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IMMUNOSTIMULATION: SYNTHETIC AND BIOLOGICAL MODULATORS OF IMMUNITY

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INTRODUCTION

In 1960 the immune system was an enigma to scientists, and treatments for immunological abnormalities sometimes reflected this lack of knowledge. Infants born with enlarged thymuses were often treated with thymic irradiation, for example. In 1961 and 1962, however, it was demonstrated (1–3), first in the chicken, that the immune system consists of two limbs, one comprised of thymus-derived lymphocytes (T cells), the other of cells that in birds derive from the bursa of Fabricius and in mammals that originate in the fetal liver and migrate to the bone marrow during early intrauterine life (B cells). By 1968, this had been documented in man; however, methods of identifying B and T cell subpopulations and enumerating them in human peripheral blood and tissues have been developed only in the last 10 years.

During the past decade it has become evident that circulating immunologically virgin B cells develop an antigen-specific antibody (membrane receptor) upon exposure to a given antigen. This stimulated B cell gradually differentiates and then produces large amounts of specific antibody molecules to the given antigen; these antibodies are secreted from the cell and exported into the serum, where quite specifically they bind the antigen, activate the complement system, and kill the "foreign" cell. Since these molecules are transported via the circulation, this limb of the immune system is termed *humoral* immunity. The humoral immune system is responsible for protection against the classic microorganisms—meningococcus, streptococcus, pneumococcus, etc. Prophylaxis is attained by vaccination with the appropriate antigen at subinfect-

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ing doses to stimulate the development of memory cells able to produce rapidly large amounts of specific antibody when the organism is reencountered later in the environment.

Upon antigen exposure, the T cell also acquires surface membrane-specific receptors. The exact nature of these receptors is still controversial; however, they appear to be unrelated in structure to any antibody molecule (4). Furthermore, T cells do not secrete classical antibody. Instead, the cells become activated and are morphologically transformed into entities with the appearance of leukemic lymphoblasts; during and after this blast transformation they appear to participate directly in a variety of protective pathways, collectively termed *cell-mediated immunity*. T cells can be identified by their ability to form rosettes with sheep erythrocytes. This arm of the immune system is responsible for resistance to protozoal, parasitic, fungal, and most viral infections. Delayed cutaneous hypersensitivity (skin test) reactions are mediated by T cells, as are reactions to genetically foreign tissues such as skin and kidney grafts. Some T cells when activated can kill foreign cells with which they come into contact by a process termed *cellular cytotoxicity;* this is an important mechanism for destroying malignant cells. Although T cells do not secrete antibody, they do make a wide variety of small information-carrying molecules with either antigen-specific or nonspecific effects on foreign cells and, interestingly, on other cells of the immune system. These informational molecules are collectively termed *mediators of cellular immunity* (MCI) or *lymphokines* (Table 1).

In the past decade our knowledge of the immune system has expanded enormously. The above classification is much more complicated than originally supposed, especially with regard to the cell-mediated immune system. We know now that T cells account for at least 80% of the lymphocytes present in normal human peripheral blood, and several functional classes and subclasses of T cells have been identified. These include, in addition to the cytotoxic T cells described above, regulatory T cells that regulate the functions of both B cells and other T cells. Helper T cells can increase antibody production by B cells, and different types of suppressor T cells exist that are collectively capable of an impressive variety of regulatory functions, including generalized suppression of immunoglobulin synthesis (5), suppression of the synthesis of a single class of immunoglobulin (e.g. only IgA), suppression of the synthesis of a single-antibody specificity (e.g. antibody against Epstein-Barr virus) (6), suppression of cell-mediated immune functions (e.g. mixed leukocyte reactivity of T cells) (7), suppression of B and helper T cell division, and others. It is increasingly apparent that each of these regulatory functions is mediated by a distinct subpopulation of T lymphocytes.

Additionally, at least two other cell types present in the peripheral circulation have a crucial impact on immune function. Some mononuclear cells lack the typical surface markers of either T or B cells and are termed *null* cells. These

Table 1 Mediators of cellular immunity elaborated by sensitized lymphocytes after addition of antigen[a]

Factor	Heuristic explanation of roles of some factors
Skin permeability factor	Dilutes capillaries in the skin, making it easier for cells involved in the immune response to reach foreign substances (antigens) that have entered the body tissue
Chemotactic factors for macrophages	Attract macrophages (which engulf and destroy foreign cells or parasites, or break them into pieces small enough for the immune system to handle)
Macrophage migration inhibitory factor (MIF)	Prevents macrophages from migrating away from the foreign cells while they are being engulfed
Macrophage activating factor (same as MIF?)	Prevents macrophages from migrating away from the foreign cells while they are being engulfed
Chemotactic factors for other leukocytes (neutrophils, eosinophils, basophils, lymphocytes)	Attract other blood cells which break down and release enzymes that attack viruses, protein, etc
Granulocytic migration inhibitory factor (LIF)	Prevents those cells from moving away
Growth inhibitory factors (clonal inhibitory factor, proliferation inhibitory factor)	Prevent "transformed" (cancer) cells or foreign organisms from dividing and thus increasing in mass
Lymphocytotoxin (toxic for all cells other than lymphocytes)	Kills transformed cells and foreign organisms
Osteoclast activating factor	Activates bone-destroying cells
Collagen synthesizing factor	Stimulates synthesis of collagen
Interferon	Inhibits, at least in animals, the growth of viruses, both those that cause cancer and those that do not
Mitogenic factor(s) for lymphocytes	Causes lymphocytes to divide and thus start the process over again—i.e. this is an amplication mechanism
Interleukin 2 (TCGF)	T-cell growth factor that allows T cells to be grown in continuous tissue culture and causes proliferation of T cells
Dialyzable leukocyte extract (transfer factor)	DLE-transfer factor; transfers delayed hypersensitivity (see text)

[a]Nearly 100 different mediators have been tentatively identified in studies of lymphocytes in cell culture (11). It appears that many of these substances might be isolated and perhaps used for the therapy of a number of different diseases. However, further basic research is required.

cells (or a subpopulation of cells) are the effectors of a cytotoxic mechanism termed *antibody-dependent cell-mediated cytotoxicity* (ADCC) that appears to be an important defense against the growth of malignant cells (8). Another null subset, the so-called natural killer (NK) cell, seems even more important in protecting against malignancies. These NK cells, unlike ADCC cells, do not need antibody to kill target tumor cells (82). A fourth, the monocyte-macrophage series, is regarded by many as one of the most important components of the immune system and has many functions. For example, macrophages and monocytes (MΦ) engulf and "process" an organism with many antigens (epitopes) into smaller bits, and present antigens on their surfaces to T and B cells; like T cells, they secrete a variety of soluble mediators involved in immunoregulation, including both helpers (9) and suppressors (10) of immune functions. Interestingly, monocytes have functional subsets (e.g. suppressor monocytes); even neutrophils and eosinophils have been shown to be capable of ADCC and NK activity in vitro. Our understanding of their in vivo role in the immunoregulatory panorama is still in its infancy.

The above summary, although necessarily incomplete due to space limitations, provides an indication of the complexity of the human immune system. Each of the two arms, humoral and cellular, is now known to have many hands and each hand many fingers. Further, the two arms are not viewed as distinct entities, since they interact extensively within a complex regulatory network. For instance, the list of soluble mediators (MCI) produced by immunocytes (Table 1) has grown to over 100 named factors (11), including antigen-specific and nonspecific activities, helper and suppressor factors, factors restricted and nonrestricted for histocompatibility antigens, and factors acting on B cells, T cells, macrophages, neutrophils, tumor cells, virus-infected cells, basophils, eosinophils, osteoclasts, and even vascular epithelium.

Clearly, further investigation is required before a clear picture of this complex system emerges. Nevertheless, within the scope of the limited knowledge available, applications to clinical situations have been possible. In particular, experimental approaches to immunotherapy have developed recently along several important lines. Many biological and synthetic immunostimulants, adjuvants, and drugs with specific or nonspecific effects on immunocytes are now being investigated, and new immunomodulators are being developed in many countries. Although most are still restricted to a few research centers, others are used widely in the therapy of a variety of disorders. Recent findings of immune defects in cancer have stimulated widespread interest in the development of immunomodulators that will be effective as adjuncts in the treatment of malignancies, and some promising results have been reported (12).

The various immunomodulators now in use undoubtedly act at different points in the immune spectrum, but the exact target cell has been identified in

only a very few instances (Table 2). Obviously, a need exists for development of agents that can selectively inhibit or enhance one specific class or subclass of immunocytes: e.g. increase suppressor T cells in systemic lupus erythematosus (13), decrease antigen-specific suppressor T cells in various malignancies (14), or increase natural cell activity in leukemia (15). At present, we can classify immunotherapeutic approaches generally as (*a*) nonspecific systemic immunostimulation, (*b*) adjuvant contact therapy, (*c*) active specific immunotherapy, and (*d*) adoptive transfer of immunity. These agents can also be classified as active on the humoral immune system, the cellular immune system, or both. Some agents employed and the results obtained in clinical trials are described briefly herein. In general, each immunotherapeutic agent is more effective when the amount of antigen (whether bacterium, virus, fungus, tumor cells, etc) in the patient is small, the bulk having been removed by appropriate antibiotics in the case of infectious agents, or the primary tumor having been removed by surgery when immunotherapy is used as an adjunct (or to prevent clinical metastases) in cancer immunotherapy.

SPECIFIC ACTIVE IMMUNOTHERAPY

In recent clinical trials, cancer patients have been treated with injections of purified tumor-associated antigens to bolster their specific antitumor immunity. The purified antigens were isolated from tumor cell surfaces by sophisticated biochemical and/or biophysical separation procedures (16, 17). Administration of purified lung tumor antigen in Freund's adjuvant by Stewart, Hollinshead, and co-workers to 28 patients with stage I lung cancer following surgical removal of the lung tumors resulted in a significant increase in survival (18). The experimental group received monthly injections of soluble antigen homogenized in Freund's complete adjuvant for three months without other therapy, whereas the third group received monthly chemotherapy followed by tumor antigen immunotherapy for three months. Eighty-three percent of the patients in both the immunotherapy and chemoimmunotherapy groups were still alive after four years, compared with only 49% in the control group, who received chemotherapy alone. The immunotherapy did not appear to prolong survival in stage II or stage III patients, indicating the importance of minimizing the antigenic burden (tumor) before immunotherapy, as stated above. However, in this trial no control was included for Freund's adjuvant alone. Thus, it is possible that the apparent beneficial effects may have been due to nonspecific adjuvant immunostimulation rather than to specific antitumor immunity against the lung tumor antigen. Additional trials in a larger number of lung cancer patients have been initiated, and trials in colonic cancer, malignant melanoma, and other types of cancer patients have just been instigated; a Freund's adjuvant alone group also will be included (A. C. Hollinshead,

Table 2 Some immunomodulators of therapeutic interest

Category	Example	Possible mode of action	Reference
Specific Active			
1. Tumor-associated (specific) antigens	Lung cancer, osteogenic sarcoma	Stimulates cell-mediated anti-tumor immunity	16–18 113
2. Killed, virus- or enzyme-treated	Neuraminidase, viral xenogenization	Increases antigenicity of tumor epitopes	19, 20 23, 25, 26
3. Cytotoxic drugs coupled to antitumor antibodies	Adriamycin, chlorambucil	Antibodies bring cytotoxic drug to tumor cells	35, 36
Adoptive Transfer			
Non-Specific			
1. Gamma globulin	Cohn Fraction II	Restores antibody levels in general	5
2. Isolated complement components	C2, 4, etc	Restores deficient complement level	12, 28
Antigen-Specific			
1. Immune-RNA	Allogeneic RNA	Stimulates cell-mediated anti-tumor immunity	34
	Xenogeneic RNA		32
2. Transfer factor	Dialyzable leukocyte extracts (DLE)	Increases T cell-specific immunity	54, 56
3. Monoclonal antibodies	Neuroblastoma, melanoma, leukemia osteosarcoma	Specific cytotoxicity	28–37
Non-Antigen-Specific			
1. Adjuvant (microbial)	BCG,	Stimulation of CMI and reticulo-endothelial system	58–60
	C. parvum,		68
	Bestatin		69
2. Thymic hormones	Facteur thymique serique	Stimulates differentiation and maturation of T cell precursors	2
	thymopoietin		9, 73
	thymosin		113
3. Anti-viral substance	Interferon	Stimulates natural killer cell and anti-viral activity	76–78
4. Chemically synthesized	Levamisole	Restores CMI	83, 85
	NPT 15392	Stimulates T and NK cells	113
	Methisoprinol	Increases CMI limb	98
	MVE	Stimulates immunocyte function	97
	NPT 16416	Thymic hormone-like effects	113
	Azimexon	T and B cell stimulation	113
5. Ig-derived	Tuftsin	Stimulates macrophage function	112

personal communication). In addition to the use in treatment of lung cancer, its efficacy in preventing lung cancer in high-risk individuals (heavy smokers, for example) merits exploration.

Another approach to specific active immunotherapy in cancer patients is the use of killed or modified tumor cells. Animal experiments demonstrate that pretreatment of tumor cells with the neuraminidase enzyme increases their immunogenicity. Many tumor cells are surrounded by a sialomucin coat, the glycocalix, which may protect from immunological attack. Neuraminidase treatment removes sialic acid and increases tumor cell antigenicity and susceptibility to cytotoxic antibody and complement. Heightened immunogenicity of neuraminidase-treated cells was demonstrated by their ability to induce antitumor immunity when injected into isogeneic animals (19). Consequently, a trial has been undertaken in human patients with acute myeloblastic leukemia (20) and other malignancies. These patients received multiple intradermal injections of neuraminidase-treated myeloblasts in combination with chemotherapy. Although trials of this nature as yet are not finalized, the preliminary results are encouraging. Significant increases in remission duration have been achieved. In a group of 85 acute myelocytic leukemia (AML) patients in which 41 received chemoimmunotherapy using neuraminidase-treated myeloblasts and another 45 were treated with chemotherapy alone, 24% of the patients in the chemotherapy group are in complete remission. This compares with 49% in complete remission in the group who received neuraminidase-treated myeloblasts. Fifty-eight percent of the patients in the immunotherapy group are alive, compared to 29% in the chemotherapy group. Considerable improvement in cell-mediated immunity occurred among AML patients immunized with neuraminidase-treated myeloblasts as measured by conversion to positive to at least three recall antigens: mumps, candida, and varidase. Thus, a progressive restoration of lymphocyte function in AML patients with this type of immunotherapy is apparent (21). In another trial, patients with lung cancer received injections of neuraminidase-treated tumor cells after surgical removal of their tumors and had longer remissions than controls who were treated only by surgery (22). However, side effects (especially high fevers) are common; indeed, thermal activation of monocytes, NK cells, etc, may be important precipitating factors in the immunity. Also, pretreatment of tumor cells with some viruses has led to cell modification and increased immunogenicity in animals (23). Preliminary trials with virus-treated tumor cells are being undertaken in humans. The induction of virally associated neoantigens (VAA) on tumor cells following artificial infection has been referred to as antigenic conversion (24), heterogenization (25), or viral xenogenization (26). It may be feasible to cause viral infection of an existing human tumor to xenogenize it, thereby causing immune-mediated tumor regression without surgical removal. Probably the nonlytic virus would have to be focused or concentrated into the

tumor tissue very rapidly. One method of attaining this might be attaching tumor-specific antibody chemically to the virus. Also, diethylaminoethyl-D (DEAE-D) can attach foreign elements to the cell surface due to change in the negative charge induced on the tumor cells. Virally infected liposomes might also be employed for producing foreign antigens on tumor cells. The future of specific active immunotherapy will depend on the isolation, characterization, and purification of those tumor cell-surface–associated or specific antigens that can be used to bolster immunological attack. Sufficient quantities of these antigens must be prepared for immunotherapy and immune monitoring.

We anticipate that specific active immunotherapy, particularly in cancer, will continue to receive careful study. For example, one form of possible treatment currently under investigation is the use of cytotoxic drugs coupled to antitumor antibodies, especially monoclonal (hybridoma) antibodies. In animals this technique has been shown to cause specific destruction of some tumor cells, presumably because the drug is delivered directly to the tumor site (27). Promising results have been reported in animal osteosarcoma models using purified antiosteosarcoma antibodies coupled to adriamycin (28). Similarly, radiolabeled anti-CEA (carcinoembryonic antigen) is being used in clinical trials for both early radiologic diagnosis and therapy of human lung cancer metastases (29).

ADOPTIVE TRANSFER OF IMMUNITY

Like specific active immunotherapy, adoptive transfer of immunity is intended to increase the patient's immune response to a specific antigen. In this case, however, the aim is to transfer such immunity from the donor to the recipient. In the case of humoral immunodeficiencies or isolated complement component deficiencies, the use of fresh frozen plasma or purified gamma globulin (Cohn fraction II) has become a standard therapeutic method (30). More recently, attempts have been made to develop techniques for the transfer of cell-mediated immunity from donors with normal or elevated levels demonstrable by in vitro assays to patients with either broad-spectrum or antigen-selective (i.e. one organism) cell-mediated immune defects. (Presumably these are due to rare immune response genes and are thus genetically determined defects.) To date, two such biologic agents have been used for clinical trials in humans: immune RNA and dialyzable leukocyte extracts (transfer factor).

Immune RNA

In recent years, several studies have produced evidence for a beneficial therapeutic effect of immune RNA (I-RNA) in animals with tumors. For example, after surgical extirpation of B16 melanoma isografts in C57BL/6 mice, administration of xenogeneic I-RNA prevented the fatal pulmonary metastases that

inevitably developed in other murine tumors; the same effect has been shown in rats and guinea pigs (31). There are two potential sources of I-RNA for cancer immunotherapy in human patients: allogeneic I-RNA derived from lymphocytes of cured cancer patients, and xenogeneic I-RNA derived from animals specifically immunized with neoplastic cells from the tumor-bearing patient or from another patient with a tumor of identical histologic type. However, repeated removal of lymphocytes from patients in remission theoretically could produce deficient cell-mediated immunity and consequent exacerbation of the disease; furthermore, there is evidence that tumor-specific immunity in such patients diminishes or disappears after several disease-free years. Therefore, initial clinical trials have employed xenogeneic (animal) I-RNA, an approach that also eliminates the problems of donor selection and availability. Two methods have been used for administering I-RNA to cancer patients: injecting it parenterally, and incubating the patient's peripheral blood lymphocytes with I-RNA in vitro and returning them to the patient intravenously.

In a phase-I trial reported by Pilch et al (32), 35 cancer patients (15 malignant melanoma, 12 hypernephroma, and 8 others) were injected with I-RNA extracts from the spleens and lymph nodes of sheep that had received three-weekly intradermal injections of viable tumor cells in complete Freund's adjuvant. No significant local or systemic toxicity was noted, pain at the site of injection was minimal, and no evidence of local irritation or febrile, allergic, or anaphylactoid reactions was recorded. The immune status of the recipients was monitored by in vitro cytotoxicity testing, and in some patients significant increases in tumor-specific cytotoxicity were observed. Clinical improvement or stabilization of the disease occurred in 17 of the 35 patients treated. The same investigators later reported promising results in nonrandomized trials of I-RNA therapy for advanced renal cell carcinoma (J. A. Mannick, personal communication). The regression of pulmonary metastases suggests that this approach to adoptive immunotherapy is ready for more broad clinical development. These preliminary results indicate that I-RNA may prove to be a valuable immunotherapeutic agent, particularly in view of the apparent absence of adverse side effects. Trials by others, not yet fully reported, suggest that immune RNA will be of value in patients with hypernephroma (33).

It has also been reported that lymphoid and myeloid cells from tumor-bearing mice, when transferred into normal syngeneic mice, imparted information that resulted in the production of tumor-specific cytotoxic cells by the recipients (34). Similar results have been reported recently in experiments using tumor-sensitized xenogeneic cells (i.e. from other species, such as rats). The production of tumor-specific cytotoxic cells in the recipients may involve the transfer of information by I-RNA in the injected cells and suggests that xenogeneic cells eventually might prove useful for passive immunotherapy of human tumors.

One of the most exciting prospects for future adoptive immunotherapy in humans may be the utilization of cell hybridization-cell cloning methodology. Because the use of interleukin 2 (T-cell growth factor) insures the ability to generate continuously proliferating clones of cytotoxic T cells, these autologous cells could be administered repeatedly in therapy. Passive immunotherapy (serotherapy), in which purified xenogeneic antibodies have been coupled to drugs or radioactive isotopes, has been used to increase contact with the tumor. Antibody coupled to chlorambucil (35) and ^{131}I (36) have been used to treat malignant melanoma and hepatoma respectively. With the development of human myeloma-lymphocyte hybridomas, the use of the cloned B-cell populations that produce highly specific high-titer monoclonal antibody against tumor-associated (or specific) antigens make this form of passive immunotherapy particularly promising. Monoclonal antibodies have been developed for therapeutic use against colorectal carcinoma, neuroblastoma, malignant melanoma, leukemias, and lymphomas (37). Also, studies with monoclonal antibodies directed against tumor-associated antigens have recently been utilized in therapeutic trials (e.g. inoperable pancreatic carcinoma) with dramatic results (37a). The clinical experiences in humans with acute lymphocytic leukemia and B-cell lymphoma demonstrate the necessity of selecting target antigens that do not undergo antigenic modulation on the tumor-cell surface and, as important, the need to select patients with minimal tumor burden and circulating tumor antigen. To date, however, the ultimate usefulness of these reagents in therapy is unknown.

Dialyzable Leukocyte Extracts (Transfer Factor)

In normal subjects, injection of dialyzable leukocyte extracts (DLE) has been shown to *transfer* both skin-test reactivity (delayed hypersensitivity) and the ability to produce various mediators of cellular immunity, such as macrophage migration inhibitory factor (MIF) and leukocyte migration inhibitory factor (LIF), in the presence of the same antigen(s) to which the donor of the leukocytes responded (38). The transfer factor prepared by the dialysis method originally described by Lawrence in 1955 (39) is in fact a crude DLE preparation and has been shown to contain approximately 160 separate moieties. Consequently, DLE is the current designation for such preparations, and the term *transfer factor* (TF) or *dialyzable transfer factor* (TFd) is now reserved for the component(s) with antigen-specific activity (40). Studies from our laboratory (41) have indicated that the TF activity in DLE resides in two distinct nucleopeptides of molecular weight 2000–2500 containing both RNA and protein but not DNA, and structural models of these putative moieties have been proposed (40). Crude DLE preparations contain both nonspecific immunomodulatory (adjuvant) activity and one or more inhibitory activities in addition to the TF components (42, 43).

The mode(s) of action of the antigen-specific moieties in DLE remain undetermined. TF may act on a naive stem cell to induce specificity for an antigen or group of antigens or, alternatively, may assist in the recruitment of specific antigen-sensitive cells. On the other hand, the adjuvant moieties in DLE appear to act nonspecifically by enhancing the preexisting reactivity of the recipient's lymphocytes. DLE is a readily available nonantigenic substance that can be lyophilized and stored indefinitely without loss of potency. It does not transmit infectious disease, has no HLA antigens, and causes no serious side effects. It does not produce graft-versus-host reactions nor transfer humoral immunity, an advantage that could be important for cancer immunotherapy in avoiding immunological enhancement—a mechanism by which antitumor antibodies may coat the tumor cells, interact via immune complexes with T cells, and exclude cytotoxic T cells from interacting with tumor-cell antigenic sites.

Various in vitro tests can be used for assessing the immune status of donors, testing DLE preparation activity, and monitoring recipients [reviewed in (38)]. These include induction of enhanced chemotaxis, increased intracellular cyclic nucleotides, augmented antigen and mitogen responses, and increased formation of active T-cell rosettes (44), either by untreated lymphocytes from patients with low levels in vivo or by trypsinized normal lymphocytes. Recent studies by several groups (45–47) showed that the leukocyte migration inhibition (LMI) assay in agarose, which measures the production of LIF, can be used to measure both nonspecific and antigen-specific effects of DLE in vitro, and this is currently the method of choice for clinical applications. It should be emphasized that careful selection of donors, testing of DLE activity (both specificity and potency), and monitoring of recipients by immunological tests are essential for optimal clinical efficacy regardless of the type of disorder being treated.

DLE has received widespread clinical use [reviewed in (48)]. Although few clinical trials have been double-blind, the results obtained in some viral, fungal, and other diseases have been striking. DLE was first applied as a therapeutic and prophylactic agent in patients with Wiskott-Aldrich syndrome (49), an X-linked deficiency of cellular immunity in which death invariably occurred before age 20. Impressive results were obtained in more than half the treated patients; it was demonstrated later that the patients who responded favorably constituted a subgroup of this syndrome characterized by a defect in monocyte receptors for IgG (50). Other genetically determined immune deficiencies treated successfully with DLE include severe combined immunodeficiency disease, ataxia telangiectasia, genetically determined antigen-selective defects with recurrent infections, recurrent shingles and refractory cytomegalovirus, candida infection, and others (48). Conflicting results reported by several different investigators probably reflect the different methods for selecting donors, preparing the DLE, and monitoring the recipients. In addition, it is

likely that, as in the case of Wiskott-Aldrich syndrome, different subgroups of patients classified within a given syndrome may respond differently to the same therapy.

A number of infectious (viral, fungal, and mycobacterial) and parasitic diseases (i.e. especially diseases due to agents handled by T cells) also have been treated with DLE (48). In viral infections, dramatic improvements have been reported by our group and others in disseminated vaccinia, measles, pneumonia, congenital *Herpes simplex, Herpes zoster,* cytomegalovirus, and other infections treated with antigen-specific DLE. In fungal infections, clinical improvement after reduction of the antigenic load has been reported in disseminated mucocutaneous candidiasis, disseminated coccidioidomycosis, and disseminated histoplasmosis refractory to conventional antifungal therapy. Several mycobacterial diseases refractory to the usual antibiotics and associated with antigen-selective defects in cell-mediated immunity, such as miliary tuberculosis, lupus vulgaris, and progressive BCG *(Mycobacterium bovis)* infection refractory to standard therapy, also have been treated successfully with DLE. Recently, human transfer factor therapy (administered orally) was seen to cause a remarkable clearing of pruitic and plaque lesions in a patient with *Psoriasis vulgaris* that had been recalcitrant to any other treatment (51). One of the few well-controlled double-blind clinical trials of DLE has been reported in the parasitic disease chronic cutaneous leishmaniasis (52). The results indicated both clinical efficacy and antigenic specificity of DLE prepared from donors with demonstrable in vitro cell-mediated immunity *Leishmania* antigens. In patients with persistent cutaneous leishmaniasis, administration of more than 100 units of specific DLE over a one-year period was usually required for complete eradication of the lesions (53).

At present it is impossible to reach any conclusion about the potential of DLE in the treatment of malignancies. A significant problem is the selection of appropriate leukocyte donors. As previously mentioned, antitumor immunity in cured patients is usually short-lived, and removal of their lymphocytes theoretically may lead to increased susceptibility to recurrence. However, it has been found that some 25–50% of the household contacts of cancer patients have specific cell-mediated immunity against the tumor type present in the patient (54), and these healthy individuals often prove to be suitable donors. For example, in one trial of DLE in osteosarcoma, the tumor in which this agent has been studied most extensively, five of seven patients were treated for 18–24 months after surgical removal of their primary tumor (and in two patients with lung metastases) with antigen-specific DLE prepared from the leukocytes of household contacts with specific cell-mediated reactivity against osteosarcoma cells in vitro (55). The patients did not receive any other therapy after surgery. All five of the recipients at last report have been in remission for more than five years, a highly significant advance over historical controls (56). DLE has been

given also in breast carcinoma, malignant melanoma, and alveolar cell carcinoma with reported clinical and immunological improvement in some cases (48).

NONANTIGEN-SPECIFIC IMMUNOTHERAPY

Most of the immunomodulating agents in clinical use are administered with the intent of general stimulation (or suppression) of the patient's immune system, without attempting to direct the activity of the modulated cell toward a given antigen. These nonantigen-specific immunotherapeutic agents can be divided roughly into two groups, those requiring a functional immune system and those with effects only or primarily on a suppressed immune system. In the first group, particulate adjuvants such as BCG or *Corynebacterium parvum* are generally most effective in patients capable of mounting normal immune responses. These two adjuvants have been utilized for both nonspecific immunostimulation and adjuvant contact chemotherapy in cancer patients. Agents with restorative effects, such as levamisole and thymosin (or other thymic hormones), are in the second group and seem to act by conferring temporary immune competence in patients with suppressed immunity. They apparently have little or no effect when administered to patients with a normal immune apparatus.

Of particular importance is the development of synthetic compounds with immunotherapeutic applications. Due to space limitations, only a few of these synthetic drugs (increasing daily in number) are discussed below. Many other agents have been or are being tested as nonspecific immunomodulators, including microbial extracts (some of which apparently increase suppressor cell activity) (57), vitamin A and several of its analogs, tilorone, L-fucose, synthetic polynucleotides (poly A-U, poly I-C), lynestrenole, thiabendazole, tolazoline, lentinen, schizophyllan, picibanil, bestatin, and others.

Bacillus Calmette-Guérin (BCG)

BCG is a viable attenuated strain of *Mycobacterium bovis* obtained by progressive reduction of virulence in a culture medium enriched with beef bile. This nonspecific immunostimulant is thought to act by stimulating the reticuloendothelial system, but this effect may be secondary to T-cell activation and mediator production. BCG also stimulates natural killer cells, which can nonspecifically kill malignant cells. Some investigators believe that BCG also cross-reacts immunologically with hepatoma, melanoma, and leukemic cells, a finding which might account for some of its apparently specific effects on these tumors. Nonviable extracts of BCG have also been shown to be immunostimulatory and to inhibit tumor growth in animals, as have smaller molecules such as the monomeric subunit of the peptidoglycan component of the cell wall. The smallest active compound derived from BCG thus far identified is

muramyl dipeptide (MDP), which is an effective nonspecific adjuvant of macrophage activation in mice but not in humans. A synthetic analog of muramyl dipeptide, SM-1213, is also under investigation. In addition, the methanol-extractable residue of phenol-treated BCG (MER-BCG) has been employed in some preliminary oncologic trials in humans, and the results appear promising for solid tumors, acute leukemias, and perhaps melanomas, especially in conjunction with chemotherapy to reduce the antigen burden.

Various routes of administration have been employed for BCG immunotherapy, including intradermal injection, oral administration, intravenous injection, intralesional injection, and intrapleural administration in lung-cancer patients after primary tumor resection. The most widely used method of administration is scarification, where about 10^8 viable organisms are applied to an area of the upper arm or upper thigh after a series of incisions about 2 mm deep have been made either with a needle or with a Heaf gun. After weekly scarifications with BCG, some patients show an absence of cutaneous reactivity to the treatment. Other tests indicate that they usually become immunosuppressed and therapy must be discontinued for a month or more, only to be resumed when immune function is restored. In addition, BCG therapy sometimes may have dangerous side effects, usually consisting of a severe hypersensitivity reaction and shock. Rarely patients have died after intralesional therapy. Complications of BCG therapy administered by scarification or with the Heaf gun are mild, and no fatalities have been reported.

The effectiveness of BCG has been shown most dramatically in trials using the scarification technique for therapy of malignant melanoma (58). Preliminary results, especially in patients with only local regional involvement, appear very encouraging provided BCG is given near the tumor site. In patients with stage IV melanoma, the combination of chemotherapy and BCG (chemoimmunotherapy) produced more remissions, longer remissions, and longer survival than chemotherapy alone (59). It has been reported that increases in urinary lysozyme excretion and active T cells are valid indicators of clinical improvement in such patients after BCG therapy, and that no improvement is seen if such increases are absent (60).

The first clinical trial of BCG was reported by Mathé and co-workers in patients with acute lymphocytic leukemia (61). A small group of patients received chemotherapy and irradiation of the central nervous system to induce complete clinical remission, followed by either BCG immunotherapy, BCG in combination with irradiated allogeneic tumor cells, or no immunotherapy. The duration of the immunotherapy was five years. Patients receiving weekly doses of BCG by scarification, either with or without specific immunotherapy (tumor cells), appear to have responded best, since 7 out of 20 such patients are still in remission 17 years after initiation of treatment. The majority of the BCG-treated patients who suffered relapse did so before 100 days, indicating that the

number of tumor cells left after chemo- and radiotherapy in these patients was greater than the maximum able to be controlled by immunotherapy. In a later trial with a newer chemotherapy regimen and with cranial irradiation rather than total central nervous system radiotherapy, the median survival duration was increased to 10 years (62). However, these results could not be confirmed by English or American investigators using different therapeutic regimens and different sources of BCG. [The French BCG (Pasteur strain) is alive, non-lyophilized, and has more activity than the British (Glaxo) or American (Tice) strains, which are killed and/or lyophilized. Therefore, the French BCG has produced the best clinical results.]

Preliminary trials of BCG therapy have been conducted also in acute and chronic myelogenous leukemia, lymphoma, breast cancer, head and neck tumors, and colon cancer with promising results, but all of these observations will require confirmation. In lung cancer, a 1977 study suggested that intrapleural BCG injection after surgical removal of bronchial carcinoma decreased the incidence of relapse (63). Later studies in malignant melanoma (64) and lung cancer (65) suggest that intralesional immunotherapy of the primary malignant lesions is feasible and can lead to increased survival. BCG has been used intravesically with documented beneficial results in bladder carcinoma (66). However, for most human clinical cancer studies in which a benefit was seen, there is another report that negates this, so that BCG effectiveness thus far must be said to be equivocal. In contrast, in adult acute myelogenous leukemias, either alone or in combination with an allogeneic irradiated tumor cell vaccine, BCG therapy has provided prolongation of survival, remission, or both (67).

Corynebacterium parvum

C. parvum, a gram-positive bacterium, is a systemic adjuvant used for immunotherapy as a heat-killed and formaldehyde-treated suspension, given orally or injected parenterally either directly into the lesion or at a distant site. It appears that *C. parvum,* like BCG, induces macrophage activation; paradoxically, it appears to depress T-cell function, especially that of splenic T cells. Many clinical trials with *C. parvum* are under way and claims have been made of increased duration of remissions in lung cancer and in metastatic breast cancer when the agent is used in conjunction with chemotherapy. Recent study in mice indicate that a synergistic effect is produced when *C. parvum* administration precedes chemotherapy with cyclophosphamide, but when the order of administration is reversed (cyclophosphamide followed by *C. parvum*) the antitumor effects of the adjuvant are delayed and toxic side effects are increased (68). Such studies are clearly of major importance for the development of optimal chemoimmunotherapeutic protocols for human neoplasias. Although *C. parvum* can cause tumor regression after intralesional injection, and in one

human study induced regression of lung metastatic nodules in diverse sites with disseminated cancers in 40% of cases, its toxicity and lack of major activity in most cases seem to preclude further use in human cancer.

The major drawback to the use of *C. parvum* for immunotherapy is its sometimes serious toxic effects. Fever up to 40°C, headache, and vomiting may follow its administration, and some patients have developed mild hypertension and/or peripheral vasoconstriction. For these reasons, we avoid it.

Bestatin

Bestatin, a metabolite dipeptide of *Streptomyces olivoreticuli,* potentiates both humoral and cell-mediated immune responses in vivo and in vitro (69) and has been effective therapeutically in controlling the metastasizing murine ESb-lymphoma. Bestatin seems to induce a macrophage-mediated tumoricidal activity even in nude mice. Oral administration to cancer patients has augmented NK activity and increased the T cells in the peripheral blood. Furthermore, Bestatin seems to induce the release of interleukin 2 from lymphocytes and to impede the turnover of lymphocyte membrane structures (70). A plethora of other augmenting agents of microbial origin and/or their components, too numerous to mention here in detail, are the subjects of extensive experimentation. Whether these agents eventually will find a niche in the clinicians' immunomodulating repertoire is unknown. For instance, Yamamura (71) employed the cell-wall skeleton of *Nocardia rubra* clinically to control malignant effusions of the pleura with considerable effectiveness. Although serious side effects were encountered, lung-cancer remission was considerably prolonged in a randomized trial. Another immunostimulating agent derived from the edible Japanese mushroom *Lentinus edodes* is a purified polysaccharide extract called lentinan (LNT). This agent in preliminary trials produced significant life prolongation in patients with advanced or recurrent cancers of the breast, stomach, and colon-rectum (72).

Thymic Hormones

Immunotherapeutic applications of thymic factors have received increasing attention in recent years, and a detailed description of clinical results and chemical studies can be found in the proceedings of several excellent symposia devoted to this topic (2, 9, 73). Many factors with thymic-hormone–like activity have been isolated and described, including thymosin, facteur thymique serique (FTS), and thymopoietin. Thymosin, a mixture of seven different peptides with different biological activities and the most widely studied of the thymic hormones, promotes T-cell differentiation and also may have other effects on cell-mediated immunity. The other thymic hormones appear to be comparable in activity yet different in structure. Although animal experiments have shown little or no toxicity, reactions to thymosin have been reported in a

few patients [see for example (74)], presumably to bovine-specific antigens since bovine thymosin is used generally for immunotherapy. Thus, thymosin should not be given to patients with histories of severe milk allergies; in our laboratory, patients receiving repeated injections are monitored by skin testing with the thymosin itself. A number of laboratories have isolated these different thymic hormones, and some of the fractions have been purified, sequenced, and chemically synthesized. In fact, thymosin has been biologically synthesized with the use of recombinant DNA.

Thymic factors have been used most extensively in patients with acquired or congenital T-cell defects, and dramatic improvements have been reported in some instances. In some patients with congenital T-cell deficiency, combined therapy using both thymosin and DLE appears to be beneficial, even though thymosin or DLE alone is without effect. Thymus extracts also are being tried in some autoimmune diseases such a systemic lupus erythematosus, since it appears that they can induce the differentiation of null cells into suppressor T cells in patients with apparent deficiencies in suppressor activity and, conversely, can cause a reduction in suppressor activity (or an increase in helper activity) in some hypogammaglobulinemic patients. Thymosin increases the percentage of T cells in vitro in some cancer patients. In clinical trials with thymus extracts for the treatment of solid tumors, clinical improvement appears to occur only in those patients who show such an in vitro response. In one controlled study, patients with lung oat cell carcinoma treated with multiple chemotherapy and thymosin demonstrated increased survival (113); however, at this point it is still too early to evaluate the efficacy of thymic hormones in cancer immunotherapy. To date, more than 300 cancer patients have been treated for periods up to four years with thymosin fraction 5 or thymosin and consequent therapy in phase I or II protocols. In one study, thymosin significantly prolonged survival in patients who showed eradication of all detectable disease by chemotherapy. The median survival for thymosin and chemotherapy was 500 days compared to 240 days for chemotherapy and placebo (75). It will be important to synthesize each component of the thymic hormones (Thymosin fraction 5 contains at least 20 different peptides), and test each component separately for immunomodulating effects in animal models before ultimately meaningful clinical trials in humans.

Interferon

Interferon, originally described and characterized as a specific antiviral substance, is now receiving widespread attention as a possible antitumor agent. Interferon preparations derived from pooled buffy coat cells of normal human blood donors currently are being tested in cancer patients. However, most of the early work with interferon has been done with preparations of dubious purity. For example, crude interferon preparations from human buffy coat cells

probably contain less than 0.5% interferon. Virus-induced type I interferons are proteins or glycoproteins of molecular weight between 15,000 and 40,000. The major effect of the active ingredient in interferon appears to be the potentiation and stimulation of natural killer cell activity (76).

A clinical trial of interferon was begun in 1971 in Sweden, using a crude preparation to treat patients with osteosarcoma. Twenty-eight patients received daily injections of 3×10^6 units of leukocyte interferon, followed by three injections per week for 17 months. Although the tumor load was reduced by surgical extirpation or irradiation before immunotherapy, no other therapy was used. The results indicated that after two and a half years the incidence of pulmonary metastases in the interferon treatment group was about 50% of that in concurrent controls, and the mortality rate was less than 50% of that for controls (77). Strander and co-workers in Stockholm also have treated patients with several other types of neoplasia (78). Preliminary results indicated some beneficial effects in patients with Hodgkin's disease, multiple myeloma, laryngeal papilloma, condyloma acuminata, and osteogenic sarcoma following surgical amputation. Partially purified human leukocyte interferon was used to treat these patients daily for a two-year period. Compared to groups of non-randomized concurrents and historical controls, significant prolongation of disease-free survival was noted. In 1979, the same workers using interferon saw complete or partial remissions in multiple myeloma (79), and others accomplished a 30% complete and partial remission rate in patients with metastatic breast cancer, non-Hodgkin's lymphoma, multiple myeloma (80), and partial regressions of both malignant melanoma and gastric carcinoma (81). The excitement surrounding interferon relates to the fact that it is a natural mediator molecule (produced in response to viral infection) that has shown antitumor activating potential in humans. The results of a handful of recent National Cancer Institute-sponsored trials have reported that, while interferon is not a cancer panacea, it indeed can counter some human cancers. Of 81 who received pure interferon made by recombinant DNA techniques, nine patients demonstrated a decrease in tumor size. In another study, 52 patients with various unresponsive cancers were treated and seven experienced tumor regression (82). Although these meager results were phase I trials in which dosage and administration schedules were being assessed, the use of sufficient quantities of pure (recombinant DNA) interferon preparations in the near future should afford a critical appraisal of its efficacy. Others have reported promising results in a few patients with cervical cancer, basal cell carcinoma, breast cancer, non-Hodgkin's lymphoma, and neuroblastoma. As already stated, however, the interferon preparations used in the earliest trials were extremely heterogeneous, containing less than 1% of interferon in an uncharacterized mixture of other leukocyte products, including, in our hands, many mediators of cellular immunity and TF as gauged by in vitro tests. Results now being obtained with preparations of greater purity seem to indicate far fewer benefi-

cial effects. Indeed, we believe that trials on cancer with more highly purified interferon will show that the beneficial effects of interferon preparations were due to the contaminants, i.e. other mediators of cellular immunity, including TF, present in the buffy coat extracts, rather than to interferon itself, and that purified leukocyte interferon will be only a weak immunomodulator (fibroblasts also make an interferon that is slightly different from leukocyte interferon).

CHEMICALLY SYNTHESIZED IMMUNOMODULATORS

Levamisole

Levamisole is a synthetic derivative of tetramisole that has been used extensively as a veterinary anthelmintic drug. It immunopotentiates the graft-versus-host reaction in experimental rats and in some animal diseases causes an apparent increase in host resistance to tumor cells. It acts on the cellular limb of the immune system and can restore impaired cell-mediated immune responses to normal levels but fails to hyperstimulate the normal functioning immune system (83). Thus, it shows true immunomodulator activity. Recent studies suggest that a metabolite of levamisole, DL-2-oxo-3-(2-mercapto-ethyl)-5-phenylimidazolidine, is the compound active on the immune system (84). The primary mechanism of action of levamisole may be to facilitate the participation of monocytes in the cellular immune response (85), apparently by enhancing monocyte chemotaxis (86). In addition, it has been reported to increase DNA synthesis of T lymphocytes and to augment their proliferative responses to mitogens, as well as their production of mediators of cellular immunity in vitro (87). NPT-16416, an immunomodulating levamisole-like purine, has been shown to have thymic hormone effects. It increases "active" T cell rosettes in human peripheral blood, augments by 30% lymphokine-induced macrophage proliferation, and induces the maturation of murine prothymocytes. It also demonstrates a restorative effect on the deficient E-rosetting capacity of peripheral blood lymphocytes of rheumatoid arthritics.

In humans, levamisole has been reported to restore delayed hypersensitivity reactions in anergic cancer patients and to be of some benefit in the treatment of aphthous stomatitis, rheumatoid arthritis, systemic lupus erythematosus, viral diseases, chronic staphylococcal infections, and breast cancer. In a placebo-controlled study of levamisole immunotherapy in resectable lung cancer, Amery (88) reported that both disease-free intervals and survival times were increased in patients who received the drug in three-day courses beginning three days prior to surgical removal of the tumor and courses every two weeks for two years. Thereafter, with adequate doses patients showed 25% versus 50% relapses and 15% versus 44% deaths respectively when compared to controls. Also, the number of hematogenous metastases was diminished sig-

nificantly. However, in the last few years, several in-depth studies of levamisole in patients with bronchogenic carcinoma, both alone and in conjuction with other therapy (e.g. BCG), were unable to demonstrate a therapeutic benefit. Indeed, in some cases the treated groups responded less favorably than the untreated (12, 89). Conflicting or negative data regarding the ameliorative effects of levamisone have been reported in breast cancer (90), colon cancer (91), head and neck cancer (92), melanoma (93), and leukemia (94). The reasons for these overall disappointing results are uncertain but may relate to the degree of tumor burden. With the emergence of more refined methods of detecting neoplasia in the early stages, levamisole may find a place in the oncological regimen.

Levamisole has a variety of side effects, and patients may complain of nausea, flu-like malaise, and cutaneous rashes that disappear after cessation of therapy. The most serious side effect is a granulocytopenia that is reversed upon therapy termination, but white cell counts should be monitored in patients taking the drug for prolonged periods. Interestingly, levamisole has been shown to suppress the formation of anti-DNA antibody in nude mice (95) and in high concentrations to produce suppression rather than augmentation of human T-cell mitogenic responses in vitro (96). Thus, clinical trials in patients should include careful immune monitoring to ensure that the immune system is stimulated rather than suppressed.

Maleic Anhydride-Divinyl Ether

MVE is a copolymer that has been studied extensively in animals for immunoregulatory capabilities. This compound and a series of similar polymers are reported to have anti-tumor activity through regulation of T, B, macrophage, and NK cell functions; they have been shown to possess antiviral activity, induce interferon, and act as adjuvants to tumor cell and viral vaccines (97). The early copolymers had extensive toxicity, but recently developed copolymer products have led to an enthusiastic reappraisal of this type of immunostimulant. Currently, phase-I cancer trials are under way with MVE-2 and preliminary results in humans affirm the animal studies.

Methisoprinol (Inosiplex) (Isoprinosine)

Methisoprinol (ISO), the p-acetamidobenzoic acid salt of N,N-dimenthylamino-2-propanol:inosine complex (3:1 molar ratio), is a synthetic immunomodulatory drug recently approved for clinical use in the United States. It appears to be effective in a wide variety of viral diseases. ISO increases cell-mediated immune functions in vitro, such as T-cell proliferative responses to antigens or mitogens, active T rosette formation, and macrophage activation. It also increases active T-cell levels in vivo in patients with low levels before treatment (98).

In vitro experiments have shown that ISO inhibits the replication of both DNA and RNA viruses in tissue culture, including *Herpes simplex,* adenovirus, and vaccinia (DNA viruses), and poliovirus, influenzae types A and B, rhinovirus, ECHO, and Eastern equine encephalitis (RNA viruses). Toxicological, teratogenicity, and carcinogenicity studies have demonstrated that ISO is safe, well tolerated, and remarkably free of side effects, even upon prolonged administration. It contains an inosine moiety, a naturally occurring purine in lymphocytes, that is metabolized via normal biochemical pathways to uric acid.

ISO potentiates cell-mediated immune responsiveness in vivo (103), and a major factor in its effectiveness against viral infections appears to be its ability to prevent the depression of cell-mediated immunity shown to occur during viral infection and to persist for four to six weeks thereafter (44). It may prove useful as an adjunct in cancer therapy as well, since there is considerable evidence for a viral etiology of some types of malignancies in humans (99), and since cancer cells produce immunosuppressive factors (100) that might be counteracted by the immunoenhancing effects of ISO. ISO has been shown to enhance production of interleukin 2, and its structure probably helps to increase the general metabolic availability of inosine. A synergistic effect with NPT 15392 was noted on mitogen stimulation, induction of suppressor-cell activity in vitro, and suppression of mixed lymphocyte reactivity; an increase of 63% over controls was achieved in lymphocyte lymphotoxin production in 60 *Herpes simplex* patients (101). Although little evaluation of ISO has been done yet in cancer patients, it has been extensively studied in a hamster model (102). Recently, in 47 human patients with primary tumors (26 lung carcinoma, 14 breast carcinoma, and 7 melanoma), ISO restored to normal all three parameters tested, including ConA-induced lymphocyte proliferation, NK cell activity, and monocyte chemotaxis (103).

The clinical efficacy of ISO has been well documented in double-blind trials; for example, ISO produces a striking decrease in both the duration of infection and severity of symptoms in a whole host of viral diseases, including influenza virus infections (104), rhinovirus infections (105), *Herpes labialis* and *Herpes progenitalis* (106), *Herpes zoster* (107), viral hepatitis (108), rubella (109), and viral otitis (E. Berthaux, unpublished observation, 1978). Of particular interest are the results of ISO therapy in subacute sclerosing panencephalitis (SSPE), a progressive disease due to a chronic measles virus infection that results in complete debilitation and eventual death of the patient. ISO has been reported to halt the progression of SSPE when given in stages I and II of the disease in 80% of the patients provided it is administered for at least six months (111). Indeed, ISO is the only agent to date with documented beneficial effects in SSPE patients. The mechanism of action is unknown, but it appears to prevent virus-induced and cancer-induced immunosuppression.

NPT 15392

This hypoxanthine analogue is a relatively new heterocyclic immunostimulating compound that possesses neutrophil, T-cell, and NK stimulatory properties. Its structure is related to inosine and its action to methisoprinol. It augments human T-cell mitogen stimulation by PHA or ConA, lymphokine-induced macrophage proliferation, and suppressor T-cell induction. When 20 cancer patients were treated with 0.4 or 0.7 mg of NPT 15392 every three days for 10 days, the numbers of blood T cells, percentages of E rosettes, and autologous T rosettes were increased; furthermore, a decrease in the OKT4+ T-cell subpopulation also was observed. This drug is appealing particularly since it has virtually no adverse side effects. Another related synthetic compound belonging to the 2-cyanaziridines is Azimexon. This agent, although active mainly on T-lymphocytes in normals, has been shown to have a B-cell activation effect on the deficient immune system in chronic lymphocytic leukemia. No clinical side effects have been reported and the patients demonstrated significant improvements in testing for in vitro cell-mediated immunity and IgM and IgG immunoglobulin synthesis. Therapeutic effects have been noticed in arthritis and tumor systems, with increases in suppressor T-cell activity and concomitant decreases in T helper cell activity. One exciting aspect of this drug is that it seems to diminish markedly the chemotherapy-induced immunosuppression seen with other neoplastic drugs, such as cyclophosphamide. While it reduces their toxicity, it induces leukocytosis and reconstitutes the nuclear cellular components of the bone marrow.

Tuftsin

Tuftsin is a biological naturally occurring tetrapeptide with remarkable immunostimulating capability. It is generated in the serum by two enzymatic cleavages from the parent molecule, a cytophilic immunoglobulin, and represents amino-acid residues 289 through 292 of the constant region of the IgG heavy chain. Tuftsin accentuates a number of activities associated with the macrophage and neutrophil, including motility, phagocytosis, immunogenic functions, augmentation of antibody production, bactericidal activity, and antitumor effects in animal systems (112). This naturally occurring immunopotentiator shows tremendous promise as an antineoplastic agent. It is anticipated that clinical cancer trials in humans soon will be attempted.

CONCLUSION

The above review provides an indication of the rapid progress made in recent years in the development of immunostimulating agents for the treatment of human disease. As stated earlier, the clinical effects of these other immunomodulators are generally more favorable in patients with a minimal antigenic

burden; this is the rationale for the use of these agents in combination with chemotherapy, radiotherapy, and/or surgery, particularly in cancer patients, an approach termed chemoimmunotherapy or immunochemotherapy. Furthermore, most of the immunotherapeutic agents now employed for cancer therapy are more effective against lymphoid malignancies than other types of cancers: for example, acute lymphatic leukemia responds far better to immunotherapy than does acute myelogenous leukemia, and lymphomas respond better than solid tumors (colon, breast, etc).

It may be also that combinations of immunomodulators will prove more effective than single agents. It can be envisioned that once the mechanisms of action of the various drugs and biologicals are known in detail, specific combinations can be used in sequence to produce a desired clinical result, such as ISO to combat viral infection, followed by DLE to provide prophylaxis against future infection with the same virus in a patient with a genetically determined antigen-specific defect in cell-mediated immunity against that particular viral agent. Studies of the type described above with *C. parvum* and levamisole should provide valuable insights into the possibilities of this sort of approach.

Finally, in regard to synthetic immunostimulants, rapid advances are accruing in the development of many new agents. Second- and third-generation compounds related to ISO and other chemically synthesized immunomodulators (e.g. azimexone) are already under investigation, and it is anticipated that future research will provide both further insights into the nature and mechanisms of action of such compounds and increasing clinical benefits to patients who receive immunotherapy.

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