased or altered taste sensation is a relatively common adverse effect for penicillamine. A monthly blood count, platelet count, and urinalysis are recommended, and also hepatic and renal function should be periodically monitored. Penicillamine is teratogenic and should not be used during pregnancy.

Sulfasalazine 3, used for many years for the treatment of inflammatory bowel disease (60), is increasingly used for RA. Sulfasalazine appears to be as effective as injectable gold and is better tolerated. Other studies (60) showed sulfasalazine was as effective as penicillamine, but not as toxic. Sulfasalazine may be more effective than hydroxychloroquine in preventing progression of joint erosion. Adverse side effects include gastrointestinal disturbances and rash, but hepatitis and blood dyscrasias are rare. Enteric coating decreases

the gastrointestinal toxicity. Monitoring for hepatitis and bone marrow suppression are recommended every 2–3 weeks during the first three months of treatment and less frequently thereafter.

Interferons (IFNs) (52, 53), a family of species-specific vertebrate proteins, confer nonspecific resistance to a broad range of viral infections, affect cell proliferation, and modulate immune responses. All three principal interferons, $\alpha$-interferon (IFN-$\alpha$) produced by blood leucocytes, $\beta$-interferon (IFN-$\beta$) by fibroblasts, and $\gamma$-interferon (IFN-$\gamma$) by lymphocytes, also have antiviral activity. The ability of interferons to inhibit growth of transplantable and carcinogen-induced tumor led to research showing the direct antiproliferative and indirect immune-mediated antitumor activities (see Chemotherapeutics, anticancer). IFNs have been found to be efficacious in certain malignancies and viral infections, eg, hairy cell leukemia (85% response) and basal cell carcinoma (86% response). However, the interferons do have adverse side effects (54).

The gene encoding IFN-$\gamma$ has been cloned and large quantities of recombinant $\gamma$-interferon (r-IFN-$\gamma$) are available for clinical testing. Preclinical *in vivo* testing of r-IFN-$\gamma$ detected no significant differences between the recombinant and natural IFNs. The lack of glycosylation does not affect the plasma half-life of r-IFN-$\gamma$. Additionally, r-IFN-$\gamma$ has important and significant *in vivo* biological effects.

Clinical trials for r-IFN-$\gamma$ in RA indicated that the drug is well tolerated (52). Consistent improvement in tender and swollen joint scores was observed, but a large number of patients were needed in the trial to show statistical significance for r-IFN-$\gamma$ treatment. In certain individuals, responses were remarkable. An additive effect between r-IFN-$\gamma$ and penicillamine was detected. Efficacy was lower when r-IFN-$\gamma$ was combined with gold therapy. Research is continuing.

Immunosuppressive agents (see Table 3), such as methotrexate 3 and azathioprine 3, are used increasingly in management of RA. These can suppress inflammation and thus allow a decrease in the doses of corticosteroids used. Oral methotrexate 3, a potent immunosuppressive, can be very effective for stopping the erosive changes associated with rheumatoid arthritis (50). The antirheumatic effect of 3 weekly, in low doses, is often apparent within 4–6 weeks of treatment and some rheumatologists consider this compound a first-line drug. Although methotrexate is often well tolerated, the drug can cause anorexia, nausea, vomiting, abdominal cramps, hepatic toxicity, bone marrow suppression, and rarely, hepatic fibrosis. Allergic pneumonitis, often severe, characterized by dry cough, fever, breathlessness, and hypoxia, occurs in 1–4% of RA patients taking methotrexate. Infections, eg, herpes zoster and *Pneumocystis carinii*, may also be more common in patients taking methotrexate.

Percutaneous liver biopsy after each 1.5 g of total accumulated methotrexate dosage to detect hepatic fibrosis or cirrhosis not reliably predicted by serum aminotransferase tests are recommended (1, 50). Concurrent use of NSAIDs may increase toxicity of methotrexate, although toxicity may be avoided if the drugs are separated by 12 h.

Azathioprine 3, a purine analogue immunosuppressive drug (51), can be as effective as gold and penicillamine in patients with refractory RA. Adverse side effects include nausea, vomiting, abdominal pain, and hepatitis. Reversible bone marrow depression can occur, but severe toxicity is uncommon with the dosage used for RA. A complete blood count, serum aminotransferase, bilirubin, and alkaline phosphatase determinations are recommended every 2–4 weeks, until the disease is under control, or adverse side effects such as cytopenia appear. Azathioprine-treated patients may have an added risk of malignancy. The drug should not be used during pregnancy.

Initially, the immunosuppressive agents, such as cyclophosphamide 3, azathioprine, and methotrexate, were developed to inhibit malignant cell proliferation. The immunosuppressant activity was discovered later and these agents were then applied to treat autoimmune diseases, where patients did not respond to high doses of steroids (51). The potential side effects associated with these agents have encouraged the search for unique immunosuppressants having more acceptable safety and efficacy profiles (62). Future approaches need to incorporate early treatment with immunotherapy (63).

### 2.3. Cancer

Cancer is a cellular malignancy characterized by loss of normal controls resulting in unregulated growth, lack of differentiation, and the ability to invade local tissues and metastasize. Most cancers are potentially curable, if detected at an early enough stage. The ideal antineoplastic agent would destroy cancer cells without adverse effects or toxicities to normal cells. No such drug exists.

Modern cancer therapy has been primarily dependent upon surgery, radiotherapy, chemotherapy, and hormonal therapy (72) (see Chemotherapeutics, anticancer; Hormones; Radiopharmaceuticals). Chemotherapeutic agents may be able to retard the rate of growth, but are unable to eradicate the entire population of neoplastic cells without significant destruction of normal host tissue. This serious side effect limits general use. More recently, the immunotherapeutic approach to cancer has involved modification and exploitation of the cellular and molecular mechanisms in host defense, regulation of tissue proliferation, tissue differentiation, and tissue survival. The results have been more than encouraging.

Natural products that are immune stimulants, eg, *Bacillus calmette guerin* (BCG), the purified protein derivative (PPD) of tuberculin, and crude lymphokine preparations, have been used to attempt regression of numerous primary or metastatic skin malignancies. This modality has also been used to treat basal cell carcinoma, mycosis fungoids, lymphangiosarcoma, reticulum cell sarcoma, and breast cancer (76). However, this local response appears to be selective for tumor cells and is relatively sparing of the normal adjacent tissues. Active nonspecific immune stimulation can be accomplished, not only using BCG, but also other intact organisms, eg, *Corynebacterium parvum*, *Bordetella pertussis*, penicillin-inactivated *Streptococcus* OK 432 endotoxin, yeast, and products extracted from yeast. It has been speculated that these nonspecific approaches may enhance specific immune responses to tumor antigens or tumor cells. However, the success of such approaches has not been reproducible.

Modern clinical immunotherapy of cancer (65) began in the 1960s with treatment of localized skin malignancies. By the 1980s human interferons were known to modulate the expression and shedding of HLA and other melanoma-associated antigens. The availability of unlimited quantities of purified interferon through recombinant DNA technology led to in-depth studies and the extensive use of interferons in the clinic. In the early 1990s immunologic treatment modalities for primary or metastatic malignant melanoma include interferons as single agents, or in combination with other cytotoxic drugs, monoclonal antibodies, and melanoma vaccines.

The concept that a weak or suppressed host immune response may be overridden by active immunization appears to be valid. Vaccines of autologous tumor extracts have been used to treat patients with malignant melanoma. Interesting and significant progress has been made in the treatment of neoplasia using interferons and interleukin-2. A particularly effective therapy for tumor patients is the infusion of lymphocyte-activated killer (LAK) cells, produced *in vitro* by the incubation of peripheral blood mononuclear cells (PBMC) from cancer patients with interleukin-2 (IL-2) (69, 70). These IL-2-induced LAK cells have been shown to lyse autologous and allogeneic tumor cells *in vitro* and *in vivo* (71, 72).

Cytokines, eg, interferons, interleukins, tumor necrosis factor (TNF), and certain growth factors, could have antitumor activity directly, or may modulate cellular mechanisms of antitumor activity (2). Cytokines may be used to influence the proliferation and differentiation of T-cells, B-cells, macrophage–monocyte, myeloid, or other hematopoietic cells. Alternatively, the induction of interferon release may represent an important approach for synthetic–medicinal chemistry, to search for effective antiinflammatory and antifibrotic agents. Inducers of interferon release may also be useful for lepromatous leprosy and chronic granulomatous disease. The potential cytokine and cytokine-related therapeutic approaches to treatment of disease are summarized in Table 4. A combination of cytokines is a feasible modality for treatment of immunologically related diseases; however, there are dangers inherent in such an approach, as shown by the induction of lethal disseminated intravascular coagulation in mice administered TNF-$\alpha$ and IFN-$\gamma$.

**Table 4. Cytokine and Cytokine-Related Therapeutic Approaches to Disease[a]**

| Cytokine/antibody[b] | Disease target | Species tested |
|---|---|---|
| IL-1 | radiation/cytotoxic injury | rodent |
| | bacterial infection | |
| TNF-$\alpha$ | autoimmune lupus nephritis | rodent |
| TNF-$\beta$ | tumor destruction | rodent |
| INFs | antiinflammatory | rodent and human |
| | immunoregulation | |
| INF-$\alpha$, $\beta$, $\gamma$ | tumor destruction, tumor and | human |
| | lymphocyte-induced angiogenesis | rodent |
| INF-$\gamma$ | rheumatoid arthritis | human |
| | lepromatous leprosy | human |
| | chronic granulomatous disease | human |
| IFN inducers | | |
| poly I:C | fibrosis; transplantation | rodent |
| tilorone | adjuvant arthritis | rodent |
| | DTH granuloma | rodent |
| IL-2$_+$ LAK cell or tumor infiltrating lymphocyte | tumor destruction | rodent and human |
| GM-CSF, G-CSF, M-CSF, multi-CSF | cytotoxic injury; bone marrow transplantation; myelodysplastic syndromes; AIDS neutropenia | rodent and human |
| CSF-1 (M-CSF) | tumor destruction | rodent |
| basic FGF (bovine) | cartilage repair | rabbit |
| GM-CSF Ab + IL-3 Ab | cerebral marlaria | rodent |
| IL-4 Ab | allergy; parasitic infection | rodent |

[a]Ref. 77.
[b]Ab = Antibody; IL = interleukin; TNF = tumornecrosis factor; INF = interferon; LAK = lymphocyte–activated killer; CSF = colony stimulating factors; and FGF = fibroblast growth factor.

The active immunotherapeutic approach is specific and based on the premise that tumor antigens are immunogenic and the host is sufficiently immunocompetent to mount an effective immune response to an autologous tumor. Theoretically, a weak or suppressed host immune system that had allowed the formation of a tumor may be overridden by active immunization or immunostimulation. In practice, vaccines composed of so-called autologous tumor extracts have been used to treat patients with malignant melanoma (73), and purified melanoma tumor-associated antigens have been used to elicit antibody responses in melanoma patients (74).

The conjugation of monoclonal antibodies (MoAbs) to radioisotopes, chemotherapeutic agents, and protein toxins has also been given consideration (65). Large amounts of human MoAbs can be produced by biotechnological means.

The combination of different therapeutic modalities, surgery, chemotherapy, or radiation, as well as a combination of different chemotherapeutic and biotechnology-engineered agents, have been used in the treatment of a number of neoplasms and led to higher rates of response (75). Investigations using biological response modifiers have produced very encouraging clinical benefits (48, 64), as seen in therapy of breast cancer, myeloma, non-Hodgkin's lymphomas, hairy cell leukemia, essential thrombocythemia, and renal cell carcinoma. Taxol 4 (see Table 3), a natural product initially isolated from the bark of the Pacific Yew tree, *Taxus breviofolia*, is an antileukemic and antitumor agent (67). It promotes assembly of microtubules and inhibits the disassembly process. The total synthesis of taxol has been achieved. Partial synthesis of derivatives using products from renewable parts of taxol sources allows preparation of a taxol-related series of antitumor agents.

Another natural product, mizoribine 4, a nucleoside antibiotic produced by the fungus *Eupenicillium brefeldianum*, has cytotoxic and immunosuppressive activity. It has been evaluated for use in renal transplantation and neoplasia (68).

### 2.4. Transplantation

Advances in surgical techniques and the availability of selective immunosuppressants, along with careful patient selection and proper post-surgery management, are factors in making transplantation the treatment of choice for organ failure. During the 1980s, the discovery of cyclosporin A 4 played a role in the significant increase in graft survival (78, 79) (see Antibiotics, peptides). But the use of transplants is still limited because of the acute immune rejection phenomenon (host vs graft reaction, or HVGR) which may destroy the transplanted tissue within days to months after the surgery. Safe and effective immunotherapeutic agents, used to control and regulate the HVGR, are crucial to the development of better transplantation methods. The primary goal is to achieve selective suppression of the recipient's immune response to the foreign antigens in the graft, ie, to attain specific immunological tolerance only to specific antigens.

Although there is no reliable method as of this writing for induction of Ag-specific unresponsiveness, some degree of tolerance has been observed by use of nonspecific immunosuppressive therapy. This conclusion is supported by a decrease in the frequency of precursor T-cells reactive with graft HLA Ags in long-term recipients of organ transplants.

Nonspecific immunosuppressive therapy in an adult patient is usually through cyclosporin 4, started intravenously at the time of transplantation, and given orally once feeding is tolerated. Typically, methylprednisone is started also at the time of transplantation, then reduced to a maintenance dose. Azathioprine 3 may also be used in conjunction with the prednisone to achieve adequate immunosuppression. Whereas the objective of immunosuppression is to protect the transplant, general or excessive immunosuppression may lead to undesirable complications, eg, opportunistic infections and potential malignancies. These adverse effects could be avoided if selective immunosuppression could be achieved. Suspected rejection episodes are treated with intravenous corticosteroids. Steroid-resistant rejection may be treated with monoclonal antibodies (78, 79) such as Muromonab-CD3, specific for the T3-receptor on human T-cells. Alternatively, antithymocyte globulin (ATG) may be used against both B- and T-cells.

A number of fungal immunosuppressives have been isolated from fermentation broths and demonstrated to have immunotherapeutic efficacy. Other than cyclosporin 4, two fungal metabolites, sirolimus 4, previously known as rapamycin (80), and FK-506 4 (81) are in various stages of development (see Antibiotics, macrolides).

Cyclosporin 4, a drug of choice in transplantation, can inhibit synthesis and release of IL-2 and other cytokines that result from interactions between an antigen presenting cell and a T-cell. Significant therapeutic effects have also been reported in patients with RA and associated autoimmune disease. Potential adverse side effects may be decreased by careful monitoring of cyclosporin blood levels, renal function parameters, liver enzymes, and blood pressure. Creative approaches to dosing, eg, every other day administrations or individualized dosing, may be very beneficial to prevent toxic effects. Upon demonstration of efficacy, gradual reduction of the cyclosporin dose reduces the frequency of cyclosporin-manifested side effects (82).

Immunosuppression induced by sirolimus 4 appears to be mediated by a mechanism distinctly different from that of either cyclosporin or FK-506. Sirolimus markedly suppresses IL-2 or IL-4-driven T-cell proliferation. The preclinical studies suggest that sirolimus is a potent immunosuppressive agent in transplantation and autoimmune disease models. The clinical potential of this agent depends on its toxicity profile (80).

FK-506 4 interferes with IL-2 synthesis and release and has a cyclosporin-like profile, but is considerably more potent *in vitro*. $IC_{50}$ values are approximately 100-fold lower. This neutral macrolide suppresses the mixed lymphocyte reaction; T-cell proliferation; generation of cytotoxic T-cells; production of T-cell derived soluble mediators, such as IL-2, IL-3, and $\gamma$-IFN; and IL-2 receptor expression (83). Structurally, FK-506 is similar to sirolimus. Mycophenolate mofetil 3, brequinar 3, and deoxyspergualin are in various phases of

clinical evaluation. Identification of therapeutic efficacy and safety are important factors in the determination of their utility as immunosuppressive agents.

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