CHAPTER 6

# IMMUNOTHERAPY OF MICROBIAL DISEASES

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## 1. INTRODUCTION

Marvels of modern medicine have apparently tamed a multitude of microbial infections. Vaccination has had a major impact in the control of important diseases including smallpox, yellow fever, polio, measles, mumps, rubella, diphtheria, tetanus, and pertusis. It is, nonetheless, enlightening to note that immunotherapeutic intervention in the form of immunization predates the postulates of infection or immunology. Edward Jenner discovered the smallpox vaccination in 1780. Since then only around 25 vaccines against various infectious diseases have been licensed and general widespread use has been restricted to about 10 vaccines. It is disconcerting that there are a vast number of diseases afflicting humans and domestic mammals for which no vaccines or specific chemotherapy will be available in the near future. In addition, infections that caused ravages in the 19th century, such as tuberculosis, are resurging with vehemence. Recent episodes of plague, diphtheria, cholera, and Ebola virus, diseases long thought to be under control, have heightened public awareness of infectious diseases.

Resistance to antibiotics and infections in immunocompromised patients would persist as problems predictably even in the next millennium. The steady progression in the longevity of the population at large will be associated with a rising number of transplantations being performed and a concurrent increase in the incidence of opportunistic infections. Extended use of immunosuppressive and cytotoxic drugs as well as diseases such as AIDS manifest opportunistic infections as one of their most

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common complication. International, governmental, and private institutions concerned with public health will have to face up to the challenge posed by existing and emerging microbial infections.

The knowledge of the intricate interaction of microbes with the immune system has engendered the realization that simple straightforward strategies may not be adequate. Antigenic analysis of the molecular structures of microbial pathogens and application of monoclonal antibodies has led to the identification of immunodominant epitopes that are germane to protection. Weak immunogens such as viral subunits, synthetic peptides, and antigenic epitopes produced by recombinant gene technology will require optimal antigen presentation and effective immunomodulators capable of potentiating protective immune responses. New concepts acting as adjunct to established therapies are urgently needed.

A wide spectrum of strategies involving immunomodulators are currently being formulated for treating infectious diseases (Masihi, 1994). The bacterial cell surface has been like a Pandora's box in yielding many immunomodulators. Active moieties have been identified for peptidoglycans of most bacterial species, cord factors of mycobacteria, endotoxic liposaccharides of gram-negative bacteria, and lipoteichoic acids of certain gram-positive bacteria. Pyrogenicity and other undesirable side effects have been observed with many bacterial immunomodulators but clinically acceptable nonpyrogenic analogues have been synthesized. This review surveys and scrutinizes the gamut of approaches being taken in developing new or improved strategies using a variety of immunomodulators for immunotherapy against infectious diseases.

## 2. IMMUNOMODULATORS IN VIRAL INFECTIONS

### 2.1. Bacterium- and Virus-Derived Immunomodulators

Viable mycobacteria, in particular the attenuated vaccine strain of bacillus Calmette–Guérin (BCG), and heat-killed or formalin-inactivated *Propionibacterium* (*Corynebacterium*) *parvum*, have been employed as nonspecific first-generation microbial immunomodulators for enhancing resistance against several viral infections. These early empirical studies have been reviewed elsewhere (Masihi *et al.*, 1989b).

One of the more promising developments has rejuvenated BCG as a vehicle for a new generation of live recombinant vaccines. New molecular genetic technology has made it feasible to introduce foreign genes into BCG. A variety of HIV type 1 polypeptides have been expressed in BCG recombinants under the control of the mycobacterial hsp70 promoter. The HIV polypeptides produced in BCG are capable of inducing antibody and T-lymphocyte responses (Aldovini and Young, 1991).

Immunomodulator *muramyl dipeptide* (MDP), *N*-acetyl-muramyl-L-alanyl-D-isoglutamine, is a small glycopeptide that represents the minimal structure essential

for mycobacterial adjuvanticity. Synthetic MDP and its analogues possess pleiotropic properties. An interesting biological activity of MDP is to enhance nonspecific resistance against microbial infections including viruses. As reviewed elsewhere (Masihi *et al.*, 1989b), MDP and certain analogues, alone or in combination with other agents, have been shown to be capable of conferring resistance against influenza, herpes simplex, Sendai, Semliki Forest, and vaccinia viruses. Mouse hepatitis virus type 3 (MHV-3) causes fatal hepatic necrosis in susceptible mice culminating in death within a matter of a few days. Hepatic necrosis liberates several enzymes that are present intracellularly within the liver into blood circulation. MDP and a non-pyrogenic analogue, Murametide, inhibited the steep elevation of serum transaminases induced by MHV-3, irrespective of whether the immunomodulators were administered before or after the infection. The histopathological examination of the liver revealed marked necrosis of the hepatic parenchymal cells and infiltration of the inflammatory cells in controls but not in MDP-treated animals (Masihi *et al.*, 1989a).

*N*-Acetylglucosaminyl-β (1–4)-*N*-acetylmuramyl tri- or tetrapeptides (GM) and lipophilic derivatives have been studied for nonspecific resistance against Sendai virus. The antiviral activity of GM derivatives was shown to increase with the chain length of the fatty acid combined with the diaminopimelyl group (Iida *et al.*, 1989). MDP-Lys(L18) (*N*-α-acetylmuramyl-L-alanyl-D-isoglutaminyl-*N*-ε-stearoyl-L-lysine) was shown to restore the resistance to HSV infection in cyclophosphamide-treated mice (Ishihara *et al.*, 1989). MDP-Lys(L18) and 6-*O*-L18-MDP(Me) could confer protection against Sendai virus infection in mice. MDP analogues 1-*O*-L18-(6-*O*-P)-MDP(Me) and 2-*N*-L18-MDP did not stimulate significant levels of cytokines or induce cytotoxic macrophages and did not protect against Sendai virus infection (Saiki *et al.*, 1988). MDP-Lys(L18) decreased murine cytomegalovirus titers in the target organs and conferred protection against systemic lethal infection (Eizuru *et al.*, 1992). MDP-Lys(L18) has been licensed in Japan under the trademark Romurtide, and has been shown to be effective for restoration of decreased neutrophils and platlets in cancer patients. The incidence of infectious diseases in the MDP-Lys(L18)-treated group has been observed to be lower than in the control groups during the clinical trials. MDP-Lys(L18) and another adjuvant, B30-MDP, were effective for the potentiation of antigenicity of Seoul-type hantaviruses strain B-1 inactivated vaccine (Azuma *et al.*, 1994) and recombinant hepatitis B virus surface antigen (Tamura *et al.*, 1995). A virosome vaccine consisting of B30-MDP, cholesterol, and influenza virus surface antigens has recently been confirmed as safe in Phase I clinical trial in humans (Azuma *et al.*, 1994). Mice administered MDP or murabutide 2 days and poly I:C 1 day prior to influenza A/Hong Kong/68 virus had reduced pulmonary virus titers and mortality (Wyde *et al.*, 1990). In earlier studies on immunomodulator-induced resistance against influenza, combination of MDP plus trehalose dimycolate (TDM) (Masihi *et al.*, 1985) and monophosphoryl lipid A (MPL) plus TDM (Masihi *et al.*, 1986b) led to a decrease in the lung virus titers on day 3 and to an earlier clearance of the virus to undetectable levels compared to controls.

An adjuvant formulation containing threonyl MDP has been shown to markedly

reduce the incidence and severity of primary herpes simplex virus infection in guinea pigs (Byars *et al.*, 1994). Another MDP analogue, MTP-PE, has been used as an adjuvant immunomodulator in a number of studies. Immunization of herpes simplex virus-infected guinea pigs with a subunit glycoprotein vaccine containing MTP-PE reduced the incidence of recurrent disease up to 80% (Burke *et al.*, 1994). Recombinant HIV envelope protein administered with MTP-PE has been shown to generate cytotoxic T lymphocytes in mice (Bui *et al.*, 1994) and has induced specific binding antibodies and lymphoproliferative responses in human volunteers (Kahn *et al.*, 1994). Influenza virus vaccine containing lipophilic MTP-PE has been reported to cause chills, fever, nausea, and transient elevation of white blood cell counts and erythrocyte sedimentation (Keitel *et al.*, 1993).

Adamantylamide dipeptide (AdDP) is a novel hybrid entity combining pertinent components of both an antiviral and an immunomodulator in a single synthetic compound (Masek *et al.*, 1984). In an innovative approach, a group of compounds were synthesized where 1 amino-amantadine moiety was linked to the essential L-alanine-D-isoglutamine portion of immunomodulator MDP. Amantadine is a primary symmetric amine with an interesting tricyclic structure that has been extensively employed in humans since 1966 for the prophylaxis and chemotherapy of influenza and Parkinson's disease.

The effect of AdDP and amantadine on the infectivity of influenza virus was investigated using the sensitive Madin–Darby canine kidney (MDCK) cells. It could be shown that 50 μg/ml of either AdDP or amantadine completely inhibited the infection and replication of influenza virus inoculated at $10^{-5}$ to $10^{-7}$ dilutions. In contrast, the same dilutions produced detectable viral hemagglutination activity in the control cultures. The efficacy of AdDP was comparable to that of amantadine and even the highest dose (150 μg/ml) that was tested did not produce toxic effects (Masihi *et al.*, 1987).

The results of preclinical studies with immunomodulator AdDP demonstrate that the homotypic immunity induced by influenza subunit vaccines can be broadened to a heterologous immune response. Subunit vaccine containing A/Sichuan(H3N2) and $Al(OH)_3$ stimulated high antibody levels. Despite the presence of high circulating antibody, animals were not protected against heterologous H1N1 influenza A/PR/8/34 infection. Mice immunized with A/Sichuan vaccine containing AdDP induced lower levels of antibody than vaccine with $Al(OH)_3$ but were significantly protected against A/PR/8/34 challenge. Similar immunization with influenza B/Beijing vaccines containing $Al(OH)_3$ or AdDP induced barely detectable antibody on day 28 but animals receiving AdDP were partially protected against A/PR/8/34 challenge. Secondary immunization greatly boosted the antibody response to A/Sichuan in animals receiving the subunits with $Al(OH)_3$, but not in the AdDP group, when compared with subunit vaccine alone. However, protection against A/PR/8/34 reached 80% in the AdDP group whereas, despite high levels of HI antibody, it did not exceed 10% in the other two groups (Masihi *et al.*, 1990a, 1992).

The stimulation of host resistance mechanisms by immunomodulators appar-

ently reduces the burden of peak amount of virus enabling the host to cope and survive. MDP analogues including murabutide, MTP-PE, and MDP-Lys(L18) are potent inducers of a variety of cytokines such as IL-1, IL-6, CSFs, TNF-α, and IFN-γ in mice and humans (Asano *et al.*, 1994; Azuma *et al.*, 1994; Bahr *et al.*, 1995).

HIV infection leads to a progressive decrease in cell-mediated immune functions which renders the patient susceptible to opportunistic infections. Common bacterial infections are increasingly being diagnosed in HIV-infected individuals. Cells of the monocyte–macrophage lineage kill invading bacterial pathogens and subsequently release immunoadjuvant components from the degraded cell walls. Since certain bacterial components possess immunomodulatory properties, an investigation of their effects on HIV-infected monocytes is of interest. MDP exhibited an inhibitory activity against HIV infection of CD4-positive H9 lymphocytes and U937 monocytoid cells. An inhibitor of viral reverse transcriptase, 2′,3′-dideoxyadenosine (ddA), produced potent inhibition in cultures that were similarly infected with HIV. MDP could partially reduce antigen production in persistently HIV-infected KE37/1 lymphocyte cultures. Although the inhibition induced by MDP on day 7 after infection did not reach the level obtained with specific retroviral reverse transcriptase inhibitor ddA, it is noteworthy that up to 67% reduction of p24 antigen could be attained. Moreover, a single application of 100 μg of MDP at the initiation of the culture containing persistently infected CD4-positive KE37/1 lymphocytes could induce an inhibition of up to 38% on day 14. The doses of MDP used in this investigation ranged from 10 to 1000 μg. The lower doses of MDP, i.e., 10 μg, were in general less effective in KE37/1 and U937 cell lines. The higher dosages were more effective and maintained their inhibitory activity even at later time points of culture (Masihi *et al.*, 1990b). Cultured monocyte-derived macrophages infected with HIV *in vitro* and treated with liposomal formulation of lipophilic MDP analogue MTP-PE were shown to have an inhibitory effect on virus production (Lazdins *et al.*, 1990). Interestingly, the MDP analogue MDP(Thr)-GDP has shown a complete lack of cellular transcription factor nuclear factor-κB (NF-κB) activation in various cell lines (Schreck *et al.*, 1992).

Cultures of lymphocytic KE37/1 cells infected with HIV and treated with AdDP or AZT inhibited production of various HIV antigens by 78 and 68%, respectively, on day 7 after infection as reflected by sensitive antigen capture ELISAs. Persistently HIV-infected KE37/1 cells cocultured with uninfected AdDP-treated or AZT-treated cells resulted respectively in 37 and 56% inhibition of p24 antigen production on day 3. Anti-HIV effects decreased to 11% inhibition by day 6 in cultures containing AZT-pretreated cells whereas in cultures containing AdDP-pretreated cells the inhibition remained stable at 38%. Inactive amounts of AdDP and AZT in the lymphocytic H9 cell line exhibited a significant synergistic effect of 60% reduction of HIV antigen production when both agents were used in combination. Treatment of monocytoid U937 cells with an inactive dose of AdDP and AZT dosage capable of inducing a 60% reduction could further increase the inhibition of HIV p24 antigen production to 92% (Masihi and Masek, 1993). A synthetic peptide derived from HIV-1 transmembrane region of glycoprotein gp41 when combined with AdDP in a liposome showed

adjuvant activity comparable to that with Freund's complete adjuvant (Turnek *et al.*, 1994). These results make AdDP a worthy candidate warranting further investigations.

Mycobacterial TDM, and detoxified endotoxin, MPL, as well as activity of antiretroviral agents, such as AZT, 2′,3′-ddA and γ-interferon, were investigated in U937- HIV infection model. Antiretrovirals strongly inhibited p24 antigen production. Immunomodulators TDM and MPL could also reduce the replication of HIV in promonocytic cells at an early stage of infection (Rohde-Schulz *et al.*, 1990). Depressed chemiluminescence activity of animals injected intravenously with peptides representing epitopes of the main structural protein of HIV core, p24 and viral envelope glycoprotein gp120 could be overcome by MPL (Pohle *et al.*, 1990).

AZT, one of the primary drugs for the treatment of HIV, is associated with bone marrow toxicity. The termination of AZT treatment results in increased levels of viral antigen and decreased numbers of $CD4^+$ cells. Recombinant GM-CSFs have been used for bone marrow salvage in immunosuppressive chemotherapy but is also associated with dose-limiting side effects that occur in the therapeutic range. *In situ* induction of physiological levels of GM-CSF and other cytokines such as IL-1 which can act synergistically would appear to be a more rational approach toward reduction of AZT toxicity. MDP is an inducer of GM-CSF and IL-1. Evaluation of the effectiveness of pretreatment with MDP or liposomal MDP-GDP showed that while MDP is able to protect against AZT-induced bone marrow toxicity at doses up to 20 mg/kg AZT, liposomal MDP-GDP confers significant myeloid protection at up to 50 mg/kg AZT (Phillips *et al.*, 1990).

Polyclonal B-cell stimulation and macrophage activation can be induced by bacterial cell wall constituents. Synthetic derivatives of N-terminal lipopeptide of bacterial lipoprotein solely consisting of palmitic acid residues are potent immunoadjuvants. Unlike Freund's complete adjuvant, lipopeptides are nonpyrogenic and do not cause tissue lesions. *Lipopeptides* bind to defined B-lymphocyte membrane proteins including MHC. Changes in the intracellular calcium concentrations obtained using lipopeptides suggest action on intracellular calcium stores or plasma membrane calcium channels without signal-transducing mechanisms involving cAMP or cGMP or phosphatidylinositol. P3C analogues can stimulate protein and RNA biosynthesis and preferentially induce IgM, IgG2(a+b), and IgG plaque-forming cells. Coupling of lipopeptides to haptens or low-molecular-weight antigens induces a marked specific antibody response which can be further enhanced by introducing haplotype-specific T-helper cell epitopes into the conjugates and *in vivo* priming of cytotoxic T cells can be achieved by coupling to epitopes presented by MHC class I molecules (Bessler *et al.*, 1994). HIV peptides covalently coupled to lipopeptides induced antigen-specific IgM and IgG antibodies. Lipopeptide conjugates of foot-and-mouth virus disease induced protection against lethal virus infection in guinea pigs (Bessler *et al.*, 1990). Covalent association of lipopeptidic lauroyl-peptides to a peptide derived from the principal neutralizing domain of HIV-1 envelope glycoprotein has been reported recently. Trimexautide was able to effi-

ciently induce a relevant virus-specific CTL response, while pimelautide was able to stimulate a strong antibody response to the linked peptide, or to a coinjected protein (Déprez *et al.*, 1995).

*Lipid A* has been identified as the active moiety exhibiting various immunopharmacological activities of bacterial lipopolysaccharide. Synthetic lipid A analogues GLA-59 and GLA-60 have been shown to confer protection against vaccinia virus infection (Ikeda *et al.*, 1990). GLA-60 could also confer complete protection to beige mice against murine CMV-associated mortality (Ikeda *et al.*, 1993). DT-5461, a synthetic lipid A-related compound, enhanced host resistance to Sendai virus infection (Yoshida *et al.*, 1994).

The novel amphiphile *BAG/LPS*, extracted from delipidated cells of *Mycobacterium bovis* BCG, has been identified in Japan. BAG/LPS is nonpyrogenic and consists mainly of sugars, such as mannose and moinositol, and fatty acids such as palmitic acid and tuberculostearic acid. Interestingly, chemical constituents such as hydroxymyristic acid, ketodeoxyoctanate, mycolic acids, muramic acids, and diaminopimelic acid which are frequently found in many bacterial immunomodulators have not been detected. BAG/LPS induced TNF and IFN-α and -β but not IFN-γ, in MDP-primed mice and showed a significant production of IL-1 in C3H/HeN mice. BAG/LPS generated tumoricidal macrophages and in combination with MDP caused hemorrhagic necrosis and complete cure in established Meth-A tumor-bearing mice (Kotani *et al.*, 1990). Effectiveness of BAG/LPS in infectious diseases such as vaccinia virus is under investigation.

Virus-derived immunomodulators have been mainly used in veterinary medicine. Inactivated poxvirus preparations such as *Baypamun* (PIND-ORF) have been shown to protect cattle against manifestation of clinical symptoms after an experimental infection with infectious bovine rhinotracheitis virus and reduce virus excretion by more than 99%; administration of Baypamun was associated with accelerated interferon synthesis (Strube *et al.*, 1989). Inactivated Newcastle disease virus mixed with endotoxin in Freund's incomplete adjuvant has been tested in field trials by treating 2782 calves and 4387 swine and comparing with a similar number of untreated animals. The clinical results showed significant reductions in the incidence and the duration of conditioned infections related to opportunistic organisms including those caused by IBR-, P13-, adeno-, and rotaviruses in the treated animals (Galassi *et al.*, 1986).

## 2.2. Fungus-Derived Immunomodulators

*Lentinan* is a chemically well-defined 1–3-β-D-glucan with 1–6-β-D-glucopyranoside branches and is isolated from an edible Japanese mushroom (Chihara, 1990; Maeda *et al.*, 1994). There are many reports on the antitumor activity of lentinan. The focus is now shifting to possible antiinfectious activities of lentinan and related yeast glucan, sulfated lentinan, and curdlan. The ability of polysaccharide immunomodulator lentinan to stimulate nonspecific resistance against respiratory

viral infections was investigated. Significant protection was conferred by lentinan administered intranasally before lethal influenza virus infection and could be corroborated by a reduction of the lung virus titers. Since the lung is the target organ of influenza virus infection, lentinan was also administered by the intravenous route. Lentinan conferred complete protection against an $LD_{75}$ challenge dose of virulent influenza virus and significantly prolonged the survival time after an $LD_{100}$ challenge (Irinoda *et al.*, 1992). Enhanced chemiluminescence (CL) activity was present at an early stage in groups receiving lentinan. Significant nitric oxide activity could also be stimulated by culturing bronchoalveolar macrophages in the presence of lentinan. TNF actvity could not be detected in lung lavage but measurable IL-6 was produced already after 6 hr in animals administered lentinan alone and in lentinan-pretreated influenza virus-infected mice (Irinoda *et al.*, 1992).

Yeast *glucan* has been shown to enhance resistance against herpes simplex virus types I and II, and murine hepatitis virus (Chihara, 1990). Polysaccharide schizophyllan conferred protection to mice against lethal Sendai virus infection (Hotta *et al.*, 1993).

Natural polysaccharides possessing antitumor activity through host-mediated reaction were not effective against HIV. For instance, lentinan enhanced the inhibitory activity against HIV only in combination with AZT (Kaneko *et al.*, 1990). In contrast, sulfated polysaccharides exerted direct inhibitory activity on HIV replication but were not active as antitumor agents. Lentinan sulfate, arabinosyl curdlan sulfate, and galactosyl curdlan sulfate exhibited significant inhibitory activity against HIV (Yoshida *et al.*, 1989). Curdlan sulfate may exert inhibitory effect on HIV-1 infection by delaying the events that precede and/or include reverse transcription and by interfering with the membrane fusion process (Jagodzinski *et al.*, 1994).

*AM3*, a polysaccharide immunomodulator, given orally for 1 year to patients with chronic active hepatitis could clear serum HBV-DNA and HBeAg in 8 of 13 patients (Villarrubia *et al.*, 1992). AM3 was among the immunomodulators found to be capable of preventing death and other disease manifestations related to Punta Toro virus, a phlebovirus related to Rift Valley fever virus (Sidwell *et al.*, 1992) and against disease induced by Friend virus complex (Sidwell *et al.*, 1993). Recently, AM3 has been used as an adjuvant to hepatitis B revaccination in nonresponder healthy persons (Sánchez *et al.*, 1995)

## 2.3. Synthetic Compounds as Immunomodulators

Immunotherapy of AIDS-related complex (ARC) and asymptomatic HIV infection with a variety of immunomodulators has been competently reviewed elsewhere (Hadden, 1992).

A new synthetic purine immunomodulator, *5'-methyl inosine monophosphate* (MIMP), is a thymomimetic immunomodulator capable of inducing in human prothymocytes the expression of T-lymphocyte differentiation markers (CD3, CD4, CD8) and IL-2 receptor (CD25). MIMP has been shown to enhance mitogen-induced proliferation of lymphocytes, augment IgM plaque-forming cells, induce delayed-

type hypersensitivity, and normalize an impaired response to IL-2. Depressed phytohemagglutinin responses of lymphocytes suppressed by an HIV-derived peptide, IFN-α, prostaglandin $PGE_2$, or lymphocytes from pre-AIDS (ARC) patients could be progressively restored by MIMP. The mean day death in mice infected with Friend leukemia virus, employed as a murine model of AIDS, could be significantly delayed by MIMP (Hadden *et al.*, 1991; Sosa *et al.*, 1994)).

*Inosine pranobex* (isoprinosine) has been shown to inhibit replication of echo-, rhino-, polio-, adeno-, herpes, and cytomegaloviruses and has been shown in two multicenter clinical trials to delay the progression of HIV infection to AIDS when $CD4^+$ counts are greater than 400 (reviewed in De Simone *et al.*, 1991).

Therapeutic approaches oriented toward improving the function of T lymphocytes include *diethyldithiocarbamate* (DTC). DTC was found to be effective in therapy of LP-BM5 murine retrovirus-induced lymphoproliferative immunodeficiency disease (Hersh *et al.*, 1991b). In multicenter double-blind placebo-controlled clinical trials in patients with ARC or AIDS, DTC was shown to decrease the occurrence of opportunistic infections (Hersh *et al.*, 1991a). However, in another trial no beneficial immunomodulatory effect of DTC could be demonstrated in HIV infection (Vanham *et al.*, 1993).

Synthetic immunomodulator *AS101* [ammonium trichloro(dioxyethylene-*O*-*O'*) tellurate] has been found to increase the secretion of cytokines such as IL-2 and CSF and to improve CD4:CD8 ratio in AIDS patients (Kalechman *et al.*, 1990). AS101 showed some activity against disease induced by Friend virus complex (Sidwell *et al.*, 1993). AS101 also restored significantly CSF and IL-6 production by BM cells in mice infected sublethally with murine cytomegalovirus (Sredni *et al.*, 1994).

Synthetic *poly A:poly U* is a complex consisting of polyribonucleotides polyadenylic acid and polyuridylic acid. It has been administered to over 1000 individuals and has shown beneficial effects in breast and gastric cancer patients. Poly A:poly U stimulates release of IFN-γ by activated T cells, induces increased levels of interferon-associated enzymes 2-5A synthetase and protein kinase, stimulates production of IL-1, TNF, IL-6, and colony stimulating activity, and activates macrophages as shown by phagocytosis, chemiluminescence, and $H_2O_2$ production. Poly A:poly U can enhance specific antibody responses to protein and polysaccharide antigens. Poly A:poly U is more potent than Freund's complete adjuvant in stimulating monoclonal antibodies to HIV-1 glycoprotein. Antibody to influenza A/New Jersey/76 monovalent vaccine was significantly enhanced by poly A:poly U. A preliminary clinical trial with human volunteers has confirmed the adjuvant effect of poly A:poly U in influenza vaccine (Tursz *et al.*, 1990). Spleen cells from poly A:poly U-treated mice inhibited the replication of murine cytomegalovirus in confluent monolayer cells of secondary mouse embryo fibroblasts at 37°C and even at hyperthermic 40°C conditions (Lee *et al.*, 1992). The antiviral activity of poly A:poly U appeared to be related mainly to the action of IFN-γ produced by T cells.

*BCH-527*, the lipophilic hydrochloride salt of octadecyl D-alanyl L-glutamine, significantly inhibited murine cytomegalovirus as shown by increased numbers of survivors and decreased titers of virus recoverable from tissues. Influenza A (H1N1)

infection was weakly inhibited, with antiviral activity seen in lowered lung scores and lung weights and less decline in arterial oxygen saturation values. BCH-527 was stimulatory to cytotoxic T cells, natural killer (NK) cells, macrophages, and splenic B cells (Sidwell *et al.*, 1995).

A glucofuranose immunomodulator, *Substance WG 209*, *N*-[*N*-(1,2; 5,6-di-*O*-isopropylidene-D-glucofuranosyl-3-*O*-methylcarbonyl)-glycyl]-D-glutamic acid, administered intranasally as an aqueous preparation provided significant protection against aerosol influenza virus infection (Gruszecki *et al.*, 1988). Substance WG 209 has been shown to lack somnogenic and pyrogenic activities (Johannsen *et al.*, 1994).

Synthetic immunomodulator *DDA* (dimethyl dioctadecyl ammoniumbromide) could prolong survival of aged mice against influenza virus, and confer protective effects against murine hepatitis virus confirmed by liver histology and undetectable levels of serum transaminases (Masihi and Rohde-Schulz, 1990).

Immunomodulator *FCE 20696* is a dibenzopyran derivative that has been shown to confer protection against several viral infections. The severity of the lung lesions caused by influenza virus was decreased and survival against HSV-1 was increased by FCE 20696. This immunomodulator is effective parenterally and even by the oral route (Trizio *et al.*, 1990)

The thiazolopyrimidine nucleoside *7-thia-8-oxoguanosine* [5-amino-3-beta-D-ribofuranosylthiazolo (4,5-*d*) pyrimidine-2,7(3*H*,6*H*)-dione] prevented death in mice infected with Semliki Forest, San Angelo, and banzi viruses when administered prophylactically. Protection was conferred against HSV-1 and murine cytomegalovirus infections and against encephalitis induced by intracerebral inoculation of a human coronavirus in mice (Smee *et al.*, 1990). Immunomodulator 7-thia-8-oxoguanosine induces interferon and potentiates NK cell activity and also provided significant protection against encephalomyocarditis virus, rat coronavirus and showed moderate effectiveness against HSV-2 and vesicular stomatitis viruses. Another nucleoside analogue, 7-deazaguanosine, was found to be highly protective in mice inoculated with lethal doses of Semliki Forest or San Angelo viruses and lesser but still significant protective activity was evident against banzi and EMC viruses (Smee *et al.*, 1991).

Immunomodulator *CL246,738* [3,6-bis(2-piperidinoethoxy)acridine trichloride] is a synthetic heterocyclic of the acridine class and is a potent inducer of interferon and NK cells. CL246,738 protected mice from lethal Semliki Forest and banzi virus infections. Spleen cells of CL246,738-treated mice produced IFN-α whereas peritoneal exudate cells produced IFN-β. Treatment of CL246,738-treated mice with antiinterferon antibodies abrogated the protection against SFV encephalitis (Sazotti *et al.*, 1989).

An immunomodulatory agent designated *R837* was investigated in a guinea pig model of genital HSV-2 infection. Topical R837 application exhibited *in vivo* anti-HSV activity and reduced both acute and latent neural infections and recurrent genital disease (Bernstein and Harrison, 1989).

The steroidal glycoside *L-644,257* [6-(5-cholesten-3β-yloxy)hexyl 1-thio-β-D-

mannopyranoside] conferred significant protection against HSV-1 when administered prior to challenge (Hagmann *et al.*, 1990).

Immunomodulator *LS 2616* (quinoline-3-carboxamide) is a potent inducer of NK cell activity. LS 2616 showed antiinflammatory effects in Coxsackie virus B3-induced myocarditis and increased the number of survivors and survival time (Ilbäck *et al.*, 1989).

Pretreatment with the synthetic peptide *FK565* [hepatanoyl-γ-D-glutamyl-(L)-*meso*-diaminopimelyl-(D)-alanine] significantly inhibited myocardial viral replication of encephalomyocarditis virus and increased survival (Sato *et al.*, 1992).

The new immunomodulator *pidotimod* [(*R*)-3-(*S*)-(5-oxo-2-pyrrolidinyl)-carbonyl-thiazolidine-4-carboxylic acid] significantly increased survival time after challenge with low doses of mengovirus, herpes simplex virus and influenza virus (Dianzani *et al.*, 1994).

An alkylpurine derivative, *9-alkylguanine*, was shown to confer protection against a lethal Semliki forest virus infection in mice (Michael *et al.*, 1994).

## 2.4. Endogenous Immunomodulators

### 2.4.1. Cytokines

Nonspecific first-generation microbial immunomodulators such as BCG and *Corynebacterium parvum* showed limited anecdotal efficacy in treatment of malignancies in early empirical studies which could not be reliably reproduced in randomized clinical trials. The general consensus among clinical investigators was that immunomodulatory treatments needed elucidation of underlying mechanisms and development of more specific immunotherapeutic agents. Recent advances in the monoclonal antibody and recombinant DNA technologies have led to availability of cytokines with defined immunomodulatory activities. The last decade has seen emergence of cytokines as promising therapeutic agents. Many adjuvant-active immunomodulators such as MDP and LPS may exert their activity, at least in part, by endogenous induction of cytokines (Asano *et al.*, 1994; Azuma *et al.*, 1994; Bahr *et al.*, 1995).

Evidence is accruing that cytokines are intimately involved in antimicrobial immune responses by modulation of the expression of major histocompatibility complex and various adhesion molecules regulating the activity of effector cells. Certain cytokines stimulate the production of other cytokines in synergistic or antagonistic networks. Synergistic and individual antiviral effects of cytokines produced by infiltrating cells appear to play an important role in control of viral infections.

*Interleukin 1* (IL-1) molecules are important regulators of immunity and inflammation. They can be induced in a wide variety of cells including monocytes, macrophages, fibroblasts, endothelial and epithelial cells in response to antigens, toxins, and other cytokines. IL-1α pretreatment of WISH cells induced an antiviral state against

VSV infection (Ruggiero *et al.*, 1989). IL-1α could transiently suppress the late erythroid colony-forming units induced by a conventional strain of Friend leukemia virus (Johnson *et al.*, 1990). Substantial elevation of spontaneous *in vitro* production of IL-1β has been observed during IFN-α therapy resulting in the clearance of HBeAg in chronic carriers of hepatitis B (Daniels *et al.*, 1990). Calves administered bovine herpesvirus-1 vaccine in conjunction with recombinant bovine IL-1β showed increased serum neutralizing antibodies, cytotoxic responses and decreased virus excretion (Reddy *et al.*, 1990).

*Interleukin 2* (IL-2) is synthesized and secreted by antigen- or mitogen-activated T lymphocytes. IL-2 acts as a potent growth factor for clonal expansion of T lymphocytes and stimulates lymphokine-activated killer cell and NK cell activities. Human and animal neonates are highly susceptible to herpes simplex virus (HSV) infection. Administration of human recombinant IL-2 protected neonatal mice from a lethal HSV infection. Protection was associated with macrophage-mediated antibody-dependent cellular cytotoxicity via helper T-cell-produced IFN-γ (Kohl *et al.*, 1989). Adoptive transfer of lymphocytes from animals infected with HSV-1 helped clear the virus more effectively when IL-2 was injected into the recipients (Rouse *et al.*, 1985). Protective effect of HSV crude extract or glycoprotein D subunit vaccines could be enhanced against HSV type 2 genital infection in guinea pigs by coadministration of recombinant IL-2 (Weinberg and Merigan, 1988). A truncated herpes simplex virus glycoprotein gene fused to the human IL-2 gene has been shown to induce superior antiherpes antibodies and to protect animals against herpes challenge (Hazama *et al.*, 1993). The potency of inactivated rabies virus vaccine could be increased by daily systemic administration of IL-2 as shown by enhanced protection following challenge with virulent rabies virus (Nunberg *et al.*, 1989). T lymphocytes from uremic patients not responding to hepatitis B vaccination produce inadequate amounts of IL-2. Administration of low-dose IL-2 with hepatitis B vaccine to uremic nonresponders resulted in a 70% seroconversion. IL-2 has also been applied *in vivo* for immunotherapy of established cytomegalovirus (Reddehase *et al.*, 1987). Human herpesvirus-6 (HHV-6) was isolated in 1986 from AIDS patients and is the etiologic agent of a childhood disease characterized by fever and skin rash, namely, exanthem subitum. Addition of recombinant IL-2 strongly inhibited the virus-induced cytopathic effect, reduced the number of infected cells, and produced an almost total absence of extracellular virions in treated cultures (Roffman and Frenkel, 1990). Several studies with IL-2 therapy in AIDS patients have generally yielded poor results despite elevation of some immunological parameters. In fact, IL-2 has been shown to increase the production within 24 hr of HIV *in vitro* by naturally infected mononuclear cells from seropositive donors (Todd *et al.*, 1991) and to enhance translocation of bacteria from intestines to other organs (Penn *et al.*, 1991).

*Lymphokine-activated killer (LAK) cells* are effector cells that are generated by culturing leukocytes in the presence of IL-2. LAK cells inoculated into suckling mice conferred protection against murine cytomegalovirus infection (Bukowski *et al.*, 1988). LAK cells have been shown to chemotactically migrate and accumulate at site

of infection by mouse hepatitis virus or vaccinia virus (Natuk *et al.*, 1989). Administration of bovine recombinant IL-2 in calves induced LAK cells and increased their resistance against bovine herpesvirus-1 challenge (Reddy *et al.*, 1989). Adherent LAK cells, generated by cultivation of NK cells with IL-2, have been shown to kill monocytes infected with HIV for up to 7 days (Melder *et al.*, 1990).

The *colony-stimulating factors* (CSFs) are intimately involved in the production and differentiation of stem cells in the bone marrow to phagocytic cells. CSFs are classified into four major types, namely, IL-3, granulocyte–macrophage CSF (GM-CSF), macrophage CSF (M-CSF), and granulocyte CSF (G-CSF).

IL-3 is a multi-colony-stimulating factor that can stimulate the proliferation and differentiation of pluripotent progenitor cells common to different hematopoietic cell lineages. Primary mouse embryonic cells infected with HSV-1 showed a 1000-fold decrease in virus titer when cultured in the presence of IL-3. Protective effect could be reversed by antiinterferon antibodies suggesting production of interferon mediated by IL-3 (Chan *et al.*, 1990).

M-CSF secreted by mononuclear phagocytes, fibroblasts, and endothelial cells provides an autocrine differentiation signal for proliferation of committed hematopoietic progenitor cells to form macrophages. M-CSF can enhance the production of other cytokines such as interferons and TNF. Murine macrophages treated with M-CSF became resistant to VSV infection (Lee and Warren, 1987). Treatment of murine macrophages with M-CSF increased macrophage survival and reduced the amount of HSV-1 virus produced in cultures. Protective effect of M-CSF could be inhibited by antiinterferon antibodies indicating that the effect was related to the induction of endogenous interferon (Lee and Warren, 1987). In contrast to the HSV-1 virus model, monocytes cultured in recombinant human M-CSF were more than 400-fold more susceptible to HIV infection (Kalter *et al.*, 1991). The significantly enhanced susceptibility of human primary macrophages also reduced the anti-HIV activity of dextran sulfate and soluble CD4 (Bergamini *et al.*, 1994). This demonstrates that M-CSF can either confer protection or exacerbate disease development.

Pretreatment and presence of GM-CSF during culture of U937 human monocytic cells provided protection against HIV infection (Hammer *et al.*, 1986). A synergistic activity of GM-CSF with AZT could be obtained (Hammer and Gillis, 1987; Perno *et al.*, 1989) and toxic effect of AZT on human myeloid progenitor cells could be ameliorated (Bhalla *et al.*, 1989). Replication of HIV in monocytes may, however, be increased under certain experimental conditions by CSF (Perno *et al.*, 1989; Gendelman *et al.*, 1988). In another model, GM-CSF enhanced influenza virus infection of monocytes even though a more rapid release of IFN-α could be induced (Bender *et al.*, 1993).

*IL-10*, a regulator cytokine of both the lymphoid and myeloid cells, is produced by the Th2 subset of T-helper cells. Recombinant human IL-10 could inhibit HIV-1 replication in infected monocytes and peripheral blood mononuclear cells (Masood *et al.*, 1994) and in human macrophages (Akridge *et al.*, 1994).

*IL-12* stimulated enhanced levels of IFN-γ but showed no protective effect in

either prophylactic or therapeutic regimens against influenza virus or encephalomyocarditis virus (Gladue *et al.*, 1994).

*TNF*-α is a pleiotropic cytokine that is mainly produced by the macrophages. TNF exhibits potent immunomodulatory activities including proliferation of B and T lymphocytes, cytotoxic T cells, enhanced expression of MHC class I and II antigens, IL-2 receptors and augmentation of IL-2-stimulated immunoglobulin production and NK cell activity. TNF can also activate neutrophils, induce differentiation of hematopoietic cells, and synergize with other cytokines such as IFN-γ and IL-1. TNF has been shown to possess protective effects against a variety of microorganisms. Recombinant human TNF-α exhibited a distinct antiviral activity on HeLa cells infected with encephalomyocarditis virus (Arakawa *et al.*, 1987). TNF induced antiviral activity against VSV in HEP-2, WI-38, and HEL cell lines (Mestan *et al.*, 1986; Wong and Goeddel, 1986). Combination of TNF with low concentrations of IFN-β or IFN-γ exerted synergistic antiviral activity against VSV (Mestan *et al.*, 1988) and HSV-1 (Feduchi and Carrasco, 1991; Feduchi *et al.*, 1989). Pretreatment of WISH cells with TNF-α or -β or IL-1 induced an antiviral state against VSV infection (Ruggiero *et al.*, 1989). *In vivo* administration of TNF-α inhibited the yield of VSV in peripheral blood mononuclear cells from patients with malignancy by 99% (Nokta *et al.*, 1991). The neurotropic pseudorabies virus (PRV) replicates efficiently in all neural cell types. TNF-α induced a state of antiviral activity in astrocytes against a low dose of PRV (Schijns *et al.*, 1991). TNF has been used to treat chronic hepatitis B virus infection (Sheron *et al.*, 1990). Positive response to IFN-α therapy in chronic carriers of hepatitis B is accompanied by the clearance of HBeAg and substantial elevation in spontaneous *in vitro* production of TNF (Daniels *et al.*, 1990).

Response to TNF can be dichotomous and may be dependent on the pathogenesis caused by the disease state. TNF has been attributed with both beneficial and harmful effects. Bacterial LPS has been found to potentiate the production of TNF from influenza virus-infected (Nain *et al.*, 1990) or influenza neuraminidase-treated (Houde and Arora, 1990) macrophages. TNF production was found to be significantly reduced in patients with chronic hepatitis B (Mueller and Zielinski, 1990). Administration of recombinant human TNF to chronic hepatitis B virus patients not responsive to interferon treatment showed an initial reduction in serum HBV DNA at lower doses (10–15 $\mu g/m^2$) of TNF. Higher doses ($> 30\ \mu g/m^2$) of TNF in some patients enhanced the viral replication and raised HBV DNA and HBsAg. Administration of human recombinant TNF to mice with severe, but clinically inapparent lymphocytic choriomeningitis virus (LCMV) infection caused rapid death. In contrast, TNF given earlier in the course of disease prevented mortality and pretreatment protected against development of lethal disease (Doherty *et al.*, 1989). In another study, treatment of mice with recombinant TNF had no effect on LCM virus clearance (Klavinski *et al.*, 1989). Simian varicella virus (SVV)-infected monkeys given rHuTNF, at doses found to be nontoxic in uninfected monkeys, showed increased mortality (Soike *et al.*, 1989). Porcine monocytes treated with TNF showed an increase of African swine fever virus production (Esparza *et al.*, 1988). C57BL/6 mice pretreated with TNF

showed prolonged survival after infection with HSV-1 (Rossol-Voth *et al.*, 1991), but persistently HSV-infected macrophages from the same strain of mice showed an increase of virus yield and cytopathic effects after treatment with TNF (Domke-Opitz and Kirchner, 1990). The exact molecular mechanisms responsible for protective or antagonistic activities of cytokines such as TNF remain to be elucidated.

TNF reduced the replication of HIV in HUT-78 cells (Wong *et al.*, 1988) but enhanced viral replication in MOLT-4 cells (Ito *et al.*, 1989). TNF treatment of the HUT-78 cell line chronically infected with simian immunodeficiency virus induced a two- to threefold increase in the viral reverse transcriptase activity (Lairmore *et al.*, 1991). Primary blood monocyte-derived macrophages treated with recombinant human TNF-α starting either before or after HIV-1 infection enhanced viral replication of both lymphocyte-tropic and macrophage-tropic strains (Mellors *et al.*, 1991). Recombinant TNF has been shown to activate HIV mRNA (Matsuyama *et al.*, 1989), increase replication (Folks *et al.*, 1989), and enhance syncytium formation (Vyakarnam *et al.*, 1990) in T cell lines. The activity of AZT against HIV can be inhibited by TNF (Ito *et al.*, 1990). Raised levels of both TNF and soluble TNF receptors are detected during HIV-1 infection in seropositive patients (Aukrust *et al.*, 1994). The role of TNF and other cytokines in HIV has been reviewed (Matsuyama *et al.*, 1991).

The evidence for a pathophysiological involvement of TNF has provided the rationale for development of potential therapeutic strategies based on interrupting the production of TNF. Antibodies against TNF and IL-6 abolished HIV-inductive capacity of B-cell cultures from HIV-infected patients with hypergammaglobulinemia (Rieckmann *et al.*, 1991). Cell surface Fas antigen is associated with TNF receptor. A cytotoxic monoclonal anti-Fas antibody selectively could kill HIV-infected cells mimicking the cytocidal action of TNF but without augmentation of HIV replication (Kobayashi *et al.*, 1990). A recent survey of several preclinical and clinical studies of pentoxifylline for the treatment of HIV infection showed that decreased TNF production correlated with changes in HIV load suggesting that pentoxifylline may inhibit HIV expression by suppressing TNF production (Dezube, 1994). Thalidomide, a selective inhibitor of TNF, also has been shown to suppress the replication of HIV in cell lines and in the peripheral blood mononuclear cells of patients with AIDS (Makonkawkeyoon *et al.*, 1993).

### 2.4.2. Prostaglandins

*Prostaglandins* are natural fatty acid products obtained as a result of enzymatic oxidation of arachidonic acid. Prostaglandin A (PGA) and related derivatives can exert inhibitory activity against viruses. The replication of HIV in a T-cell line was inhibited by PGA as shown by the number and size of virus-induced syncytia and the amount of viral antigen (Ankel *et al.*, 1991). PGA has shown interferon-independent antiviral activity against encephalomyocarditis virus (Ankel *et al.*, 1985) and inhibition of primary transcription of vesicular stomatitis virus (Bader and Ankel, 1990). Herpes simplex virus replication could be inhibited by PGA (Yamamoto *et al.*, 1987)

and viral gene expression of vaccinia virus (Benavente *et al.*, 1984) and virus glycosylation in Sendai virus-infected cells (Santoro *et al.*, 1989) were selectively inhibited by PGA. A synthetic long-acting 16,16-dimethyl-PGA analogue exhibited activity against influenza virus in mice as shown by the increased survival of infected mice and by 40% decreased virus titers in the lungs (Santoro *et al.*, 1988). These studies indicate that PGA and analogues might be useful for therapy of viral disease, but further evaluation of the safety and antiviral efficacy *in vivo* are needed.

## 3. IMMUNOMODULATORS IN BACTERIAL INFECTIONS

### 3.1. Bacterium-Derived Immunomodulators

Heat-killed *Lactobacillus casei*, LC 9018, enhanced phagocytic functions and IL-1 production, and led to elimination of *Mycobacterium fortuitum* and *M. chelonae* at the site of infection when administered intramuscularly six times a week (Saito *et al.*, 1987). Combination of ofloxacin with *L. casei* in mice infected with *M. fortuitum* led to delay in incidence of spinning disease, decreased renal lesions, and an increase in the rate of elimination of organisms from the kidneys (Tomioka *et al.*, 1990). DEODAN, a lysozyme from *L. bulgaricus*, has been shown to increase the phagocytic activity, IL-1 and provide protection against *K. pneumoniae* and *L. monocytogenes* infections (Popova *et al.*, 1993).

*TDM* has been shown to enhance resistance against diverse bacterial infections including *E. coli*, *L. monocytogenes*, and *S. enteritidis* infections (reviewed in Masihi *et al.*, 1988) and against *K. pneumoniae* in neutropenic animals (Madonna *et al.*, 1989). TDM administered in combination with MDP could induce significant resistance against virulent *M. tuberculosis* (Masihi *et al.*, 1985).

Immunomodulator *GF 787* is a particulate fraction of *Propionibacterium acnes* consisting of cell wall peptidoglycan with attached glycoprotein. Treatment with GF 787 prolonged the survival of susceptible BALB/c mice expressing the *ity*$^{s}$ gene on macrophages against *Salmonella typhimurium* infection. Kupffer cells from treated mice displayed resistance to *in vitro* lethal effects of *S. typhimurium* (Delfino *et al.*, 1990).

A natural immunomodulator, *L. monocytogenes factor Ei*, could induce nonspecific resistance against *K. pneumoniae* infection (Franek and Malina, 1990).

Administration of synthetic *MDP* analogue muramyl tripeptide, MTP-PE, induced resistance against *K. pneumoniae* infection in mice; liposomal encapsulation of MTP-PE reduced by twofold the amount of immunomodulator required and decreased the toxic side effects by tenfold (Melissen *et al.*, 1992, 1994). Pretreatment of pigs with MTP-PE protected against development of septic leukocytopenia caused by experimental pneumococcemia, enhanced bacterial clearance, and significantly decreased mortality (Izbicki *et al.*, 1991). Nonspecific activation of the host defense system can be a helpful adjunct in supporting the failing antibiotic treatment in certain

infectious diseases. Prophylactic treatment of mice with five doses of liposomal MTP-PE or IFN-γ increased survival from 0 to 65% in a model of *K. pneumoniae*-induced septicemia in mice. Administration of MTP-PE and IFN-γ coencapsulated in liposome resulted in 100% survival in this model (Ten Hagen *et al.*, 1995).

A recent study reported on a placebo-controlled double-blind clinical trial of GMDP for immunotherapy of septic complications arising after abdominal surgery (Khaitov *et al.*, 1994). Prophylactic administration of GMDP and postoperative treatment of patients who acquired infections showed decreased frequency of septic complications and reduced mortality. In a different investigation, the core body temperature of Sprague–Dawley rats was regulated at 32–40°C, and 24 hr after administration of MDP, a sublethal challenge with *E. coli* was given; high-dose MDP significantly accelerated peritoneal bacterial clearance but no interaction between MDP and core body temperature was observed (Stellato *et al.*, 1988). In another study, killed *E. coli* was given multiple times with MDP to cynomolgous monkeys in an attempt to enhance the effect of vaginal immunization against urinary tract infections; levels of urinary and serum immunoglobulins were reduced and no protective effect on induced cystitis was observed (Uehling *et al.*, 1990). An extracorporeal filter consisting of polystyrene fiber-bound polymyxin B has been used experimentally in treatment of gram-negative bacterial infection. Significant improvements in rats infected with *E. coli* occurred in groups pretreated with MDP and in animals treated with a combination of MDP, polystyrene fiber-bound polymyxin B, and gentamycin (Cheadle *et al.*, 1991). The growth of leprosy bacilli in the hybrid nude mouse strain Jcl:AF-nu could be completely inhibited by combination of the bacteriostatic DDS and either MDP or water-soluble lipoidal amine, CP-46665, mixed with the chow (Gidoh and Tsutsumi, 1989). Nonspecific resistance induced by MDP to bacterial infections has been reviewed elsewhere (Parant *et al.*, 1992).

Soluble protective antigen (SPA) extracted from *Salmonella enteritidis* can enhance the chemotactic activity and superoxide generation greater than that induced by MDP or LPS and has been evaluated as a bacterial-origin immunomodulator. SPA reduced the number of bacteria in liver and afforded increased resistance to *L. monocytogenes* infection (Uchiya and Sugihara, 1989).

Acetone-killed *Salmonella typhimurium* vaccine containing synthetic *lipopeptide* derivative of bacterial lipoprotein, Pam3Cys-Ser-Ser-Asn-Ala, provided protection against intraperitoneal challenge with *S. typhimurium* even when 90% of the bacterial component was replaced by the lipopeptide (Schlecht *et al.*, 1989). WS1279, another novel lipopeptide, has been shown to augment host resistance to *Staphylococcus aureus* in normal and immunosuppressed mice (Tanaka *et al.*, 1993).

## 3.2. Fungus-Derived Immunomodulators

*Lentinan* can confer protection against *Listeria* and prevent relapse of *Mycobacterium tuberculosis* (Chihara, 1990; Maeda *et al.*, 1994). *S. cerevisiae* glucan induced nonspecific resistance against *K. pneumoniae* infection and yeast glucan can protect

patients from sepsis, bacteremia, and peritonitis resulting from *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* infections (reviewed in Chihara, 1990).

The safety and efficacy of PGG-glucan in surgical patients at high risk for postoperative infection who underwent major thoracic or abdominal surgery were recently studied. Patients who received PGG-glucan had significantly fewer infectious complications (3.4 versus 1.4 infections per infected patient), decreased intravenous antibiotic requirement (10.3 versus 0.4 day) and shorter intensive care unit length of stay (3.3 versus 0.1 day). PGG-glucan appears to be effective in the further reduction of the morbidity and cost of major surgery (Babineau *et al.*, 1994).

Polysaccharide "*RBS*," an α-glucan, administered intraperitoneally to mice led to increased IL-1 production, an enhanced elimination of bacteria and protection against *L. monocytogenes* and *E. coli* infections (Takeda *et al.*, 1990).

A protein-bound polysaccharide, *PSK* (Krestin), induced significant activity against *E. coli* when mice were pretreated by intraperitoneal, subcutaneous, or intramuscular routes and also after repeated oral administration (Sakagami *et al.*, 1991).

## 3.3. Synthetic Compounds as Immunomodulators

A novel synthetic immunomodulator designated *FCE 24578* [2-cyano-3-(1,4-dihydro-1-phenyl-(1)-benzothiopyran(4,3-*c*)pyrazol-3-yl)-3-oxo-*N*-phenylpropanamide] was identified during a screening program. FCE 24578 can induce protection in normal mice against *Listeria monocytogenes* and *Shigella flexneri* and in cyclophosphamide-immunodepressed mice against opportunistic infection by *Pseudomonas aeruginosa* (Verini and Ungheri, 1989). Immunomodulator FCE 20696 showed some activity against *M. tuberculosis* when it was given orally twice weekly for 5 weeks during the course of the disease (Verini and Ungheri, 1989).

A glucofuranose immunomodulator, *Substance 209*, administered as a squalane-in-water preparation 4 weeks before aerosol challenge with virulent *M. tuberculosis* significantly restricted bacterial growth and reduced the number of viable counts by 3 $\log_{10}$ in pretreated mice (Gruszecki *et al.*, 1988).

Synthetic immunomodulators *AdDP* and *ML 310* also induced nonspecific resistance against *K. pneumoniae* infection (Franek and Malina, 1990).

Cross-bred calves vaccinated against *Pasteurella multocida* and administered *levamisole* by oral, subcutaneous, or transdermal route produced highly significant antibody titers during the primary humoral response (Sharma *et al.*, 1990).

The steroidal glycoside *L-644,257* [6-(5-cholesten-3β-yloxy)hexyl 1-thio-β-D-mannopyranoside] provided protection to cyclophosphamide-immunosuppressed mice against *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* (Hagmann *et al.*, 1990).

## 3.4. Endogenous Immunomodulators: Cytokines

Treatment with recombinant murine *IL-1α* significantly enhanced resistance of mice to *Listeria monocytogenes*. Maximal antibacterial resistance was observed when

intravenous IL-1 was given concurrently or intraperitoneal IL-1 was injected 48 hr before listerial challenge (Czuprynski and Brown, 1987). Recombinant human IL-1α administered 3 days and 1 day before infection with *Pseudomonas aeruginosa* most effectively protected animals from death (Ozaki *et al.*, 1987). Mice rendered granulocytopenic by cyclophosphamide, infected with *Pseudomonas aeruginosa* and given gentamicin 6 and 23 hr later showed increased survival when IL-1β was administered 24 hr before infection (Van der Meer *et al.*, 1988). Recombinant human IL-1α given intraperitoneally 24 hr before *P. aeruginosa* infection enhanced survival of neutropenic mice (Van der Meer *et al.*, 1989). Recombinant human IL-1α administered simultaneously and 1 day after infection with *Klebsiella pneumoniae* conferred maximal protection (Ozaki *et al.*, 1987); the protective activity of IL-1 was dose-dependent. Intravenous administration of recombinant human IL-1α to mice depressed the growth of *Brucella abortus* 19 in spleen and liver when given 4 hr before infection (Zhan *et al.*, 1991). IL-1 given to C3H/HeJ mice after but not prior to infection increased resistance to *Salmonella typhimurium* (Morrissey and Charrier, 1991). These studies show that IL-1 pretreatment prolongs survival in lethal infection in normal and in neutropenic mice. In a recent study, IL-1 could reduce circulating TNF-α and IL-6 as well as LPS-stimulated production of IL-1α and TNF-α (Vogels *et al.*, 1994). Upregulation of mRNA for the IL-1 receptor antagonist (IL-1Ra) was observed in several organs of IL-1-pretreated mice, suggesting that IL-1Ra could attenuate deleterious IL-1 effects. In addition, IL-1 pretreatment downregulated steady-state mRNA for the type I IL-1R and the type I TNFR in several organs at the time of infection, suggesting desensitization of target cells as an additional mechanism of IL-1-induced protection (Vogels *et al.*, 1994).

The lethal effects of endotoxin or septicemia are mediated mainly by TNF. Since large quantities of IL-1 are released soon after TNF in response to bacteremia or endotoxin, IL-1 has been suggested to be a comediator of endotoxin lethality (Alexander *et al.*, 1991). A receptor antagonist to IL-1 produced from IgG-adherent human monocytes has been sequenced and expressed in *E. coli* vector. Mice given a lethal *E. coli* endotoxin (LPS) challenge exhibited improved survival when treated with human recombinant receptor antagonist protein to IL-1 (Alexander *et al.*, 1991). The growth of virulent, but not avirulent, *E. coli* has been observed to be enhanced by IL-1β but can be blocked by IL-1 receptor antagonist (Porat *et al.*, 1991).

Recombinant human *IL-2* significantly reduced the total *Mycobacterium lepraemurium* counts in the footpad, lymph nodes, and liver of infected mice by 6 months (Jeevan and Asherson, 1988). A lack of local lymphokine production could account for the inability of infected macrophages to eliminate *M. leprae* in patients. Administration of recombinant human IL-2 in clinical studies induced an influx of T cells, enhanced cell-mediated immunity, and led to significant decrease in the total burden of *M. leprae* and degradation of leprosy bacilli (Kaplan, 1991). Administration of recombinant human IL-2 significantly reduced the viable counts of *Mycobacterium bovis* in the spleen by 60 days after BCG infection (Jeevan and Asherson, 1988). The number of viable *Mycobacterium avium* in splenic macrophages decreased when cultured in the presence of autologous sensitized T cells and recombinant IL-2

(Hubbard and Collins, 1991). Exposure of human monocyte-derived macrophages to IL-2-treated NK cells before infection with *M. avium* induced significant mycobactericidal activity (Bermudez and Young, 1991). Recombinant human IL-2 administered intravenously significantly enhanced resistance of mice against *L. monocytogenes*; IL-2 was protective when injected concurrently or up to 24 hr prior to listerial infection (Haak-Frendscho *et al.*, 1989). Administration of recombinant IL-2 daily for 7 days reduced *K. pneumoniae* counts in a dose-dependent manner and enhanced the clearance of bacteria from the lungs after aerosol exposure (Iizawa *et al.*, 1988).

Recombinant human IL-2 given prophylactically enhanced survival and conferred complete recovery from an otherwise lethal *E. coli* type O2 infection (Chong, 1987). Cell-mediated protection against fatal *E. coli* septicemia was enhanced by treatment with IL-2 (Goronzy *et al.*, 1989). However, patients who receive high doses of IL-2 acquire a profound defect in neutrophil chemotaxis and show a frequent complication of bacterial sepsis (Klempner *et al.*, 1990). A higher incidence of bacterial infections has been observed in AIDS patients receiving IL-2 (Murphy *et al.*, 1988). Recombinant human IL-2, even in concentrations as low as 1 U/ml, and recombinant human GM-CSF have been shown to enhance growth of a virulent, but not avirulent, strain of *E. coli* in tissue culture medium by two- to threefold suggesting that certain cytokines may act as growth factors for some virulent bacteria (Denis *et al.*, 1991).

*IL-4* has been shown to increase the bacteriostatic activity of murine peritoneal macrophages against *M. avium* infection (Denis and Gregg, 1991).

Recombinant human *IL-6* given intraperitoneally 24 hr before *P. aeruginosa* infection showed some protective effect in neutropenic mice but only when a high dosage of 800 ng was used (Van der Meer *et al.*, 1989). IL-6 was 10–100 times less potent than IL-1 in protecting mice in this infection model. Since IL-6 can produce minute amounts of IL-1 *in vivo*, the protection induced by IL-6 may be mediated by IL-1.

Increased levels of IL-6 have been observed in various disease states including patients with bacterial infections (Bauer and Hermann, 1991). *Listeria monocytogenes* induces IL-6 production which shows direct correlation with the severity of the infection in mice (Havell and Sehgal, 1991). Treatment with bioengineered IL-6 receptors or anti-IL-6 antibodies may be a promising therapeutic approach. Anti-IL-6 monoclonal antibody pretreatment in a mouse model of septic shock by a lethal dose of intraperitoneal *E. coli* has been shown to protect animals from death (Starnes *et al.*, 1990).

*IL-8* is a potent neutrophil-activating peptide-1. Microbial invasion induces migration of neutrophils to the site of infection and subsequent phagocytosis. Polymorphonuclear leukocytes showed induction of IL-8 mRNA on exposure to commonly encountered stimuli such as bacterial endotoxin at sites of infection and release of IL-8 after phagocytosis (Bazzoni *et al.*, 1991). Production of IL-8 by phagocytosing neutrophils strengthens the notion that neutrophils may enhance antimicrobial defense by facilitating recruitment of new cells.

*IL-10* is a cytokine synthesis inhibitory factor produced by Th2 lymphocytes and is capable of suppressing IFN-γ production by Th1 cells. Release of reactive oxygen and nitrogen intermediates and cytokines such as TNF, which are important in antimicrobial defense, can also be suppressed by IL-10. This has led to the suggestion that macrophages treated with IL-10 may become permissive for growth of pathogens through inhibition or deactivation of IFN-γ (Bogdan *et al.*, 1991).

Effects of *IL-12* have been shown to be dependent on the invading organism. Animals pretreated with IL-12 had higher mortality following challenge with *E. coli* but enhanced survival and clearance after *L. monocytogenes* and *S. aureus* infections (Gladue *et al.*, 1994).

Administration of recombinant *TNF* has been shown to confer protection against *Klebsiella pneumoniae* and *Streptococcus pneumoniae* infections in mice (Parant, 1988). The clearance of *Legionella pneumophila* in mice could be speeded up by TNF-induced activation of polymorphonuclear leukocyte function (Blanchard *et al.*, 1988). TNF has been used for the treatment of experimental disseminated *Myobacterium avium* infection in mice (Bermudez *et al.*, 1989) and anti-TNF treatment during an early stage accelerated multiplication of *M. bovis* (Kindler *et al.*, 1989). Significant inhibition of growth of *M. avium* complex could also be observed in human macrophages treated with TNF (Suzuki *et al.*, 1994). TNF has been shown to be involved in early protection against *M. avium* (Appelberg *et al.*, 1994). Protective effect of recombinant TNF has been observed in murine salmonellosis (Nakano *et al.*, 1990). Injection of anti-TNF-α antiserum to resistant A/J mice exacerbated sublethal *Salmonella typhimurium* infection; anti-TNF-α treatment did not accelerate bacterial growth but the colony counts continued to increase past the plateau point indicating that TNF-α is important in mediating the plateau phase in a salmonella infection (Mastroeni *et al.*, 1991). Human recombinant TNF-α treatment enhanced resistance to *Listeria monocytogenes* infection in mice (Roll *et al.*, 1990) whereas anti-TNF antibody injection exacerbated sublethal infection with *L. monocytogenes* (Nakane *et al.*, 1988a).

Measurable levels of circulating TNF are observed in patients with septic shock syndrome (Casey *et al.*, 1993). Clinical trials in sepsis patients and in patients at risk have shown anti-TNF antibodies to be well tolerated (Saravolatz *et al.*, 1994). Further trials on efficacy and clinical utility of anti-TNF antibodies in sepsis are ongoing. Soluble TNF receptors capable of specifically inhibiting TNF, namely, soluble type I (p55) and type II (p75) TNF receptors (TNFR), are found circulating in blood. Elevated levels of soluble TNFR have been observed in patients with a clinical diagnosis of sepsis (van der Poll *et al.*, 1993) but the concentrations of TNFR are inadequate to prevent toxic reactions. Administration of TNFR to raise plasma concentration 1000-fold can prevent TNF-mediated damage (Moldawer, 1993).

Resident peritoneal macrophages treated with *M-CSF* exhibited enhanced phagocytosis and killing of *Listeria monocytogenes* (Cheers *et al.*, 1989). Recombinant murine *GM-CSF* administered to mice could confer protection against listerial infections (Magee and Wing, 1989; Shinomiya *et al.*, 1991). Administration of GM-CSF to mice enhanced clearance of *Salmonella typhimurium* (Morrissey *et al.*, 1989; Mor-

rissey and Charrier, 1990). Significant inhibition of growth of *Mycobacterium avium* complex could be observed in human macrophages treated with GM-CSF (Suzuki *et al.*, 1994). *G-CSF* induced resistance in neutropenic animals to infections by *Pseudomonas aeruginosa* (Mooney *et al.*, 1988), *Staphylococcus aureus*, *Serratia marcescens*, or *C. albicans* (Cohen *et al.*, 1988; Matsumoto *et al.*, 1987).

Mice immunosuppressed with cyclosporin A can be protected against fatal infection by *L. monocytogenes* when treated with *IFN-γ* (Nakane *et al.*, 1988b) and endogenous production of IFN-γ is required for the resolution of listerial infection (Buchmeier and Schreiber, 1985). Bone marrow-derived macrophages treated with IFN-γ showed high bactericidal activity against *L. monocytogenes* (Denis, 1991a). Administration of IFN-γ can enhance resistance to *Francisella tularensis* (Leiby *et al.*, 1992). However, treatment with IFN-γ led to enhancement of growth in macrophages of *M. lepraemurium*, another intracellular pathogen (Denis, 1991b). Patients with chronic granulomatous disease are unable to generate oxidative respiratory burst. As a consequence, they develop recurring catalase-positive bacterial infections such as *Staphylococcus aureus*, *Pseudomonas cepacia*, and *Chromobacterium violaceum*. Multicenter clinical trials have shown that sustained administration of IFN-γ to chronic granulomatous disease patients markedly reduced the relative risk of serious infection (Gallin, 1991).

## 4. IMMUNOMODULATORS IN PARASITIC AND FUNGAL INFECTIONS

### 4.1. Bacterium-Derived Immunomodulators

Avirulent *Salmonella typhimurium*, strain SL3235, is blocked in the aromatic pathway. Three different C3H mouse strains, including defective C3H/HeJ, when treated with a single injection of *S. typhimurium* SL3235, but not viable BCG, induced the generation of activated macrophages capable of killing *L. major* (Schafer *et al.*, 1988).

Induction of immunity using soluble leishmanial antigen from *L. major* required concurrent injection of *Corynebacterium parvum* (Scott *et al.*, 1987).

Trehalose dimycolate (*TDM*) extracted from mycobacterial cell walls is a potent immunomodulator. Significant resistance against oral *Toxoplasma gondii* infection was induced by intraperitoneal pretreatment of mice with TDM (Masihi *et al.*, 1985) and corroborated by a significant reduction in the number of cysts in brains (Masihi *et al.*, 1986a). Partial protection comparable to that induced by specific immunization with nonpathogenic trophozoites against lethal intranasal infection by *Acanthamoeba culbertsoni* could be conferred by intravenous pretreatment with MDP (Masihi *et al.*, 1986a).

Multilamellar liposomes containing encapsulated MTP-PE and IFN-γ significantly reduced *Leishmania donovani* parasites in spleens of treated animals (Hockertz

*et al.*, 1991). CDRI compound 86/448, a glycopeptide structurally related to MDP, was shown to be more potent in inhibiting *L. donovani* infection in hamsters and in improving the efficacy of sodium stibogluconate (Pal *et al.*, 1991).

A cloned protein containing sequences of the circumsporozoite antigen of *Plasmodium falciparum* induced strongest antibody responses in rabbits and monkeys when immunization was performed with alum-adsorbed liposomes containing *lipid A* and antigen (Richards *et al.*, 1988). Role of immunomodulators such as MDP analogues and lipid A have been reviewed (Siddiqui, 1990). Human antibody responses to *P. falciparum* circumsporozoite protein vaccine were shown to be superior when MPL and mycobacterial cell wall skeleton were used as adjuvants (Rickman *et al.*, 1991). In another study, a *P. falciparum* circumsporozoite protein recombinant fusion protein formulated with monophosphoryl lipid A, cell wall skeleton of mycobacteria, and squalane was administered to 12 volunteers. After the third dose of vaccine, significant amounts of antibodies were observed in sera of some volunteers who also did not develop parasitemia when challenged by the bite of mosquitoes carrying *P. falciparum* sporozoites (Hoffman *et al.*, 1994). Recently, complete protection from lethal *P. yoelii* malaria was produced by three immunizations with whole killed blood-stage parasite antigen in copolymer P1004 and detoxified RaLPS as adjuvants. The protection lasted for 9 months and was associated with an anamnestic rise in antibody titer of the IgG2a isotype during the challenge infection (Hunter *et al.*, 1995).

## 4.2. Fungus-Derived Immunomodulators

*Lentinan* can induce granuloma formation against *Schistosoma mansoni* and *S. japonicum*, and show a therapeutic effect against *Mesocestoides corti* (Chihara, 1990). Particulate glucan administered with glutaraldehyde-killed *T. cruzi* culture forms, but not glucan or killed trypanosomes alone, provided significant protection up to 275 days postchallenge (Williams *et al.*, 1989). Yeast *glucan* can prolong survival against parasitic infections by *Plasmodium berghei* and *Leishmania donovani* and exert antifungal activity against *Candida*, *Cryptococcus*, and *Sporotrichum* (Chihara, 1990). Glucan administered in combination with soluble *P. berghei* antigen (Maheshwari and Siddiqui, 1989) or fractions of *L. donovani* promastigotes (Obaid *et al.*, 1989) developed well-defined cell-mediated and humoral immune responses and conferred protection against challenge with live parasites.

## 4.3. Synthetic Compounds as Immunomodulators

Treatment with synthetic immunomodulator *LF-1695* increased oxygen metabolite production and cytotoxic activity of effector macrophages and platelets against *Schistosoma mansoni* and induced a higher degree of protection against parasite challenge (Thorel *et al.*, 1988).

The steroidal glycoside *L-644,257* [6-(5-cholesten-3β-yloxy)hexyl 1-thio-β-D-

mannopyranoside] provided protection against *C. albicans* in cyclophosphamide-immunosuppressed mice (Hagmann *et al.*, 1990). An analogue of deoxyspergualin, N-563, was also shown to significantly prolong the survival time of cyclophosphamide-immunosuppressed mice against *C. albicans* (Aoyagi *et al.*, 1994).

Prophylactic administration of *isoprinosine* to mice could confer protection against *Trypanosoma cruzi* (Abath *et al.*, 1988).

Combination of pentostam with *levamisole* induced enhanced activity against *L. donovani* infection in hamsters and mice (Rifaat *et al.*, 1989). Guinea pigs infected with *L. enriettii* and treated with levamisole did not develop ulcerative lesions and showed no metastases, eosinophilia, or leukopenia (Rezai *et al.*, 1988). The severity of *L. major* infection in mice receiving levamisole was lower in comparison with controls (Rezai *et al.*, 1988). Levamisole has been reported to be active against *Strongyloides venezuelensis* infection in rats (Campos *et al.*, 1989). Levamisole administered prophylactically to mice could confer protection against *Trypanosoma cruzi* (Abath *et al.*, 1988). Mice infected for 45 days with *Schistosoma mansoni* and treated with levamisole exhibited increased resistance, which could be enhanced further by combination with praziquantel, to challenge with schistosoma cercariae (Botros *et al.*, 1989).

## 4.4. Endogenous Immunomodulators

### 4.4.1. Leukocyte-Derived Immunomodulator

Mucocutaneous candidal infection is frequently associated with a deficiency in cell-mediated immunity and oral candidiasis is an important clinical manifestation of HIV infection. Treatment with *IMREG-1*, a leukocyte-derived immunomodulator, improved control of oropharyngeal candida infection and decreased the occurrence of opportunistic infections. IMREG-1 can augment delayed-type hypersensitivity to recall antigens, enhance production of leukocyte migration inhibition factor, IFN-$\gamma$ and expression of IL-2 receptors on $CD4^+$ helper lymphocytes (Gottlieb and Gottlieb, 1990).

### 4.4.2. Cytokines

Low doses of *IL-1* have been shown to inhibit parasitemia and protect mice against cerebral malaria caused by *Plasmodium berghei* (Curfs *et al.*, 1990). The growth of *C. albicans* in mice immunosuppressed with either cyclophosphamide or irradiation was significantly reduced when recombinant human IL-1$\alpha$ was administered 24 hr before, given simultaneously with or 6 hr after infection (Kullberg *et al.*, 1990). Prophylactic treatment with recombinant human IL-1$\beta$ enhanced resistance of normal and cyclophosphamide-treated mice to systemic infection with *C. albicans* (Pecyk *et al.*, 1989).

Administration of *IL-2* has been shown to increase survival time of mice infected

with *Trypanosoma cruzi* (Choromanski and Kuhn, 1985) and *Toxoplasma gondii* (Sharma *et al.*, 1985). Macrophages cultured with IL-2 and IFN-γ before infection with *L. major* develop resistance to infection which can be abrogated by anti-TNF antibodies. This suggests that IL-2 may act as a cofactor with IFN-γ for the induction of macrophage antimicrobicidal activity with TNF as the effector molecule (Belosevic *et al.*, 1990).

*IL-4* has multiple biologic activities. Resistance against *Leishmania donovani* induced by IFN-γ in human monocytes is abrogated by IL-4 (Lehn *et al.*, 1989). Administration of anti-IL-4 monoclonal antibodies to neutralize IL-4 led to an attenuation of an otherwise fatal infection by *L. major* (Sadick *et al.*, 1990). IL-4, however, plays an important role in immunity to nematode parasites (Urban *et al.*, 1992).

*In vivo* injection of recombinant *TNF* has been shown to inhibit experimental infections with *Plasmodium* species (Taverne *et al.*, 1987; Clark *et al.*, 1987) and anti-TNF treatment is followed by an increase of *Plasmodium vinckei* parasitemia (Neifer *et al.*, 1989). Human recombinant TNF has an inhibitory effect on both virulent and nonvirulent strains of *Entamoeba histolytica* (Ghadirian, 1990). Administration of TNF-α can mediate protection against murine cutaneous leishmaniasis (Titus *et al.*, 1989; Liew *et al.*, 1990) although TNF does not exert direct leishmanicidal effect *in vitro* (Moll *et al.*, 1990). The Th1 subset of $CD4^+$ T cells secreting IFN-γ are protective in murine cutaneous leishmaniasis, whereas the Th2 subset capable of producing IL-4 exacerbate the infection (Liew, 1991). Subcutaneous immunization induces Th2 cells while intraperitoneal or intravenous immunization generates Th1 cells. Immunization with a *Leishmania major* peptide together with TNF-α prevented disease enhancement by the subcutaneous route and led to the desirable induction of Th1 cells (Liew *et al.*, 1991).

Recombinant TNF induced protective effects against *Trypanosoma cruzi* and *Toxoplasma gondii* infections in mice (Black *et al.*, 1989). Murine recombinant TNF-α has been shown to enhance the expression of antimicrobicidal activity by IFN-γ primed macrophages cultured under low endotoxin conditions to kill or inhibit *Toxoplasma gondii* (Sibley *et al.*, 1991). Protection afforded by IFN-γ and TNF to mice infected with *Toxoplasma gondii* could be abrogated by anti-TNF antibodies (Chang *et al.*, 1990).

There is growing evidence from a number of studies that *IFN-γ* plays a central role in protection against *Toxoplasma gondii* (reviewed in Subauste and Remington, 1991). Administration of IFN-γ to athymic nude mice also prevented proliferation of the parasite in various organs and prolonged the survival of treated animals (Suzuki *et al.*, 1991). Mice rendered immunodeficient by selective depletion of $CD4^+$ cells and administered an aerosol of recombinant murine IFN-γ showed reduced intensity of *Pneumocystis carinii* infection (Beck *et al.*, 1991). IFN-γ is essential for the resolution of *Plasmodium berghei* infection in rodents (Schofield *et al.*, 1987) and human neutrophils treated with IFN-γ significantly augmented the killing of asexual blood forms of *Plasmodium falciparum* (Kumaratilake *et al.*, 1991). Mice infected with

*Cryptosporidium* showed greatly enhanced oocyst shedding when treated with anti-IFN-γ antibody (Ungar *et al.*, 1991). IFN-γ plays an important role in the control of pneumonia caused by *Chlamydia trachomatis* (Williams *et al.*, 1988) and represents a crucial host defense against *Rickettsia conorii* (Li *et al.*, 1987).

Treatment with *M-CSF* of human monocytes (Wang *et al.*, 1989) and elicited murine macrophages (Karbassi *et al.*, 1987) induces killing of *Candida albicans.* Human monocytes treated *in vitro* with recombinant human *IL-3* or *GM-CSF* were capable of inducing antifungal activity against *C. albicans* (Wang *et al.*, 1989). GM-CSF stimulates the oxidative respiratory burst and secretion of hydrogen peroxide in phagocytic cells (Sullivan *et al.*, 1989; Reed *et al.*, 1987), a host defense mechanism that could augment antimicrobicidal activity. Macrophages activated by GM-CSF exhibit enhanced capacity to kill *Leishmania donovani* (Weiser *et al.*, 1987), *L. tropica* (Handmann and Burgess, 1979) and *Trypanosoma cruzi* (Reed *et al.*, 1987). In other studies, human macrophages did not show killing activity against *Toxoplasma gondii* or bacterial infection by *Legionella pneumophila* (Nathan *et al.*, 1984; Jensen *et al.*, 1988).

Treatment of mice with GM-CSF increased the parasite burden and led to an exacerbation of leishmanial infection (Greil *et al.*, 1988). Antibodies to GM-CSF and IL-3 have been shown to dramatically decrease the incidence of neurological symptoms in cerebral malaria (Grau *et al.*, 1988).

*IL-12*, also known as NK cell-stimulating factor, is a heterodimeric cytokine produced by monocytes and B cells. It has multiple effects on T and NK cells and is a potent inducer of Th1 cytokine IFN-γ. Anti-IL-12 antibodies completely inhibited *T. gondii* or endotoxin-stimulated IFN-γ by NK cells. Treatment with IL-12 prolonged survival of SCID mice infected with *T. gondii* (Gazzinelli *et al.*, 1993). IFN-γ-dependent activation of macrophages by NK cells in SCID mice is essential for the development of natural immunity. Neutralization of IL-12 showed that heat-killed *L. monocytogenes* require IL-12 to stimulate IFN-γ production by SCID splenocytes (Tripp *et al.*, 1993). Treatment with murine IL-12 during the first week of infection with *L. major* cured the majority of the normally susceptible BALB/c mice and provided durable resistance against reinfection (Heinzel *et al.*, 1993). In contrast, administration of anti-IL-12 antibody at the time of infection to resistant C57BL/6 mice exacerbated disease (Sypek *et al.*, 1993). These observations suggest that IL-12 may prevent Th2 responses that are deleterious in certain infections and promote protective IFN-γ based Th1 responses.

*Transforming growth factor beta* (TGF-β) belongs to an ancestral evolutionary family of regulatory cytokines with potent growth inhibitory activities. It is produced at high levels by activated T lymphocytes, monocytes and macrophages among other cells, and during morphogenesis. Suppressive molecules such as TGF-β can down-regulate host response to intracellular pathogens. Production and release of TGF-β appears to be an important parasite escape mechanism. Impaired activity of macrophages has been observed in association with TGF-β in infections with *T. cruzi* (Silva *et al.*, 1991), *Leishmania* (Barral-Neto *et al.*, 1992), and *T. gondii* (Bermudez *et al.*,

1993). Anti-TGF-β antibody can partially inhibit TGF-β-induced downregulation and may represent an interesting intervention strategy. Host defense mechanisms within the brain, however, may vary. Microglial cells located within the brain are considered as ontogenic and functional equivalents of macrophages. Activation of microglia with IFN-γ plus TGF-β showed dose-dependent anti-*T. gondii* effect. Anti-TGF-β antibody inhibited antitoxoplasma activity of IFN-γ plus LPS-treated microglia suggesting a role for TGF-β in neural host defense function (Chao *et al.*, 1993).

## 5. CONCLUDING REMARKS AND FUTURE PROSPECTS

Immunomodulators are rapidly evolving to become a viable adjunct to established therapeutic modalities. This review highlights the recent application of a wide array of natural products of microbial origin, chemically synthesized molecules, and recombinant cytokines in prophylaxis and treatment of diverse diseases.

Therapeutic value of cytokines in infectious diseases is increasingly being recognized. IL-2, IL-12, TNF-α, and IFNs may be useful in potentiating host antimicrobial defense via stimulating the host effector cells. Other cytokines such as IL-1, IL-3, and hematopoietic growth factors alone and in combination are considered as being beneficial in treatment of infections associated with neutropenia, neonatal septicemia or in prevention of infections accompanying aplastic anemia, chemotherapy, immunodeficiency, or burn injury. Overall, the use of cytokines as therapeutic tools in the setting of infections has given rise to an optimistic view of the use of such reagents. Approaches based on neutralization of immunosuppressive cytokines in infectious diseases are also an area of considerable promise. Limitations of therapy with exogenous cytokines, however, have to be recognized. These are associated with the inherent toxicity of such material, their unclear pharmacological behavior, and their pleiotropic effects. Efficacy of exogenous cytokines capable of potentiating normal host defense mechanisms may be curtailed in immunocompromised patients lacking pertinent effector cells or containing disease-related factors preventing lymphocyte activation. Parasite, bacterial, and viral adaptations to the presence of cytokines pose new problems and approaches based on cytokine intervention will have to take these factors into account.

T-helper lymphocytes can be distinguished into two subtypes based on their response and ability to secrete a variety of mediators. Cytokines such as IFN-γ and TNF secreted by Th1-type cells are potent agents capable of limiting viral replication and controlling the multiplication of intracellular pathogens. Cytokines such as IL-4, IL-5, and IL-10 produced by Th2-type cells are capable of downregulating cell-mediated host defense effector mechanisms and can even exacerbate certain infections. On the other hand, IFN-γ has been shown to suppress protective mechanisms and IL-4 to play an important role in immunity to certain nematode parasites. Treatment regimens capable of manipulating the repertoire of cytokine cascade will be valuable in establishing effective immunotherapy. Selective stimulation by suita-

ble immunomodulators of discrete lymphocyte subpopulations and cytokines important in protective effector mechanisms against a given infection is predicted to play an increasingly important role. Certain immunomodulators, MDP analogues for example, possess adjuvant activity whereas others more efficiently can induce CSFs and other cytokines. Identification of immunomodulators which can enhance the immunogenicity of antigens for both class I and class II MHC-restricted responses will also be important. Tailor-made immunomodulators produced by practical application of the advances in molecular biology and peptide synthesis will permit development of products based on scientific evidence rather than empiricism.

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