Review

Immunostimulating agents: what next?
A review of their present and potential medical applications

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Many chemical entities, either from natural sources or prepared by synthesis, are known to exert stimulating activities on various functions of the immune system, such as antibody production, resistance to infections, rejection of malignant cells, etc. In this review, the origin, chemical structures and main activities of several immunostimulants are described, with special emphasis on their present or potential medical usefulness. An attempt is made to envisage the future of this type of pharmacological agents, excluding however from the presentation the endogenous modulators of the immune system (cytokines), the production and activities of which are influenced by the immunostimulants themselves.

Keywords: immunopharmacology; immunostimulant; immunomodulator; adjuvant; biological response modifier.

It is convenient and probably also justified to adopt the apparently finalistic concept that, in the course of evolution, the immune system of Vertebrates arose primarily in response to the threat posed by infectious agents. This immune system is characterized by a learned self-nonself discrimination mechanism that distinguishes it from all other protective strategies [1]. There are marked resemblances, however, between the immune system, which has been called 'a mobile brain' [2] and the central nervous system, and bidirectional interactions between these systems have been recognized: they are the object of intensive investigation, in a discipline called psychoneuroimmunology [3]. The various cell populations of the immune system (macrophages, neutrophils, monocytes, lymphocytes) are organized in a highly complex and intricate network, in which connections are operated through direct interactions between cells and by communication through intercellular chemical signals, the monokines and lymphokines globally called cytokines (interferons, interleukins, transforming growth factors, chemotactic factors). Differentiation and maturation of the bone-marrow-derived precursor cells of the immune system are under the influence of a series of hematopoietic growth factors (colony-stimulating factors).

Whereas this 'sophisticate' immune system has been a major weapon in the struggle between Vertebrates and an enormous diversity of parasitic organisms, it is by no means perfect. Attempts to 'domesticate' the immune system for the benefit of man and domestic animals have been a major endeavour of medicine during the last two centuries: specific vaccines and anti-infectious immunoglobulins illustrate this point. More recently, it was recognized that several pathological conditions are caused by inadequate functioning and regulation of the immune system: immunological deficiencies (congenital or acquired, dramatically exemplified by AIDS) and a significant number of autoimmune diseases, in which an immune response is aberrantly mounted against the individual's own cells or tissues. It is also recognized that certain tumor cells express specific antigens that distinguish them from non-malignant cells, thus giving rise to the hope that immunological intervention can play a role in the treatment of certain cancers. On the other hand, the normal function of the immune system in distinguishing nonself from self represents a serious obstacle to transplantation of tissues and organs from not fully histocompatible donors and this has justified the search for agents that could temporarily and selectively suppress some components of the immune system. Early in this century, it was recognized that the protective efficacy of vaccines could be enhanced by adding to them substances called adjuvants that nonspecifically stimulate the humoral and cell-mediated responses to the antigens [4].

Even earlier, at the end of the last century, Metchnikoff suggested [5] that nonspecific stimulation of the phagocytic cells through the injection of aseptic inflammatory substances could increase patients' resistance against infections and Coley [6] used bacterial culture filtrates to enhance rejection of tumor cells by the patients' immune system. Progressively, therefore, and very rapidly over the last two decades, a new scientific and medical discipline has emerged that deserves the name of immunopharmacology. Immunopharmacological agents or immunomodulators, can conveniently be divided between immunosuppressive drugs and immunostimulating drugs (including adjuvants), which correspond to the therapeutic needs outlined above.
The major issue facing immunopharmacological research in general is to define from which sources effective immunostimulating, adjuvant and immunosuppressive agents are better obtained: should one exploit the rich arsenal of endogenous molecules (mostly peptides), which serve as communication factors between cells of the immune system or look for immunomodulators (or biological response modifiers, as they are frequently called) among the almost infinite resource of natural or synthetic chemical substances, which can be tested in the laboratory for their possible immunomodulating activities?

As the title of our review implies, we shall restrict our discussion to the so-called immunostimulants, the medicinal purpose of which may be, according to the case, to enhance resistance against infectious agents, to stimulate rejection of cancer cells or to increase the immunogenicity of vaccines. There can be little doubt that several endogenous polypeptides of the immune system (cytokines and related factors) will play a role in various modes of immunostimulating therapy: examples are provided by interferon \( \alpha \) in the treatment of chronic hepatitis B virus infection, by interleukin-2 in the treatment of metastatic renal cell carcinoma, and by colony-stimulating factors (GM-CSF and G-CSF) which have been shown to accelerate neutrophil recovery following chemotherapy of malignant diseases, including very intensive therapy used with bone marrow transplantation. Interleukin-12 is showing remarkably broad potential powers against a variety of infectious diseases (AIDS, leishmaniasis, malaria, tuberculosis, schistosomiasis) and possibly some malignant conditions [7]. On the other hand, the therapeutic potential of cytokine manipulation includes also a great variety of illnesses in which a number of cytokines (tumor-necrosis factor, interleukins-1, -4, and -6) are implicated as part of the pathophysiological process: inflammation, rheumatic diseases, AIDS. Antagonists of such cytokines, including monoclonal antibodies against them, soluble receptors, receptor antagonists, are being developed [8–10] and immunomodulation by cytokine antiserum oligonucleotides is being contemplated [11]. Direct administration of cytokines or of their antagonists as well as in vitro treatment of immune cells that are reinfused in the patient’s circulation, and gene transfer (for instance the gene coding for the tumor-necrosis factor) are some of the strategies used in this expanding approach. It must be realized, however, that cytokines are definitely pleiotropic in their activities and that side effects (including fatalities) can be anticipated and are indeed observed in many of the clinical trials involving cytokine or anti-cytokine therapies. In the healthy individual, the various cytokines collaborate efficiently, in spite of their pleiotropism and redundancy, but artificial introduction of additional amounts of a given cytokine may disturb this equilibrium, which explains why cytokine therapy still poses difficult problems of dosage and timing of administration.

Another therapeutic approach, to which we shall devote the main part of this review, is to look for immunostimulating and adjuvant activities in exogenous molecules, either natural (of microbial origin for instance), semi-synthetic or totally synthetic. The possibilities there are unlimited and search for such agents is facilitated by the wide array of in vitro and in vivo tests that are available for the detection and analysis of immunostimulating activities, such as enhancement of resistance to infections, rejection of transplanted tumors, augmentation of antibody production or delayed type hypersensitivity against various antigens. Of course it should be realized that the activities of the various immunostimulating substances of exogenous origin which we shall review must be, to a large extent, mediated through their interactions (either already demonstrated or still awaiting clarification) with the cytokine network and that therefore there is no contradiction between the direct and the indirect approach. One distinct advantage, however, of the exogenous immunostimulants is that, in contrast to the polypeptides of the cytokine system, several of them are of small molecular mass, synthetic and readily accessible to chemical synthesis, thereby endowed with easily recognized pharmacokinetic characteristics, making it possible to administer them orally, for instance; they may also be stable in dry form at room temperature and precise dosage fractionation may be easier than with the cytokine molecules. Finally, many of them may be cheaper to produce, with simpler analytical controls than biotechnological products. One additional advantage is that, in the case of synthetic or semisynthetic immunostimulants, there is a wide open possibility of designing chemical analogues with improved activity and benefit-risk ratio.

The decision to pursue biological and clinical investigations of exogenous immunostimulants finds another justification when we look into the area of their counterparts, the immunosuppressive drugs. Whereas the field of pharmacological immunostimulation is still at a very early stage of development, immunosuppressive therapy has become part of everyday medical practice, either to prevent allograft rejection in patients receiving kidney, heart, lung, liver transplants or in treating a variety of autoimmune disorders, such as rheumatoid arthritis, insulin-dependent diabetes, lupus erythematosus, Crohn’s disease and probably other conditions in the future [12–14]. With the exception of a monoclonal antibody against the CD-3 marker of T lymphocytes (OKT 3), all the immunosuppressive drugs used in medical practice or undergoing clinical investigation are exogenous molecules, either microbial secondary metabolites (such as cyclosporin, the macroolides rapamycin and tacrolimus, 15-deoxyxyspergualin, mycophenolate mofetil) or synthetic, such as leflunomide or Brequinar sodium.

We shall now start a detailed review of the various immunostimulating agents and vaccine adjuvants that are either already used in medicine (actually a small number of them) or under preclinical and clinical development. We will make no attempt to exhaustively cover this field and the reader’s attention is called to some reviews that have appeared recently [15–18].

**Bacteria and bacterial products**

**Whole bacteria.** About 40 years ago, Biozzi et al. [9] showed that inoculation of live bacillus Calmette-Guérin (BCG, the antituberculosis vaccine) induced in the mouse a sustained hyperactivity of the phagocytic cells. BCG-inoculated mice were more resistant than control animals to infection with virulent staphylococci and Biozzi et al. [21] demonstrated that in BCG-inoculated mice there was an inhibition of growth of the Ehrlich ascite tumor. These experimental findings were the basis of the clinical use of BCG for the active immunotherapy of certain leukemias and subsequently of various solid tumors, an approach which was pioneered by Mathé et al. [22]. BCG has been employed, with various degrees of success, in the treatment of malignant diseases, alone or in combination with other therapies (surgery, chemotherapy, radiotherapy) but, at the present time, the most important and clearly beneficial application of BCG is in the treatment of bladder cancer [23, 24], in which live bacilli suspensions are administered intravesically. Bladder cancer is the sixth most common cancer in men and the fourteenth most common cancer in women; in 1985, it accounted for 182000 new cases and 82000 deaths worldwide. The first clinical trials with intravesical BCG showed a remarkable decrease in the rates of recurrence of bladder cancer and in the last 20 years, many tens of thousands of patients have benefited from this therapy, which involves repeated intravesical instillations of \( 4 \times 10^9 \) bacilli every
week for six weeks, followed by two more at six and twelve months after the beginning of treatment. The most frequent complication is cystitis or more distant infections (controlled by intravesical administration) and occasional fatalities due to anaphylaxis or sepsis. Progress is being made toward optimization of BCG therapy for bladder cancer [25]. Jackson and James [26] have carefully analyzed the mechanism of action of BCG in immunotherapy of bladder cancer. They have shown that several cytokines are identified in patients' urine following repeated instillation of BCG (interleukins-1, -2, -6, and -8, tumor necrosis factor a, interferon y) and that actually BCG interacts also directly with the tumor cells and exerts potent effects both on the growth of the tumor cells and on their phenotype. Their observations have led them to postulate a two-stage mechanism for an optimal clinical response to BCG therapy: stage 1, the tumor system; stage 2, the immune system. Simultaneous activation of the immune system and a response of the tumor system are necessary: the tumor cells must be induced to display some molecules (such as intercellular adhesion molecules, like intercellular adhesion molecule-1) which predispose them to cell-mediated cytotoxicity by the stimulated immune system. In conclusion, one of the earliest known immunostimulants, namely live BCG, has a recognized beneficial role in cancer immunotherapy. Indeed, many years before the work of Biozzi, Dubos and Mathé, the immunostimulating activities of tubercle bacilli (mycobacteria) had been experimentally demonstrated and led to the development of Freund's complete adjuvant in 1937 [27]. We shall come back later to the mycobacteria-derived chemically defined substances as important immunostimulants.

**Bacterial extracts.** While BCG is the only live bacillus clinically employed as an immunostimulant, a significant number of other bacterial products, of various degrees of purification, have found medical applications. One of them is OK 432 (Picibanil), produced by a low virulence strain of human Streptococcus pneumoniae treated with penicillin G; it is widely used in Japan in cancer immunotherapy, in combination with chemotherapy or radiotherapy. This preparation augments NK (natural killer) cell activity, activates macrophages to exert tumoricidal activity, enhances cytotoxic T lymphocytes and induces the production of various cytokines (interleukin-1, interleukin-2, interferon gamma, tumor necrosis factor, colony-stimulating factor GM-CSF). In Japan, this immunostimulant is part of the management of head and neck cancer, colorectal and gastric cancer, small cell cancer of the lung and brain tumors [28, 29].

It must be emphasized at this point that western medicine in general does not appear to share the optimism of Japanese investigators and clinicians concerning the usefulness of bacterial immunostimulants in the management of malignant diseases (with the exception of BCG in bladder cancer). In Europe, for instance, several preparations of bacterial origin are on the market exclusively as anti-infectious immunostimulants: these include a preparation composed of two glycoprotein fractions (molecular masses of 95 and 350 kDa, respectively) from Klebsiella pneumoniae and a preparation consisting of ribosomal and proteoglycan fractions from several common respiratory pathogens. These bacterial immunostimulants are administered either orally or intranasally (taking advantage of the mucosal immunity, through lymphoid areas present in the nasopharynx): placebo-controlled studies have shown that repeated administrations during the winter period provides a rather modest but statistically significant protection against respiratory infections in children or in elderly subjects (with chronic bronchitis), as evidenced by reduced numbers of infectious episodes, shorter duration and milder symptoms of such episodes, and decreased need to institute antibiotic treatment. Lyophilized lysates of Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus pyogenes, and Neisseria catarrhalis enter into the composition of another orally active anti-infectious immunostimulant, whereas fractions (primarily high-molecular-mass membrane proteins) from Escherichia coli are claimed to be an effective immunostimulating agent, always by the oral route, against recurrent urinary infections. Surprisingly, the same preparation exhibits immunomodulating activities that indicate its potential usefulness in the treatment of an autoimmune disease, namely rheumatoid arthritis [30].

Apart from immunopharmacological considerations, it is noteworthy that bacteria of the physiological microflora (digestive tract) exert a natural and permanent stimulus on certain immune functions: Beuth et al. [31] have shown that antibiotic decontamination of laboratory animals resulted in immunodepression; these authors found that certain members of the mouse gastrointestinal microflora (e.g. Bacteroides, Clostridium, Lactobacillus, Propionibacterium) actually liberate low-molecular-mass peptides (molecular mass less than 6.5 kDa) which are apparently essential for adequate immune responses of the host.

**Chemically defined bacterial products.** Of all the bacterial products that exert a stimulating effect on the functions of the immune system through their interaction with macrophages, monocytes and lymphocytes, the most potent ones, are probably the endotoxins, or lipopolysaccharides of gram-negative bacteria. Chemically, lipopolysaccharides (LPS) are heteropolymers consisting of polysaccharides covalently bound to a nitrogen-containing phospholipid called lipid A. LPS exert a wide spectrum of biological activities, lipid A being the active part in most of these effects. LPS preparations and purified lipid A have been shown to stimulate antibody production (LPS are polyclonal activators of B lymphocytes), to enhance the resistance of laboratory animals to bacterial, viral, fungal, and parasitic infections. To exert a necrotizing effect on tumors, all these effects being concomitant with increased production and release of various cytokines, such as interleukin-1, interleukin-6 and tumor-necrosis factor. Bocci [32] has formulated the interesting and plausible hypothesis that minute amounts of LPS constantly liberated from the gram-negative flora of healthy individuals and animals behave as natural immunostimulants, which play a crucial role in protection against pathogenic microorganisms. As the author stated: 'Human intestine, by harboring the bulk of gram-negative bacteria, contains more than enough to kill the host in a few hours, should they enter the circulation (as happens in septic shock). Luckily, this potential bomb rarely harms the host and actually, most of the time, has two beneficial effects. The first is mainly local, on the gut-associated lymphoid tissue, with consequent induction of IgA and a localized production of cytokines, which activate mononuclear cells. The second beneficial effect is due to traces of carrier-bound LPS which, by entering the circulation, reach organs such as liver, lungs, spleen, and bone marrow and elicit focal immunological reactions'. On the other hand, it is quite clear that exogenous administration of LPS or lipid A for therapeutic purposes would be a much too dangerous procedure, in view of the marked and pleiotropic toxicity of these molecules. Takada and Kotani [33] have performed a thorough study of structural requirements of lipid A for endotoxicity and other biological activities, with the purpose of designing novel compounds that would present an adequate balance between endotoxocities and beneficial bioactivities. Such a goal has been partly achieved by Ribi et al. [34] who showed that the minimal structure required for toxicity was a bisphosphorylated diglucosamine moiety, to which long chain fatty acids were attached: this was called diposphoryl lipid A. Several of the nontoxic or less toxic analogues differed from diphos-
phoryl lipid A by the simple fact that they lacked a phosphate group on the reducing end of the disaccharide; such material was called monophosphoryl lipid A. Bioassays on monophosphoryl lipid A showed that, while it was 1000 times less potent on a molar basis in eliciting toxic and pyrogenic responses, it was comparable to diphosphoryl lipid A (and endotoxin itself) in immunostimulating activities. Monophosphoryl lipid A has been shown to cause regression of certain grafted tumors in laboratory animals, to enhance production of colony-stimulating factors, to enhance resistance against infections, and to confer protection was comparable to diphosphoryl lipid A (and endotoxin itself) in immunostimulating activities. Monophosphoryl lipid A has been shown to cause regression of certain grafted tumors in laboratory animals, to enhance production of colony-stimulating factors, to enhance resistance against infections, and to confer protection against X-radiation. At the present time, monophosphoryl lipid A is being marketed as an immunity adjuvant for laboratory animals, to enhance production of colony-stimulating factors, to enhance resistance against infections, and to confer protection against X-radiation. At the present time, monophosphoryl lipid A is being marketed as an immunity adjuvant for laboratory animals, particularly within the framework of the production of monoclonal antibodies. Recently, a rather surprising finding was reported by Yao et al. [35], namely that monophosphoryl lipid A could represent a new approach for cardioprotection. Brown et al. [36] had observed that a low dose of endotoxin, given as a 24-h pretreatment, increased endogenous myocardial catalse activity and decreased ischemia-reperfusion injury in isolated rat hearts. Yao et al. performed in vivo studies of this phenomenon, by substituting much less toxic monophosphoryl lipid A to the endotoxin used by the previous authors. They found that this glycolipid induced functional protection against myocardial ischemia-reperfusion injury in rabbits and rats and that the same preparation reduced myocardial infarct size in dogs. The mechanisms by which monophosphoryl lipid A produces a cardioprotective effect when administered 24 h prior to ischemia is not clear, but the observation that pretreatment with monophosphoryl lipid A produced a marked decrease in myeloperoxidase activity (an index of polymorphonuclear leukocyte infiltration in the border zone surrounding the necrotic tissue) suggests that an effect of monophosphoryl lipid A on neutrophil function and/or traffic may play a role in the cardioprotective action of this preparation. Neutrophils are a major contributing factor in the pathogenesis of myocardial ischemia-reperfusion injury. In conclusion, monophosphoryl lipid A could be administered as preventive therapy 24 h prior to such procedures as angioplasty and coronary bypass surgery. According to Yao et al. [35], a phase II clinical trial with monophosphoryl lipid A is ongoing. As this example shows, a phase II clinical trial with monophosphoryl lipid A is ongoing. As this example shows, a particular aspect of its mechanisms of action, an immunostimulant may have quite unexpected applications.

Next to the gram-negative bacteria and their lipopolysaccharides, the mycobacteria represent a remarkable source of immunostimulants, as already illustrated by the case of BCG.

Polar glycopeptidolipids extracted from the cell wall of Mycobacterium chelonae (a microorganism isolated from the turtle) were shown to exert in the mouse a reversal of the doxorubicin-induced leucopenia, an effect comparable in its intensity to that exerted by the colony-stimulating factor GM-CSF [37]. The same preparation was also shown to enhance resistance of mice to disseminated infection with Candida albicans, an experimental model resembling the invasive and life-threatening fungal infections in immunocompromised patients [38].

On the other hand, attempts at fully characterizing the molecular entities responsible for the immunopotentiating activities of mycobacteria (such as those of BCG or of Freund's complete adjuvant) culminated 22 years ago [39] with the identification of N-acetylmuramyl-L-alanyl-β-isoglutamine (muramyl dipeptide, MDP) as the minimal essential structure required for adjuvant activity. Many publications have appeared concerning the activities and therapeutic potential of MDP and the numerous chemical analogs that were synthesized, and we shall not attempt to give here a comprehensive review of this field. The reader is referred to an excellent review published six years ago [40]. We shall only deal here with possible clinical applications of the MDP analogs that appear to be particularly interesting, namely murabutide (N-acetyl-muramyl-L-alanyl-ß-glutaminyl-n-buty1 ester), temurtide (threonyl-MDP), romurtide (MDP-Lys(18)N'-(N-acetylmuramoyl)-L-alanyl-α-ß-isoglutaminyl)-N'-stearoyl-L-lysine) and MTP-PeEtn (muramylpeptidase phosphadiylethanolamide).

Parenteral administration of MDP and its analogs in laboratory animals (mouse, guinea pig, rabbit) induces a complex range of biological effects: increased production of antibodies to various antigens, enhanced resistance to infections, enhanced tumoricidal activity of macrophages, restoration of myelopoiesis, fever, stimulation of slow wave sleep, induction of meningee inflammation, etc. On the other hand, oral administration of muramylpeptides has been reported to induce certain other biological effects that are not observed following parenteral ad-
administration, such as a downregulation of anamnestic antigen-specific IgE response. Bahr et al. [41] have reported the efficacy of a lipophilic derivative, N-acetyl-Mur-L-Thr-d-isoGln-sn-glyceryl-dipalmitoyl-MDP, incorporated into liposomes, in suppressing polyclonally induced serum IgE levels in anti-IgD-treated mice. The anti-allergic activity of certain muramylpeptides, exerted through their effects on gut-associated lymphoid tissues, may be one of the possible therapeutic applications of this family of compounds. The same authors have reported, on the other hand, that murabutide has been administered (in phase I and phase IIa studies) to 200 healthy volunteers and cancer patients: good tolerance was observed at doses up to 200 μg/kg injected subcutaneously. Synergistic activity between murabutide and interferon-α was demonstrated in vitro and in vivo models (protection of mice against endotoxic shock and against infection with the murine encephalomyocarditis virus) and such results gave support for assessment of a combination therapy between murabutide and interferon-α, in malignancies against which the latter cytokine has already shown some activity. Preliminary studies in healthy volunteers demonstrated excellent tolerance of murabutide when injected in association with interferon-α; interestingly, the incidence of side-effects caused by a high dose of interferon-α (6 X 10^6 International Units) was reduced upon association with murabutide.

Temurtide is an MDP analog, in which alanine has been replaced with threonine, that was selected from approximately 20 MDP analogs because of its superior adjuvant activity (on both humoral and cell-mediated immunity) and its lack of side effects at adjuvant-active doses (pyrogenicity, induction of adjuvant arthritis or uveitis) and which is now being developed as a vaccine adjuvant, as reviewed by Lidgate and Byars [42]. The dilution vehicle for threonyl-MDP (highly soluble in water) is composed of a finely dispersed metabolizable oil in an aqueous continuous phase. The oil is squalane and the emulsifiers are polysorbate 80 (Tween 80) and a nonionic surfactant (Pluronic L 121). Virtually all of the adjuvant remains in the aqueous phase of the emulsion, which is an oil-in-water emulsion instead of the water-in-oil emulsion of Freund’s adjuvant. Studies in laboratory animals have shown that temurtide exerts superior adjuvant activities toward the following antigens: influenza virus hemagglutinin; hepatitis B surface antigen; herpes simplex type 2 virus glycoprotein; Epstein-Barr virus, simian immunodeficiency virus, HIV-1 glycoprotein 120, as well as melanoma and β lymphoma tumor antigens.

Another MDP derivative, romurtide [N^2-(N-acetyl[muramoyl]-L-alanyl-D-isoglutaminyl)-N^6-stearoyl-L-lysine] is, since 1991, on the pharmaceutical market in Japan [43, 44]. Experimental data have shown this stearoyl-MDP to stimulate resistance of laboratory animals to bacterial and mycotic infections and to be a potent inducer of several cytokines, such as interleukin-1, interleukin-6, tumor-necrosis factor, interferon-α and of the colony-stimulating factors G-CSF and GM-CSF. The hematopoietic stimulation observed in monkeys receiving subcutaneous administrations of romurtide is attributable to the augmenting effect of this agent on the production of cytokines, and notably the colony-stimulating factors. In mice, romurtide promoted the recovery from a leucopenic state induced by cyclophosphamide treatment or X-irradiation and in X-irradiated guinea pigs, the same agent enhanced platelet recovery. Clinical trials have been performed in lung cancer patients undergoing chemotherapy with cisplatin, vindesine, and mitomycin C. Subcutaneous treatments with low doses of romurtide (200 μg) led to rapid recovery of white blood cells counts and to recovery of platelet counts, in comparison with a slower recovery in control patients that received chemotherapy alone. A similar restorative effect of romurtide was observed in cancer patients submitted to radiotherapy. Romurtide is now widely utilized in Japan for this therapeutic application. Fever is the most common side effect of romurtide treatment. However, whereas this drug induces the production of several cytokines, the severe toxic manifestations reported following injection of recombinant human interleukin-1, interleukin-6 and tumor-necrosis factor were not observed in the course of treatment with romurtide.

Another synthetic MDP analog of considerable interest is the lipophilic muramyltripeptide phosphatidylethanolamine (MTP-PtdEtn). This lipophilic MDP analog was synthesized to obtain a stable association of the compound with liposomes (because MTP-PtdEtn can be inserted into the phospholipid bilayer of the latter), following the observation that repeated systemic administrations of liposomal MDP, but not of the free form of MDP, caused in mice eradication of pulmonary and lymph node metastases from a subcutaneous murine melanoma [45, 46]. Systemic administration of liposomes containing MTP-PtdEtn has been shown to eradicate spontaneous metastases in several animal tumor models, including dogs with autochthonous osteogenic sarcoma metastases. Indeed, activating macrophages and monocytes to a tumoricidal state is one of the possible immunotherapeutic approaches to the immunological treatment of cancer. After several preclinical and phase I clinical trials in cancer patients had given the necessary information concerning the biological parameters influenced by liposomal MTP-PtdEtn and its side effects as well as acceptable doses, a phase II trial of this
agent was initiated in patients with osteosarcoma who developed pulmonary metastases during adjuvant chemotherapy or who presented pulmonary metastases that persisted despite chemotherapy. Liposomal MTP-PtdEtn was infused at a dose of 2 mg/m² twice weekly for three months. Histological examination of the tumor nodules recurring after this period showed unique morphological changes marked by peripheral fibrosis surrounding the tumor and inflammatory cell infiltration and/or a change in malignant characteristics from high grade before therapy to low grade after therapy [47]. Killion et al. [48], on the other hand, have shown that in mice, oral administration of lipophilic MTP-PtdEtn prevents the monocytopenia induced by chemotherapy (doxorubicin) or whole body X-irradiation, thus adding another aspect to the therapeutic potential of this agent in cancer patients.

The last three examples have illustrated the possible prophylactic or therapeutic applications of synthetic MDP analogs: potent adjuvant effect on the immunogenicity of vaccines in the case of temur tide, recovery from chemotherapy or radiotherapy-induced leukopenia in the case of romurtide, activation of tumoricidal state of monocytes as well as prevention of chemotherapy or radiotherapy-induced monocytopenia in the case of MTP-PtdEtn. The last two effects take us away from the original medicinal profile of MDP, based on its activities in experimental models, namely an enhancement of the host’s resistance to infections. However, as O’Reilly and Zak rightly stress [49], muramylpeptide immunostimulators could also be used clinically to enhance the effectiveness of conventional chemotherapies of bacterial, fungal, parasitic and viral infections, possibly by allowing the use of lower doses of the antimicrobial drugs or shortening the duration of treatment or simply in promoting faster patient recovery through combination of two attacks on the pathogenic microorganisms: the antimicrobial agent and the immune system (phagocytes and lymphocytes). It should be pointed out, however, that in the experimental models of infections that are used to analyze the activities of MDP and its analogs, treatment must be initiated before inoculation of the infectious agent (i.e. prophylactically) to exhibit significant activity, even when associated with post-infection chemotherapy.

The title of the present section is Bacteria and bacterial products and, as is evident from the discussion concerning MDP derivatives, many of the so-called bacterial products do have a structure basically reminiscent of the structure of a natural bacterial product (cell wall constituent, for instance) but are in fact synthetic preparations (MDP analogs such as murabutide, temurtide, romurtide, and MTP-PtdEtn).

Semi-synthetic molecules of bacterial origin. A similar approach was followed by our team about 15 years ago, when we became interested in the immunostimulating activity of crude water-soluble extracts of a Streptomyces strain and attempted to identify their active component. A tetrapeptide, L-Ala-D-Glu(L-L-A-pm(GLy))NH₂ was isolated, but was found to be inactive in immunostimulation tests. Following a lead provided by our earlier work showing that chemical conjugation with fatty acids of water-soluble fragments from strains of M. tuberculosis modified the immunoadjuvant properties of these substances by rendering them active in the absence of mineral oil [50], the inactive tetrapeptide was conjugated with vaccin acylation. The compound thus obtained (lauroyltetrapeptide) exhibited marked in vitro and in vivo immunopotentiating activities [51, 52], which justified its preparation by total synthesis as well as the synthesis of close to 100 analogs. The biological activities of the synthetic lauroyltetrapeptide and of several analogs have been described in several publications [53–58] and may be summarized as follows: adjuvant activity on antibody production and on delayed type hypersensitivity reactions against various antigens, enhancement of the resistance of mice against bacterial infections (including intracellular microorganisms, like L. monocytogenes), increased resistance of mice to the lethal effect of γ-ray irradiation (radioprotective activity), decrease of the amount of hepatic microsomal cytochrome P-450 and of the level of CCL₂-induced lipid peroxidation. Among the likeliest mechanisms of action of pilemulide and its analogs, one can quote a stimulation of phagocytosis and of interleukin-1 and tumor-necrosis factor production by macrophages and monocytes, an enhancement of interleukin-2 production by lymphocytes, a stimulation of cytolytic T lymphocytes and the production of the colony-stimulating-factor-like factors in the serum of mice. It was also realized that the synthetic lauroyltetrapeptide is a mixture of two stereoisomers, of which the active component was the molecule containing dianinopimelic acid in its L,L form (pilemulide, RP 44102). The presence of glycine in the molecule is not essential for the immunopharmacological activity of lauroyltetrapeptide, since the lauroyltripeptide N₃₋(N-(N-lauryl-L-alanyl)-γ-D-glutamyl)-L-L,L-2,6-diaminopimelamic acid (RP 56142, trimexautide) exhibited activities comparable to those of the tetrapeptide. Recently, Dépréz et al. [59] described the synthesis of analytically pure immunogens, in which a hexadecameric peptide (V3) derived from the principal neutralizing domain of the envelope glycoprotein of HIV-1 was associated with either pilemulide or trimexautide. The in vivo (in Balb/C mice) immunogenicity of these compounds (built-in adjuvants) was evaluated according to two criteria: the ability to elicit a T-lymphocyte cytotoxicity and the ability to stimulate antibody response. The results indicated that the trimexautide conjugate was able to induce an efficient and relevant HIV-1-specific T-lymphocyte cytotoxicity response, whereas the pilemulide conjugate stimulated a strong antibody response to the linked viral peptide. Such a chemically defined model of peptide vaccines against HIV-1 may be used to selectively stimulate subpopulations in immunocompetent cells and, obviously, a similar strategy could be used in designing vaccines against other viruses against which both cell-mediated and humoral immune responses are important in terms of protection.

One should also mention the discovery in 1981 in Japan of the marked immunostimulating activities of compounds belonging to the same general family as the lipopeptides described above, the acyloligopeptides. The parent compound in this case was a natural metabolite of Streptomyces olivaceoroseus: D-lactoyl-L-alanyl-γ-D-glutamyl-(L)-meso-2,6-diaminopimelylglucose (FK-156), of which several analogs were synthesized [60–63]. One of the most active analogs appears to be the acyloligopeptide FK-565: heptanoyl-γ-D-Glu-(L-meso-α,β-A-pm(GLy)-
Ala-OH. Acyloligopeptides were found to enhance resistance against bacterial or viral infections in animals with either compromised or competent immune systems and FK-565 did exert a stimulation of NK (natural killer) cells [64].

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the lauroyltetrapeptide molecule. One of the compounds thus synthesized is

MeAla-D-Glu-NH₂ Gly-Lys-NH₂.C₆H₄O₇(n),

which was shown to exert in mice a partial protective effect against infection with Leishmania donovani. Recently, Sidwell et al. [66] reported that a lipophilic desmuramyl dipeptide analog, the hydrochloride salt of octadecyl-D-alanyl-L-glutamine, enhanced the resistance of mice to the infection with murine cytomegalovirus and exerted a modest protective effect on the infection with mouse-adapted human influenza A virus.

Bessler and his coworkers have concentrated their research efforts on a totally different class of synthetic ligopeptides with immunoajvant activities, the chemical structure of which is derived from the lipoprotein of gram-negative bacteria such as Escherichia coli [67-70]. One example is provided by the palmitoylpeptide that is identical to the N-terminus of the bacterial lipoprotein with respect to the S-glycerylcysteine and the peptide part, but differs in the composition of the fatty acid part (in this case palmitic acid). These ligopeptides exert potent adjuvant activities, when administered in combination with particulate or soluble antigens to various laboratory animals; they potentiate both the cellular and the humoral responses. These adjuvants, which are devoid of pyrogenicity (like some but not all muramylopeptides), can be coupled covalently to low-molecular-mass haptenes (peptides or toxins) and the resulting conjugates are able to elicit high hapten-specific antibody titers in mice and rabbits. Conjugates containing B or T helper cell epitopes constitute novel synthetic vaccines that protect against viral infections by inducing the production of virus-specific antibodies. When coupled to T-lymphocyte cytotoxicity epitopes, the conjugates can induce the appearance of cytotoxic T lymphocytes, which in vivo may eliminate virus-infected cells. For instance, a vaccine against foot-and-mouth virus disease was prepared by coupling a lipopeptide adjuvant to suitable VP-1 segments of the viral protein: this preparation gave full protection to guinea pigs against challenge with the homologous virus type. The technique of in vitro immunization using a lipopeptide adjuvant can be used for the production of monoclonal antibodies. Lipopeptide-antigen conjugates were also used to produce in vivo (mice) and in vitro (human or murine cells) HIV-specific antibodies.

Substances of fungal origin

Numerous polysaccharides from various biological origins (yeasts, algae, bacteria, higher plants and especially fungi) have been investigated for anti-tumor and immunomodulating activities. Active polysaccharides have been shown experimentally to exert anti-tumor effects against allogenic, syngenic and even autologous tumors and the general consensus at the present time is that these antitumor activities are mediated to a large extent by stimulating effects on the immune system (macrophages, NK cells, cytokine induction).

Several recent reviews have been devoted to the biochemical and pharmacological characteristics of glucans endowed with immunostimulating activities [71-74]. These glucans consist of a linear backbone of β(1,3)-linked D-glucopyranosyl groups with varying degrees of branching from the C6 position. Reported degrees of branching (number of branches/main chain residues) should be considered average values. Branches are usually only a single glucose residue, although more than one glucose unit may be present in some glucans. The immunostimulating glucans which were first discovered (and are used clinically) in Japan, namely schizophyllan (an extracellular polysaccharide from the culture filtrate of Schizophyllum commune) and lentivan (a cell wall glucan from Lentinus edodes) present interestingly a triple helical conformation, as shown by viscosity measurements, NMR and X-ray diffraction studies. Triple helical parallel strands of glucan are hydrogen bonded via the C2 hydroxyls, with higher order structures arising through hydrogen bonding between C4 and C6 hydroxyls, the latter being present on the external surface of the triple helix. β-Glucans can also adopt single chain, and single helix forms as assessed by NMR spectroscopy. The physicochemical properties and biological activities of glucans can be modulated by chemical modification; for instance, insoluble yeast glucan was converted to water soluble glucan sulfate, or glucan phosphate, with a significant decrease of its toxicity (hepatosplenomegaly, granuloma formation, formation of microembolisms), thus providing safer compounds for parenteral administration. The immunostimulating activities of glucans have been demonstrated in many experimental models, in addition to those involving tumors: stimulation of resistance to bacterial, fungal and viral infections, stimulation of hematopoiesis (likely through induction of colony-stimulating factors), stimulation of wound healing. Enhancement of the production of various cytokines (interleukin-1, tumor-necrosis factor, interleukin-6) was reported in vitro and in vivo, as well as stimulation of cytotoxic T lymphocytes. Tumor regression in various animal models can be ascribed to vascular damage to tumor blood flow and to necrosis caused by T cells and local tumor-necrosis factor production. When injected into one of a pair of double-grafted tumors, in mice, soluble scleroglucan (an exopolymer produced by Sclerotium glucanicum) remained localized in the injected tumor but exerted shrinking effects on the distal tumor, presumably through stimulation of the immune response [75]. Scleroglucan was also found in the blood, liver and spleen when administered to normal mice, with significant amounts remaining in the liver and spleen for up to four weeks.
Clinical studies in man of various glucans have been performed essentially by Japanese investigators. Lentinan (500 kDa molecular mass, composed of a linear \( \beta(1,3) \)-glucan with \( \beta(1,6) \)-linked branches) is being used in association with various chemotherapy regimens, in the treatment of gastric, colorectal, and breast cancers. Schizophyllan (450 kDa molecular mass) is used in conjunction with radiotherapy in the management of cervical cancer. Increased disease-free survival and improved quality of life for the patients are reported both for lentinan and schizophyllan, but it does not appear that these interesting results have convinced oncologists outside of Japan about the usefulness of combining this immunostimulating approach with conventional anti-cancer chemotherapy.

In the United States, clinical development of a genetically modified (engineered) glucan from \textit{Saccharomyces cerevisiae} PGG-glucan (Betafectin) is in progress. This compound is a triple-helical \( \beta(1-3)\beta(1-6) \)-linked glucose polymer with a unique branching structure [76]. Its superior in vitro biological activity is reflected in its increased glucan receptor-binding affinity as compared to naturally occurring \( \beta \)-glucan. Through \( \beta \)-glucan receptor binding, PGG activates macrophages, inducing a cascade of interactions mediated by the release of monocyte-derived cytokines. Administration of PGG to mice enhances resistance to bacterial and fungal infections and accelerates recovery from drug-induced neutropenia in mice treated with cyclophosphamide [77]. In \textit{in vitro} cells treated with PGG generated significantly increased levels of hydrogen peroxide and the glucan also stimulated nitric oxide production by rat neutrophils. Recently, double-blind placebo-controlled randomized phase II clinical studies examined the safety and efficacy of PGG-glucan in preventing postoperative infection in patients undergoing major thoracic or abdominal surgery [74]. The patients received multiple intravenous doses of PGG (from 0.5 mg/kg to 2.0 mg/kg). Infection incidence and severity was lower in the PGG-glucan-treated patient group, as was the incidence of postoperative antibiotic usage and duration of hospital stay. Two other independent studies reported efficacy of PGG administration to trauma patients: intravenous injections every 12 h decreased the incidence of pneumonia and sepsis in a group of 21 patients, in a randomized, double-blind placebo-controlled trial. Slight increases in interleukin-2 levels, but not tumor-necrosis factor levels, in the blood were transiently observed.

It is interesting to note that several studies have demonstrated the biological activity of glucans administered orally to laboratory animals. For instance, Nicoletti et al. [78] have shown that a glucan extracted from \textit{Candida albicans} enhanced, upon oral administration of low doses to mice, their resistance to systemic infection with either \textit{Staphylococcus} or \textit{Candida albicans} and stimulated interleukin-2 production in the blood. This anti-infectious activity is of prophylactic nature, since treatment of the mice must be initiated ten days before the infectious challenge. The fact that a high-molecular-mass substance can exert immunostimulating activities when administered orally is not altogether surprising in view of what is presently known concerning the existence and activity of a system of mucosal immunity, such as the gut-associated lymphoid tissue and the lymphoid tissue associated with the upper respiratory tract.

Even more complex than the glucans described above, is the protein-bound glucan extracted from the edible mushroom \textit{Coriolus versicolor} and used in clinical oncology in Japan under the name of Krestin, or PSK. Krestin (average molecular mass 100 kDa) is obtained by hot water extraction from the cultured mycelium; it contains 18–38% protein and the main fraction of the polysaccharide part is a \( \beta \)-glucan, with main chain 1–4 bonds and branches at the 3 and 6 positions, in a proportion of one per several residues. This interesting substance has been shown, in various experimental models, to exert significant antitumor mechanisms mediated through stimulation of macrophages, monocytes, NK cells and various T lymphocyte populations; it also enhances the resistance of laboratory animals to bacterial, fungal and viral infections and exerts a chemopreventive effect against carcinogenesis [79]. In models with various methylcholanthrene-induced tumors, there was a good correlation between the antitumor activity and the antigens of the tumor. There is clear evidence that Krestin is mostly effective in host-tumor systems, in which tumor-induced immunosuppression can be observed. The compound has also been shown to exert antimetastatic effects [80] and to induce the production of monokines such as tumor-necrosis-factor-\( \alpha \) and interleukin-1.\( \beta \). Activities are exerted following various of routes of administration, including the oral route.

Krestin is extensively used clinically in Japan, as an anticancer immunotherapeutic agent, in association with conventional anticancer chemotherapy (for instance 5-fluorouracil or mitomycin). Randomized controlled trials of the compound have been performed, following primary tumor resection, in patients with gastric cancer [81] and in colorectal cancer patients [82], comparing the disease-free periods and the overall survival in patients receiving chemotherapy alone and in those treated in addition with Krestin (3 g/day per os for prolonged periods). In both sets of trials, the disease-free period and the five-year survival were significantly increased in the patients receiving the combination in comparison with those treated with chemotherapy alone. Side effects (diarrhea, nausea) were rarely observed. A plausible explanation of the clinical efficacy of Krestin is that this immunostimulant antagonizes humoral immunosuppressive factors that are produced in tumor-bearing hosts: quality of life and resistance to infections are improved and antimetastatic effects may also be observed.

Immunostimulating activities have also been reported for polysaccharides extracted from higher plants and, in some cases, have led to the pharmaceutical use in certain countries of complex extracts of plants such as \textit{Echinacea purpurea} or \textit{Vaccinium album} [83, 84]. These preparations are administered, generally by the oral route, to young children or to elderly individuals to increase their natural resistance to bacterial or viral infections of the upper respiratory tract; their widespread use in some areas gives indirect indication of their efficacy in this context. The active ingredients in these complex preparations appear to be water-soluble polysaccharides, such as xylglucan, arabinogalactan and pectin, and probably also glycoproteins as well as flavonoids.

Immunostimulating peptides have, on the other hand, been isolated from soybean, and the following amino acid sequences have been reported for the active peptides: Ala-Glu-Ile-Asn-Met-Pro-Asp-Tyr, Ile-Gln-Gly-Asn, and Ser-Gly-Phe-Ala-Pro [85].

**Substances of mammalian origin**

Strictly speaking, the thymus-derived hormones (thymic peptides) and tuftsin (a tetrapeptide derived from the Fc portion of the immunoglobulin molecule) should not be discussed here, since they are not the exogenous substances (to which the present review restricts itself), but can be considered as natural endogenous immunomodulators. They will however deserve brief mention, inasmuch as several such peptides are prepared by chemical synthesis or extracted from bovine thymus and used in various clinical applications.

The nature and therapeutic uses of synthetic thymic peptides have been reviewed fairly recently [17, 18, 86]. The pentapeptide thymopentin (TP-5), corresponding to the Arg-Lys-Asp-Val-
Tyr sequence of thymopoietin has found clinical use in the treatment of autoimmune diseases, such as rheumatoid arthritis. Thymosin α1 is a 28-amino-acid recombinant peptide derived from thymosin fraction V; current trials are in cancer immunotherapy and in the treatment of chronic hepatitis B virus infection [87]. Thymic humoral factor is the synthetic octapeptide leucyl-glutamyl-aspartyl-glycyl-prolyl-lysyl-phenylalanyl-leucine; in an open nonrandomized pilot clinical study of patients with immunological defects associated with anticancer chemotherapy or severe infection, thymic humoral factor augmented lymphocyte populations in 70% of the patients, with a trend toward CD4/CD8 ratio normalization [88]. It is also in phase II clinical trials, in combination with azidothymidine, in HIV-seropositive individuals.

Thymomodulin and thymostimulin are purified extracts from calf thymus and are rather extensively used clinically, especially in Italy. Thymomodulin contains a mixture of low-molecular mass (less than 10 kDa) proteins. It has been claimed to improve the course of chronic infectious diseases, such as chronic bronchitis and recurrent pediatric respiratory infections [89]. Braga et al. have reported restoration of polynucleonuclear leukocyte functions in elderly subjects treated with thymomodulin [90]. Thymostimulin is another bovine thymic extract that has been reported to show efficacy, following repeated intramuscular injections, in the treatment of recurrent respiratory infections in small children [91]. Periti et al. [92] have conducted a prospective controlled multicenter study showing the benefit of chemoinmunoprophylaxis (the antibiotic cefotaxim and thymostimulin) in patients undergoing colorectal surgery: abdominal abscesses and respiratory tract infections were significantly less frequent in patients submitted to this chemoprophylactic combination than in those receiving a placebo.

The tetrapeptide tuftsin, Thr-Lys-Pro-Arg, is known to enhance phagocytosis, bactericidal, and tumoricidal activities of macrophages and to enhance the release of interleukin-1 and tumor-necrosis factor; numerous analogs of tuftsin have been synthesized (polytuftsins, glycotuftsins derivatives, O-glycosylated tuftsins), but as far as we know, very little has been achieved so far in terms of delineating the potential clinical usefulness of these molecules, which seems surprising when considering that the biological activities of this natural tetrapeptide were first described almost 30 years ago.

Switching from the thymus and the immunoglobulin molecule, we may turn now to the mammary gland as a source of immunostimulating substances. Casein has been described as a milk protein with diverse biologic consequences [93]; casein fragments exert a rather wide range of immunopharmacological, hematological and neuropharmacological effects. Several years ago, our team observed that enzymatic fragments obtained from human casein (maternal milk being frequently and advantageously man’s first food) exhibited immunostimulating activities, such as stimulation of phagocytosis by macrophages and enhancement of resistance of mice to a bacterial infection [94]. The purification, sequence, synthesis and further biological activities of an immunostimulating hexapeptide (Val-Glu-Pro-Lle-Pro-Tyr) from human β-casein were described [95]. Furthermore, the presence of some other biologically active short peptides has been detected in human as well as in cow milk caseins, such as Gly-Leu-Phe and Leu-Leu-Tyr [96, 97]. Whether such peptides, released from milk by enzymes in the gut, do play a role in the natural resistance of breastfed infants to infections (in addition to the obvious role of maternal antibodies) and could find therapeutic applications remains to be investigated.

**Vitamins**

As stated in a comprehensive review on vitamin A status and its relationship to immunity [98], vitamin A (retinol), a fat-soluble vitamin, plays an essential role in several biological processes, many of which concern growth, cellular differentiation and cell-cell or cell-substrate interactions. Vitamin A is important in maintaining the functional integrity of epithelial and mucosal surfaces and in the production of mucous secretions. Clinical and experimental data have shown that vitamin A deficiency is associated with impaired resistance to infections. Vitamin A supplementation can decrease morbidity in preschool children in populations that are at high risk for vitamin A deficiency; some of the benefit of vitamin A is due to restoration of normal epithelial barriers, resulting in improved resistance to respiratory and gastrointestinal infections but stimulation of the immune system must also be taken into account. For instance, moderate increases in dietary vitamin A were reported to enhance host resistance to infection with bacterial or fungal pathogens and experiments in chicks, rats and mice have shown that administration of nontoxic doses of retinol or retinoic acid stimulates phagocytic functions of macrophages and antibody production as well as NK cell activity and antibody-dependent cell-mediated cytotoxicity. Vitamin A can function as an adjuvant in immunization experiments with tetanus toxoid, cholera toxin, pneumococcal polysaccharide or other antigens. Vitamin A and retinoic acid have been also shown to stimulate the rejection of certain immunogenic tumors in mice [99]. Several studies on antibody production and phagocytosis support a role of retinoids in immunostimulation in animals in which vitamin A nutritional status is normal, so that retinoids can be considered as bona fide immunostimulating agents, and not just immunorestoring agents in deficient hosts. Even in developed countries, vitamin A dietary intake may in many individuals (especially women using low-fat diets and poorly nourished elderly subjects) be definitely suboptimal and supplementation (at least 3000 IU/day) is advisable to avoid the immunosuppressive effects of this deficiency.

Turning now to another vitamin, it is known that, in addition to its established role as a calcium regulating factor, the active metabolite of vitamin D, namely the sterol 1,25-dihydroxyvitamin D₃, exerts anti-proliferative, predifferentiating and immunosuppressive properties. Lemire has shown [100] that this compound inhibits the production of interleukin-2 and interleukin-12 as well as that of interferon γ and prevents in vivo the development of spontaneous or induced models of autoimmunity; Alroy et al. [101] have reported that other cytokines, such as interleukin-4 and GM-CSF (granulocyte-macrophage colony-stimulating factor), seem to be downregulated by vitamin D3 in T lymphocytes, in which promoters have the same binding sites for activators. The question then arises about a possible physiological role of vitamin D in the immune system; in this respect, it is worth noting that suboptimal levels of vitamin D are frequently observed in elderly individuals, due to a deficient diet and insufficient exposure to sunlight.

Vitamin E (z-tocopherol) has also been claimed to be endowed with immunomodulating activities: Shkalar et al. [102] have reported that treatment with vitamin E of hamsters, in which buccal pouches were repeatedly painted with dimethylbenzanthracene, inhibited the formation of the gross tumors observed in the control animals; instead, the small tumors seen in the treated hamsters were densely infiltrated with lymphocytes and macrophages. On the other hand, Wang and Watson [103] have reviewed the available evidence on the immunosuppressive effect of excessive alcohol consumption and the immunoenhancing activities of vitamin E and proposed that...
supplementation with this vitamin could provide a useful therapeutic approach to enhance the resistance of alcoholics to infections.

Antibiotics

The concept of a possible interaction of clinically effective antimicrobial agents with various functions of the immune system is presently the subject of intensive research, which has been lucidly reviewed by Labro [104]. Obviously antibiotics capable of exerting immunosuppressive effects (such as chloramphenicol and rifampicin) should not be used in immunocompromised patients, whereas antibacterial agents endowed with an immunostimulating activity, in addition to their antimicrobial activity, could represent a most welcome therapeutic strategy in several infectious situations. Apart from such a direct potentiating effect on immune reactions it is clear that the alterations induced in bacterial metabolism and morphology by most antibacterial agents, at concentrations prior to their minimal inhibitory or bactericidal values, may facilitate phagocytosis by macrophages and polymorphonuclear leukocytes; furthermore, several antibiotics (such as the macrolides) have the ability to penetrate phagocytes in infections caused by intracellular bacteria (such as Listeria monocytogenes). On the other hand, a great number of in vitro experiments have shown some antibiotics to exert stimulating or inhibitory activities on several functions of phagocytic cells and T and B lymphocytes, including production of cytokines. Whether such immunomodulating activities have a real significance in vivo situations is open to question, inasmuch as the in vitro effective concentrations are quite different from the blood levels reached following in vivo administration of effective antibacterial doses. Nevertheless, it is now established that some antibacterial agents are indeed truly capable to directly modify the immune responses. As mentioned by Labro [104], cepimizole was the first molecule to be proposed as a bona fide immunomodulating antibiotic, capable of stimulating monocytes to release neutrophil-activating factors. More recently, the oxymino-amino-2-thiazolyl cephalosporin cefodizime has been shown to enhance and/or restore some immune functions in vitro andex vivo and to be effective in various ex vivo experiments. We must also stress the fact that many of the compounds from natural sources described in the preceding sections are obtained by chemical synthesis, but their structure is identical to or inspired from the structure of natural molecules.

Compounds from mineral chemistry.

Compounds from mineral chemistry have been shown to exert immunostimulating activities. For instance, dietary supplementation with selenium (as sodium selenite) restores in mice the age-related decline in immune cell function, probably through an increase in the number of high-affinity interleukin-2 receptors on T lymphocytes [108] and enhances internalization of interleukin-2 [109]. Supplementation with Se in vivo or in vitro results in an earlier expression of high-affinity interleukin-2 receptors, whereas Se deficiency results in a delayed expression of lower numbers of receptors [110].

Propagermanium, i.e. 3-oxygermylpionic acid polymer, was shown, upon oral administration in mice, to enhance resistance to infection with herpes simplex virus type 1, through stimulation of cytotoxic T lymphocytes and NK cells and the same compound enhanced interferon production in BCG-sensitized and in influenza-virus-infected mice [111]. Clinical studies are under way, following reassuring toxicological data in laboratory animals and in healthy volunteers [112].

AS-101, or ammonium trichlorodioxymethylene-O-O′cellulurate was developed in Israel and was shown to stimulate the production of a variety of cytokines. Phase 1 clinical trials in cancer patients showed an enhancement of the secretion of tumour-necrosis factor, interferon and interleukin-2. This compound was shown to have radioprotective activities, when injected into mice prior to sublethal or lethal doses of radiation and to protect mice from hemopoietic cell damage caused by cyclophosphamide [113].

Miscellaneous synthetic molecules

We shall review here a rather large number of synthetic compounds exerting immunostimulating activities (often leading to clinical applications) but the chemical structures of which could not a priori allow prediction of such activities, in marked contrast with molecules originating from microbial, fungal or mammalian sources. Several years ago, Georgiev [16] published a comprehensive review on synthetic immunomodulating agents, but we shall restrict the present review to those synthetic compounds exhibiting a sufficiently wide range of in vitro immunostimulating activities, without mentioning the many molecules of no practical potential may exert various types of effects that are not confirmed by in vivo experiments. We must also stress the fact that many of the compounds from natural sources described in the preceding sections are obtained by chemical synthesis, but their structure is identical to or inspired from the structure of natural molecules.

Tetramisole and levamisole.

The imidazothiazole tetramisole and its levorotatory isomer levamisole have been known for many years for their marked effectiveness as anthelmintics (especially against nematode infestations, such as ascaridiosis) in man and domestic animals. In 1971, Renoux and Renoux [114] observed that mice immunized with a poorly immunogenic

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anti-Brucella vaccine were nevertheless protected against challenge with live Brucella bacilli if they had been treated with levamisole or tetramisole at the time of immunization. It was the first time that a simple synthetic compound was shown to potentiate an immune response, in other words to behave as an adjuvant. The mechanisms of the immunopharmacological activities of levamisole have been investigated by many immunologists and thus far the picture is still quite complex; stimulation of recruitment and functions of macrophages, monocytes and various classes of T lymphocytes, especially in conditions of suboptimal immune responses, can be referred to when dealing with the global effects of levamisole. But what is more important is that, after many rather inconclusive clinical trials of levamisole in a wide variety of illnesses possibly associated with impaired immune functions (recurrent infections, rheumatoid arthritis, Crohn’s disease, leukemia, aphthous stomatitis, etc.), this drug is now used, in combination with the anticancer agent 5-fluorouracil, in the treatment of colorectal cancer in patients with stage C (Duke’s classification) disease and the results in terms of prolongation of disease-free interval and overall survival have been so superior to those observed in patients receiving 5-fluorouracil alone, that the Food and Drug Administration has approved the use of levamisole and 5-fluorouracil as the standard therapy for stage C colon carcinoma [115, 116].

The mechanism(s) by which levamisole exerts this beneficial effect are still inadequately elucidated. Kimball [117] states that this agent may be able to promote antitumor responses by increasing the production of cytokines that can be directly cytotoxic to tumors (in synergy with 5-fluorouracil) and that can promote and sustain cell-mediated host responses to cancer cells. In addition, levamisole may serve to render certain tumors more susceptible to immune responses of the host, by increasing the exposure of their surface recognition molecules, but these are mainly hypotheses. On the other hand, recent reports involving the use of levamisole in the treatment of steroid-dependent pediatric nephrotic syndrome demonstrated encouraging results [118], a somewhat paradoxical finding inasmuch as the alternative to corticosteroids for the treatment of this syndrome has generally been immunosuppressive agents, such as cyclophosphamide.

**Nucleic acid derivatives.** Nucleic acid derivatives provide another source of immunostimulating agents, as exemplified by pyrimidinones, guanosine derivatives, inosine-5′-methylmonophosphate and hypoxanthine derivatives. The immunostimulating properties of pyrimidinones can be summarized as follows [119]: (a) induction of interferon-α, tumor-necrosis factor a and interleukin-2; (b) stimulation of the expression of receptors to interleukin-2; (c) induction of proliferation and differentiation of B lymphocytes; (d) stimulation of macrophage and NK cell activities; (e) restoration of levels and function of T, lymphocytes in immunosuppressed mice. All these studies were performed *in vivo*, very few direct effects being demonstrable *in vitro*. It appears that the primary effectors cells activated by pyrimidinones are the macrophages and the NK cells. Of all the pyrimidinones synthesized, the most interesting one is bropirimine, which exerts in animal models a wide variety of antiviral and antitumor activities. The antiviral activities of this agent (in a large number of animal models) can be explained by its interferon-inducing property as well as the stimulation of NK-cells. The antitumor activity of bropirimine and the pyrimidinones in general has been in models involving its association with chemotherapeutic agents (cyclophosphamide, doxorubicin, mitomycin C, cisplatin, etc.), especially with those exerting no suppressive effects on NK cells functions. As a result of these extensive observations on the antitumor potential of bropirimine, clinical studies have been performed with this agent in bladder cancer patients, to whom the drug has been administered by the oral route [120]. The phase I trial indicated that bropirimine had significant single-agent activity in carcinoma *in situ* of the bladder, a good number of patients demonstrating complete or partial responses. It appears too early to demonstrate that, in bladder cancer patients, the efficacy of bropirimine approaches that of BCG, administered intravesically: if this was indeed the case, bropirimine might have several advantages over BCG (more convenient route of administration and less side effects); the total daily dose of 4.5 g for three consecutive days each week was well tolerated. Trials of combination of bropirimine with BCG are also under way. Phase II trials also showed possible efficacy of bropirimine in nodular lymphoma and hepatoma, but renal cell carcinoma was unresponsive to this therapy.

Guanosine derivatives have been extensively studied with respect to their immunomodulating activities. Anderson and Cater [121] described the activities of 7-allyl-8-oxoguanosine which, in various experimental models, exhibited adjuvant-like effects in enhancing antibody production in response to several antigens, even in the absence of T lymphocytes, while being devoid of pyrogenicity and unable to exacerbate autoimmune manifestations. More recently, several related analogs of 7-allyl-8-oxoguanosine (loxoribine) have been synthesized by Chen et al. [122]: 2′,3′-ketal ofloxoribine display a significant activity on their own, apparently without being cleaved to the free nucleoside.

Hypoxanthine derivatives, such as ST-789, a 9-pentylarginine-hypoxanthine compound, were shown to enhance resistance of immunosuppressed mice to infections and to exert antitumor activities, likely mediated through stimulation of NK cell activity and of production of interleukin-6, a pleiotropic cytokine [123].

Inosine-5′-methyl monophosphate was described as a thymomimetic immunomodulator, acting directly on T lymphocytes (induction of T-cell differentiation markers and interleukin-2 receptors in human pro-thymocytes), increasing T-cell dependent antibody formation and delayed type hypersensitivity (DTH)
responses, enhancing resistance of laboratory animals to bacterial and viral infections and exerting antitumor activities [124]; it is active both orally and parenterally.

**Unrelated structures.** A number of unrelated chemical structures have been shown to provide new leads for the development of promising immunostimulating agents. One of those is imiquimod (R-837 or S-26308), i.e. (1-(2-methyl propyl)-1H-imidazo(4,5-c)quinolin 4-amine), which has been found to be an effective antiviral and antitumor agent in animal models, without exerting such activities in vitro but acting through the induction of various cytokines, such as interferon-\(\alpha\), tumor-necrosis factor-\(\alpha\), interleukin-1 \(\alpha\) and \(\beta\), interleukin-6 and interleukin-8, monocytes being largely responsible for the cytokines produced [125]. Imiquod protects guinea pigs from infection by herpes simplex virus when administered intravaginally, intramuscularly, intraperitoneally, and orally, and reduces recurrences caused in guinea pig by latent infection with herpes simplex virus [126]. The same agent is active prophylactically and therapeutically against cytomegalovirus infection and arbovirus infection in mice. On the other hand, imiquimod has been shown to inhibit the growth of a number of murine tumors, including a tumor induced by a chemical carcinogen. Phase I studies in healthy adult males indicated that imiquimod administered orally induced detectable serum concentrations of interferon-\(\alpha\). Imiquimod also exerts adjuvant activities versus a herpes simplex virus type 2 glycoprotein vaccine in guinea pigs [127]. Recently, the cellular requirements for cytokine production in response to imiquimod have been characterized [128]: the cell population responsible for the majority of cytokine release in human peripheral blood monocytes in response to this agent appears to be E rosette-, CD14+, CD 36+, HLA-DR+ monocyte.

Pyrazolo[3,4-f]quinoline derivatives have been reported to exert in vivo anti-infectious immunostimulating activities, using a model infection of mice with a pathogenic strain of *Escherichia coli* and structure-activity relationships in this chemical series have been precisely determined [129-131].

**Schiff base-forming molecules.** A quite novel approach to therapeutic potentiation of the immune system has recently been reported by Rhodes et al. [139]. These authors have shown that small Schiff base-forming molecules, such as tucaresol, can substitute for the physiological donor of carbonyl groups (constitutively expressed on antigen-presenting cells) and thus interact with the amines on T-cell receptors, thereby providing a co-stimulatory signal to T lymphocytes, by activating transport of Na\(^+\) and K\(^+\), leading to phosphorylation of key signalling proteins. Tucaresol enhances CD4 T-cell responses, selectively favoring a Th-1-type profile of cytokine production. In vivo, tucaresol potently enhances CD4 T-cell priming and CD8 cytotoxic T-cell priming to influenza virus peptide antigens by parenterally administered tucaresol was demonstrated and, at immunopotentiating doses, this agent was shown to be therapeutically effective in a murine model of cytomegalovirus infection as well as in a model of syngeneic tumor growth (murine colon adenocarcinoma). Oral administration of tucaresol to mice caused an increase in production of interleukin-2 and interferon-\(\gamma\) and a decrease in the production of interleukin-4 and interleukin-6.

A distinct therapeutic advantage of small Schiff base-forming molecules over cytokine therapy is likely to be the avoidance of toxic effects mediated through cytokine cascade events. Promotion of a Th-1-like profile of cytokine production and selective enhancement of cell-mediated responses can be therapeuti-
Stimulators of nonspecific suppressor cell activity induction.

Finally, it is worth mentioning that compounds stimulating the induction of nonspecific suppressor cell activity are capable of exerting interesting immunopharmacological effects: one example is provided by the azaspirane SKF 105685 (N,N-dimethyl-8,8-dipropyl-Z-azaspiro(4,5)decane-2-propanamine dihydrochloride). This compound exerts therapeutic activities in animal models of autoimmune disease [140] while, at the same time, stimulating myelopoiesis and enhancing survival from lethal irradiation in mice [141].

The special case of adjuvants

Several compounds described in this review have been reported as exerting a particular type of immunostimulating activity, namely an adjuvant activity, that is the power of enhancing significantly the humoral and/or cell-mediated immune responses to particulate or soluble antigens. This is the case of monophosphoryl lipid A, of muramylidipeptide (MDP) and its derivatives such as murabutide, of temur tide (threonyl-MDP), of MTP-PE (muramyl-tripeptide phosphatidyethanolamine), of the lipopeptides palmitoleuamide and trimexautide and of other lipopeptides such as palmitoyl-lipoelipeptides.

An adjuvant activity is of distinct practical interest for the development of human and veterinary vaccines, inasmuch as the highly purified antigens that are presently incorporated in vaccines tend often to be poorly immunogenic per se. Furthermore, some individuals are weak responders to certain vaccines, as in the case of hepatitis B vaccine or of influenza vaccines in elderly people.

Adjuvants are also very useful when attempts are made to immunize laboratory animals with various substances to prepare high titer antibodies against these molecules for research purposes. Freund's complete adjuvant (i.e. killed mycobacteria in a water-in-oil emulsion) provided biochemists and immunologists, almost 60 years ago, with a powerful tool, which is still used today and has led to the discovery of the adjuvant activities of muramylidipeptides, but the use of this adjuvant is restricted to laboratory animals, in view of its multiple side effects.

At the present time, the most common adjuvants for human vaccines are aluminium hydroxide and aluminium phosphate, although calcium phosphate is also being used in some vaccines and may have some advantages over aluminium-based adjuvants. Aluminium adjuvants have an extensive record of efficacy and safety and any new type of adjuvant will have to be endowed with distinctive superior properties to be considered for development. The aluminium adjuvants seem to act mainly by depot formation, allowing the slow release of antigen and thereby prolonging the time for interaction between antigen and antigen-presenting cells as well as lymphocytes. Contrary to the other potential adjuvants mentioned in this review, aluminium hydroxide or phosphate and calcium phosphate do not exert the overall immunostimulating activities that the former exhibit.

Several excellent reviews have been devoted recently to the topic of adjuvants for human and veterinary vaccines [142–145] and we refer to them for details. It is, however, important to note that several adjuvants have been shown to selectively modulate the immune response to elicit humoral and/or cellular immune responses. With their use, this immune response can be either an MHC class I or an MCH class II response. An MHC class I response is usually directed against intracellular pathogens (viruses, for instance) leading to the induction of cytotoxic T lymphocytes (CTL); it is normally not observed with protein or peptide antigens, but some adjuvants are capable of promoting this response with the latter. The MHC class II response is usually elicited against protein antigens or inactivated microorganisms and leads to antibody production: most adjuvants are efficient in eliciting a MHC class II response. We have mentioned earlier in the text that two lipopeptides of very close chemical structure, pimelautide and trimexautide, are capable when conjugated with an HIV-1-derived peptide, to stimulate a specific cytotoxic T lymphocyte response in the case of trimexautide and an antibody response, in the case of pimelautide. This example also illustrates the potential usefulness of built-in adjuvants, i.e. chemical conjugation between an antigen and an adjuvant.

Adjuvants can also modulate the immune response to different T-helper cells (Th-1 and Th-2). Stimulation of the Th-1 type response leads to a cell-mediated immune response and production of IgG2a antibodies (in mice), whereas stimulation of the Th-2 type response leads to the production of IgG1 and IgE antibodies. MDP, temur tide, monophosphoryl lipid A stimulate the Th-1-type response; aluminium adjuvants are known to stimulate a Th-2-type response.

As things stand presently in a fast moving field, temur tide (threonyl-MDP) may well be one of the most promising adjuvants for possible use in human vaccines, as indicated above in the section devoted to this compound [42], probably because of its effective formulation in a stable oil-in-water emulsion. Furthermore, temur tide appears to be devoid of other immunostimulating activities beside adjuvanticity: it is nonpyrogenic and does not stimulate resistance to infections. This selectivity of action pleads in favor of a minimal potential of causing side effects. As stated by Gupta et al. [143] the search for vaccine adjuvants is guided by a balance between toxicity and true adjuvanticity and we have seen in this review that many immunostimulants do exert some toxic effects. Lack of toxicity is an essential prerequisite for vaccine adjuvants since most vaccines are administered to young children.

There are several other possible strategies for the design of adjuvants which have not been mentioned in our review on immunostimulants and which we shall briefly describe here, based mainly on the paper by Gupta et al. [145] and a few other publications. Saponin, for instance, isolated from the bark of Quillaja saponaria, can be purified and give an adjuvant-active fraction called Quil A, which is used in several veterinary vaccines. But its hemolytic activity and local reactions make it unsuitable for human vaccines. Further purification of Quil A led to the discovery of QS 21, a water-soluble substance with potent adjuvant activities and minimal toxicity, which in mice elicits a Th-1-type response. Clinical studies in man have established the adjuvanticity and acceptable toxicity of QS 21 [146]. Iscom (immunestimulating complexes) is the name given to noncovalently bound complexes of Quil A adjuvant, cholesterol and amphiphatic antigens: in the Iscom, the antigens are attached as multimers to a 40-nm cage-like particle with a built-in adjuvant; the antigens in Iscoms are swiftly transported from the injection site to the draining lymph node. Iscoms, which stimulate both humoral and cell-mediated immune response to amphiphatic antigens (hepatitis B virus surface antigen, influenza virus hemagglutinins, herpesvirus glycoproteins, for instance) are now used
in veterinary vaccines but have not yet been approved for human vaccines.

Another approach to adjuvanticity of vaccines is the encapsulation of antigens in liposomes; furthermore, substances such as monophosphoryl lipid A or MDP show enhanced adjuvanticity and reduced side effects when encapsulated in liposomes. Adjuvanticity of liposomal formulations is probably due to depot formation at the site of injection and efficient presentation of the antigens to macrophages.

Biodegradable polymer microspheres containing vaccine antigens receive also much attention due to their potential as a vehicle to target the antigen to antigen-presenting cells and for the controlled release of antigens, allowing the reduction of the number of doses for primary immunization. The microspheres are made from various polymers such as poly(lactic)/glycolic acid.

Gjeta et al. [147] have described the adjuvant activity of polar glycopeptidolipids from Mycobacterium chelonae on the immunogenic and protective effects in mice of an inactivated influenza virus vaccine.

As could be expected, several interleukins have been examined from the point of view of their possible adjuvant activity on vaccines. For instance, Alfonso et al. [148] have described the adjuvant activity of interleukin-12 in the vaccination of mice against the protozoan parasite Leishmania major. In leishmaniasis, protection requires the induction of Leishmania-specific CD4+T helper lymphocytes; mice vaccinated with a soluble leishmanial antigen and injected simultaneously with interleukin-12 were resistant to a subsequent normally lethal challenge with the parasite. Duits et al. [149] have shown the adjuvant activity of interleukin-6 entrapped in liposomes and injected into mice immunized with a heat-shock protein used as a model antigen. The multiple, pleiotropic activities of most known interleukins may however represent an obstacle to their routine use as adjuvants for vaccines. A more subtle and probably safer approach, but applicable only to live microorganisms, is engineering to express a particular cytokine, thus enhancing the immune response, as demonstrated very recently by Murray et al. [150] who engineered the BCG strain of tubercle bacillus to express several murine cytokines, such as interleukin-2, interleukin-4, interleukin-6 or interferon-γ. There is an obvious need for a more potent BCG vaccine than the one presently used, in the face of the current threat posed by widespread infection of certain populations with antibiotic-resistant strains of Mycobacterium tuberculosis.

We can also mention the demonstration by Dempsey et al. [151] that vaccination of mice with a recombinant model antigen (hen egg lysozyme) fused to the C3 component of complement caused a 10000-fold higher humoral and cell-mediated immune response than when the antigen alone was administered, enabling the authors to conclude that the third complement protein, which is a molecular adjuvant of innate immunity, can profoundly influence an acquired immune response.

Thus far most experimental data on adjuvants and adjuvant activity have been obtained in systems in which both the antigens and the adjuvants are administered by parenteral routes, inasmuch as most human or veterinary vaccines are ordinarily injected either subcutaneously or intramuscularly, with the notable exception of live poxviruses which are administered orally. Nevertheless, with the present recognition of the importance of mucosal immunity involving lymphoid cells present on diverse mucosal surfaces (gut-associated lymphoid tissue, bronchi-associated lymphoid tissue, Langherans-dendritic cells in the skin), the trend will be to preferentially stimulate mucosal immunity with vaccines, especially when the primary site of infection in the natural pathological process is indeed a mucosal surface (skin, respiratory tract, digestive tract). So-called mucosal vaccines are probably going to be developed in the near future against respiratory syncytial virus, influenza virus, adenoviruses, measles and rubella viruses, caries-causing streptococci, Hemophilus pertussis, Shigella, pathogenic Escherichia coli strains, Vibrio cholerae and possibly HIV-1 and HIV-2. Whether these mucosal vaccines will contain live or inactivated infectious agents, there will be a need for adjuvants (of humoral and cell mediated immunity) which will be active when administered together with the vaccines on a mucosal surface (orally, intranasally). There is therefore a definite need for experimental systems which will be able to demonstrate an immunoadjuvant activity under such conditions of administration.

Furthermore, a novel and likely promising approach to anti-infectious immunization is represented by the DNA vaccines, in which the DNA sequence coding for a particular antigen, involved in protective mechanisms, is injected directly in the host. Whether or not there will be, in such a strategy, a need for adjuvants, to boost the immune response against the endogenously produced antigen, is open to question.

Conclusions and perspectives

Table 1 is a list, hopefully not too incomplete, of the immunostimulants that are being used as such presently (1996) in various clinical applications. Note that only two immunostimulants have become part of a standard therapeutic scheme in several countries: BCG (a live bacillus) for cancer of the bladder, leptomisole (a simple synthetic molecule) for colorectal cancer. Nothing that was known about the immunostimulating activities of BCG and of levamisole would have allowed a reasonable guess that these agents would indeed find their special usefulness in those two particular pathologies (although intravesical treatment was a rather obvious choice in the case of bladder cancer). Immunotherapy of cancers with the other complex preparations listed in the table (picibanil, schizophyllan, lentinan, krestin) is performed exclusively in Japan and, for a number of reasons, which are not altogether clear, has not been adopted by Western oncologists and probably never will.

The other possible applications of immunostimulants are in the field of infectious diseases, especially when such infections episodes occur in individuals whose immune system does not function optimally: young children, elderly persons, patients submitted to major anesthetic and surgical procedures. In this domain, we find various bacterial lysates (some of which may in part behave as vaccines), chemically pure bacterial glycoproteins, plant extracts and only one chemically characterized molecule, namely pidotimod. Also used in this context are the thymic extracts thymomodulin and thymostimulin. At this point, one may however ask the pertinent question: do we really need anti-infectious immunostimulants? There is no doubt that recurrent ear-nose-and-throat infections are a real problem in immunologically immature young children, most of whom finally outgrow this situation. In these children, some of the immunostimulants listed in the table appear to reduce to a certain extent the number and duration of infectious episodes and to allow a less frequent recourse to antibiotics, but these immunostimulants need to be administered rather frequently throughout the cold season. With respect to elderly individuals, the situation is more complex, inasmuch as adequate immune functions are practically preserved in many quite old persons, whereas they decline in others. Here again, the existence of recurrent respiratory (and possibly urinary) infections may justify the use of immunostimulants, as in the case in chronic bronchitis.

On the other hand, it is often claimed that major surgical interventions requiring prolonged and deep anesthesia, exert a
Table 1. Immunostimulants presently used in clinical practice (1996).

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Therapeutic application</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bacille Calmette-Guérin (BCG)</td>
<td>bladder cancer</td>
<td>standard therapy</td>
</tr>
<tr>
<td>2. Streptococcus pyogenes strain (Picibanil)</td>
<td>various solid tumors</td>
<td>in some countries</td>
</tr>
<tr>
<td>3. Bacterial lysates (from various respiratory pathogens)</td>
<td>recurrent respiratory infections</td>
<td>in some countries</td>
</tr>
<tr>
<td>4. Glycoproteins from Klebsiella pneumoniae</td>
<td>recurrent respiratory infections</td>
<td>in some countries</td>
</tr>
<tr>
<td>5. Escherichia coli lysate</td>
<td>urinary infections</td>
<td>in some countries</td>
</tr>
<tr>
<td>6. Extract from Echinacea purpurea</td>
<td>recurrent respiratory infections</td>
<td>in Germany</td>
</tr>
<tr>
<td>7. Schizophyllan (glucan)</td>
<td>gastric, colorectal, breast cancers (association with chemotherapy)</td>
<td>in Japan</td>
</tr>
<tr>
<td>8. Lenitnin (glucan)</td>
<td>cervical cancer (association with chemotherapy)</td>
<td>in Japan</td>
</tr>
<tr>
<td>9. Krestin, PSK (protein-bound glucan)</td>
<td>gastric and colorectal cancers (association with chemotherapy)</td>
<td>in Japan</td>
</tr>
<tr>
<td>10. Thymomodulin purified extract from bovine thymus</td>
<td>chronic or recurrent infections, post-surgical infections</td>
<td>in Italy</td>
</tr>
<tr>
<td>11. Thymostimulin</td>
<td>colorectal cancer (stage C) (in association with 5-fluorouracil)</td>
<td>in Japan</td>
</tr>
<tr>
<td>12. Levamisole (synthetic)</td>
<td>chronic or recurrent respiratory infections, post-surgical infections</td>
<td>in Italy</td>
</tr>
<tr>
<td>13. Pidotimod (synthetic)</td>
<td></td>
<td>standard therapy</td>
</tr>
<tr>
<td>14. Romurtide (synthetic)</td>
<td>Recovery from chemotherapy or radiotherapy-induced leukopenia</td>
<td>in Japan</td>
</tr>
</tbody>
</table>

transient immunosuppressive effect, thereby favoring the appearance of severe nosocomial infections. In this case, the association of anti-infectious immunostimulants with wide spectrum antibiotics may become a favorable strategy; further evidence of the usefulness of PGG-glucan in this context is of course awaited with considerable interest. On the other hand, and although such an approach appears to be scientifically founded, the day has probably not yet come when the treatment of infectious diseases in general will consist in an association between an immunostimulant and antibiotics, thereby allowing a reduction in the amount of antibiotic and the duration of its administration. Is it utopian to think that anti-infectious immunostimulants might play a role in the fight against emergent pathogens and/or against antibiotic-resistant microorganisms? Remember at this point that some antibiotics may possess immunomodulating activities per se.

If there is one infectious pathology in which therapeutic immunostimulation would clearly appear necessary it is indeed the acquired immunodeficiency syndrome, i.e. AIDS. But, as far as things stand nowadays, the immunopathological mechanisms of this syndrome appear so complex and still insufficiently known, that the use of immunostimulants, either to treat the HIV infection itself or to prevent the occurrence of opportunistic infections, is generally considered much too hazardous (fear of reactivating virus replication, etc.). The results of the various current protocols in which certain cytokines are administered to AIDS patients will probably provide the necessary guidelines in the future.

Turning now to the immunotherapy of cancer, present trends of fundamental and clinical research in cancer immunology may cautiously help to predict the future. Progress continues to be made in characterizing various specific tumor antigens, as summarized recently by Boon [152]. This opens the way to the possible use of therapeutic cancer vaccines and, in such a strategy, the association of a tumor antigen with one or another of the immunological adjuvants mentioned in this review may turn out to be advantageous. Another approach, currently the object of extensive investigation, is to engineer tumor cells to make cytokines (such as interleukin-2 or interleukin-4) and to reinject those engineered cells into the tumor bearing host [153]; the well-defined cytokine-inducing properties of some immunostimulants (such as bropirimine or imiquimod, for instance) might make those useful in such therapeutic schemes.

There is however still room in the future for the direct utilization of an immunostimulant in a more conventional type of cancer immunotherapy, as suggested by the recently reported efficacy of liposomal muramyltripeptide in the treatment of relapsed osteosarcoma [154], justifying an ongoing multicenter phase III clinical trial of this agent in patients with newly diagnosed osteosarcoma. Just as in the case of levamisole and colorectal cancer, liposomal muramyltripeptide looks like an effective immunostimulant against a precise type of malignancy.

To the general question 'do exogenous immunostimulants of the type described in this review have a real future in human medicine?', the answer can be only cautiously affirmative and only for some of them. This will depend, to a large extent, on what will be achieved therapeutically with the endogenous immunomodulators represented by the various cytokines, now extensively investigated in several areas, since it is clear that all known immunostimulants do exert their activities through the in vivo induction of one or several cytokines. For instance, romurtide is used clinically in Japan to help cancer patients to recover from chemotherapy or radiotherapy-induced leukopenia: this drug does act indeed through induction of colony-stimulating factors, but its use may be more practical and economical than that of the factors themselves.

It appears also likely that some of the adjuvants described in this review will be used in the future to boost the immune responses to various anti-infectious vaccines.

Finally, it is not impossible to envisage the practical use of certain immunostimulants in therapies that do not appear, at least superficially, to involve the immune system, as exemplified by the cardioprotective activity of monophosphoryl lipid A (MPL). The recognized existence of bidirectional interactions between the neuroendocrine system and the immune system may well constitute the basis of still unknown therapeutic applications of immunomodulating agents. In a very recent paper, Sigel and Rosenbaum [155] have reviewed the evidence showing that many cytokines (such as interferon-γ, colony-stimulating factor GM-CSF, tumor-necrosis factor, interleukin-1, interleukin-6) promote neuronal survival in cultured cell populations, suggesting that some cytokines (and the immunomodulators that are able to induce their production and/or their activity) might ultimately have a potential in the treatment of stroke, trauma and dementia.

One should however always remember the caveat of Lewis Thomas [156]: 'Good applied science in medicine requires a
high degree of certainty about the basic factors at hand, and especially about their meaning, and we have not yet reached this point for most of medicine'. Such a cautious view is indeed appropriate when dealing with molecules acting on the immune system.

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REFERENCES


