ENHANCEMENT OF HOST DEFENSE MECHANISMS BY PHARMACOLOGICAL AGENTS

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INTRODUCTION

The current use of pharmacologic agents in the treatment of infectious or neoplastic diseases is based, for the most part, on the ability of such drugs to interfere with the metabolism of the invasive organism or cell. This may be successful in the case of bacterial and protozoal infections because of metabolic differences from host cells that allow selective attack. In the case of neoplastic and virus-infected cells, metabolic pathway differences from normal host cells may be subtle, a fact that may at least in part explain the frequent toxicity of agents found to be active against these abnormal cells. Occasionally, this toxicity is manifested by suppression of host defenses which are important for prevention or cure. Therefore, as new pharmacologic agents are developed, it is important to define their effects on host defenses, and to influence these defenses favorably. This review has been organized along the lines of traditional immunologic and nonimmunologic host defenses in order to provide a rational basis for understanding the effects of some of the newer agents under development (Table 1). This task is difficult because in some cases the mechanism of action of an agent may not be well defined; in other cases multiple effects may make it difficult to delineate the primary role of a drug. Nevertheless, an attempt at synthesis is appropriate at a time when enhancers of various host defenses are either being developed or are becoming available for clinical trial.

Several general points should be made in regard to manipulating host defenses. In vivo, they function as a cooperative, rapidly occurring, and closely interrelated set of responses. Hence, agents influencing them may have critical requirements in terms of timing and extent of the disease process, route of administration, and exact

Table 1 Enhancement of host defense mechanisms by pharmacological agents

Host defense	Agent	Potential uses	Side effects
Reticuloendothelial system			
Macrophage function			
Phagocytosis	Adjuvants: (BCG, LPS, polyribonucleotides, *B. pertussis*	Immunization Cancer immunotherapy	Local inflammatory reactions, uncontrolled BCG infection
Killing of microorganisms	Clofazimine	Intracellular infections	
Opsonization	Human plasma	Lenier's disease	Transfusion reaction
	Human gammaglobulin	Agammaglobulinemia	—
Immunity			
Humoral	Amantadine	Influenza Type A	CNS irritability
	Tilorone	?	?
Cellular	Transfer factor	Immunological deficiency states Infectious disease with anergy	—
	Levamisole	Malignant disease with anergy	—
	Lentinan	Malignant disease with anergy	?
Cellular resistance	Interferon and inducers	Antiviral and antitumor	Crude preparations pyrogenic immunosuppression? allergenic?
Nutritional factors			
Competition for iron	Desferrioxamine	Listeriosis? tuberculosis?	Allergenic
Epithelial keratinization, Lysosome labilization	Vitamin A	Adjuvant	Vitamin A toxicity
?	Vitamin C	Common cold	—

dosage. The agents described in this review also may have differing effects on host defenses depending upon how soon the immune or nonimmune function is studied after administration. For example, injections of endotoxin have diphasic effects, e.g. they may first depress and then enhance reticuloendothelial (RE) function. Hence, exact results in any species, or with a given dosage, may differ depending upon the kinetics under those particular conditions. It is conceivable that immunopathology could be a result of the improper use of host defense modifiers. In addition, immunosurveillance may not always be beneficial. For example, Prehn and his associates (1) have advanced the concept that under certain conditions immunoenhancement might potentiate rather than suppress tumor development. Hence, we require knowledge of host defense mechanisms in infectious and neoplastic diseases in order to use these enhancing agents skillfully.

RETICULOENDOTHELIAL SYSTEM

The classic concept of the reticuloendothelial system (RES) evolved in reference to a widespread system of fixed and migratory mononuclear macrophages which have the common functional property of being able to ingest and clear the circulation of foreign colloidal and particulate matter. The clearance of colloid serves as a quantitative measure of RE activity which can be modified by a number of endogenous or exogenous factors (2). The activation of the RES during bacterial infection (3,

4), neoplastic disease (5), and autoimmune diseases (6) suggests its importance as a host defense in these conditions. Depression of the RES, as in circulatory failure, may also be an important factor in determining the outcome of disease processes (7). The physiology of the RES has been studied by the blockade technique in which a suppression of RE activity is produced by the intravenous injections of large amounts of foreign colloidal material. As a result of altered function during the state of blockade, the RES has been shown to play a role in the metabolism of iron, bilirubin, cholesterol, steroids, and certain drugs, in addition to its role in clearing the blood of exogenous material (2). The definition of the component functions of the RES and its interactions with the immune system has provided the opportunity to influence certain of these host defenses pharmacologically.

Macrophages

Because of the potential value of the ability to alter and direct the function of the RES, much current effort is devoted to the study of agents that appear to act via this system. Phagocytic activity can be enhanced by the administration of glucan (8), zymosan (9), estrogen (10), endotoxin (11), BCG (12), or *Corynebacterium parvum* (13), while cortisone (14), methyl and ethyl palmitate (15, 16), antilymphocyte serum (17, 18), and silica particles (19) diminish RE activity. Recently, a phenazine derivative, Clofazimine, has been shown to enhance the ability of human macrophages to kill Listeria. The enhanced bactericidal effect was prevented by anaerobiosis and it was tentatively concluded that increased hydrogen peroxide generation was responsible for the enhanced killing. However, the macrophages exposed to Clofazimine contained crystalline deposits, which raised the question that stimulation of phagocytosis may have been involved (20).

ADJUVANT EFFECTS ON MACROPHAGES It has been known for some time that adjuvants can be employed to increase antibody titers and that their use enhances development of the delayed hypersensitivity response. A growing body of evidence indicates that the macrophage plays a key role in the afferent limb of the immune response, not only in the concentration and processing of antigen, but also in the activation of lymphocyte functions as well. In syngeneic mice utilizing hemocyanin as antigen and *Bordetella pertussis* as adjuvant it has been shown that adjuvant-treated peritoneal mononuclear cells were required for an increased antibody response (21). Moreover, the adjuvant action did not require the presence of adjuvant and antigen in the same cell because peritoneal mononuclear cells could be exposed separately to each component with a resulting enhanced response. Similar results were obtained using bovine serum albumin as antigen and *Escherichia coli* lipopolysaccharide (LPS) as adjuvant (22). The effects of double-stranded polyribonucleotides on the response of mice to sheep red blood cells (RBC) appear to involve macrophages in the same manner as the previous adjuvants (23, 24). It now appears that for at least some antigens, the production of antibody requires the interaction of three cell types; macrophages, thymus-derived helper cells, and bone marrow–derived, immunoglobulin-synthesizing lymphocytes or plasma cells. In addition to a nonspecific effect on macrophages, some adjuvants *(B. pertussis)* require prior

sensitization (25). Also, supernatants of spleen cells exposed to adjuvant were able to enhance the primary immune response (25). Macrophages exposed to endotoxin in vitro have been shown to liberate a factor that stimulates DNA synthesis by T-lymphocytes (26). Direct stimulatory effects of double-stranded RNA on T-lymphocytes in vitro have also been reported (27). Thus it appears that adjuvant effects may be mediated by at least two mechanisms: a T-cell stimulatory factor produced by macrophages and a B-cell (or macrophage) stimulatory factor produced by adjuvant specific stimulation of T-cells. The development of adjuvants for use in man that are potentially effective and safe, such as adjuvant 65 (28), increases the importance of understanding the exact mechanisms involved in their action.

MACROPHAGE ACTIVATION In addition to their role in the afferent limb of the immune response, mononuclear phagocytes are now recognized as important effector cells of both the humoral and cellular immune responses. These functions are manifested by enhanced phagocytosis after opsonization by specific antibody and by enhanced bactericidal and cytotoxic effects. It has become clear that one manifestation of cellular immunity, the delayed hypersensitivity response, is mediated by both mononuclear cells (which may be obtained from unimmunized donors) and immune lymphocytes (29–31). There is a similar dependence upon monocytes for the expression of cellular immunity against listeria and toxoplasma microorganisms. Macrophage activation may result from the antigen-specific stimulation of immune lymphocytes; however, once activated, macrophages will exhibit increased antitumor cell (31a) and bactericidal properties to antigenically unrelated microorganisms (32–34). Increased macrophage-mediated resistance to intracellular infection can also be induced by poly-L-glutamic acid (35). Further studies of this system have revealed that supernatants from cultures of macrophages, sensitized spleen cells, and antigen could activate normal macrophages (33, 36) and that thymus-derived lymphocytes were the responsible cell type for such activation (37). The resulting enhanced phagocytosis and bactericidal activity of macrophages is associated with an increase in the rate of glucose oxidation (38, 39).

In addition to possessing enhanced bactericidal capabilities, macrophages can be rendered cytotoxic by a factor released from specifically stimulated immune lymphoid cells. The cytotoxic macrophage response can be specifically triggered by lymphocytes immune to unrelated microorganisms or to antigens of the cells that are ultimately destroyed (40). This finding reinforces the concept that the cytotoxic properties of activated macrophages are nonspecific despite their triggering by an immunologically specific event (31). It has also become apparent that macrophages have important antiviral functions. Newborn mice can be protected against systemic disease with herpes virus by macrophages from nonimmune adult animals suggesting that immature macrophages are more difficult to activate (41). The activated macrophage has been shown to be an important effector mechanism in the recovery from ectromelia virus (42) and Venezuelan equine encephalomyelitis virus (43) infections in mice. The production of interferon by stimulated macrophages may contribute to the protection observed in these model infections (44).

Opsonization

The coating of "foreign" particles by immunoglobulins and complement is an important regulator of the phagocytic activity of the RES (45). Either a particulate antigen can combine with specific antibody and be ingested, or the antigen-antibody combination may fix the first four components of complement, which then combine with receptor sites on macrophages. The second mechanism involves a heat-labile system which appears to be nonspecific and is found in nonimmune and germ-free animals (46). This system utilizes a nonspecific 7S immunoglobulin and also results in the fixation of the third component of complement—produced by the so-called alternate pathway involving properdin.

The clinical significance of opsonins is shown by a number of conditions in which decreased serum-opsonizing activity is associated with increased susceptibility to infection (45). This association is found in the case of newborn infants (47–49) and sickle cell disease (50, 51). An acquired defect of heat-labile opsonins in the serum of patients with active systemic lupus erythematosus has been related to the presence of concomitant infection (52). Disorders of the complement system that result in decreased opsonin activity include increased C3 catabolism, also associated with deficient bactericidal and chemotactic activity (53, 54), and C5 dysfunction (55–57). The decrease in circulating opsonin activity has been shown to be a major factor mediating the state of RE impairment in colloid-induced RE blockade (58, 59), starvation (60), and surgically induced RE depression (splenectomy) (61, 62). Restoration of normal function may be possible by the use of plasma transfusions in Lenier's disease (C5 dysfunction) and the administration of immune globulin to patients with agammaglobulinemia.

IMMUNITY

Humoral

The role of humoral immunity as a host defense is readily apparent from the clinical problems that arise in states of acquired and congenital agammaglobulinemia. These patients suffer from recurrent infections with encapsulated bacteria presumably as a result of defective opsonization and phagocytosis. The specific nature of this defect is indicated by its reversibility upon administration of human gammaglobulin.

Pharmacologic agents that either act in concert with or stimulate the humoral immune system include amantadine and tilorone. The antiviral properties of amantadine appear to be due to the ability of this drug to impede cellular penetration of A_2 strains of influenza. This allows a cooperative function with antibody which has been demonstrated both in tissue culture (63) and in field studies (64) where protection is maximum in individuals with preexisting antibody.

Another example of cooperation between pharmacologic agents and host factors that has recently been summarized (65) illustrates the synergism between aminocyclitol antimicrobics and serum factors on the inhibition of the growth of *E. coli* in vitro. The clinical relevance of this finding is unknown at present; however, in mice,

additive protective effects have been observed between carbenicillin and immune serum against *Pseudomonas aeruginosa.*

Tilorone was initially described as having antiviral activity in mice, activity that was associated with the induction of interferon (66, 67). Subsequently, antitumor activity (68), stimulation of the RES (69), and enhancement of the primary humoral immune response (70) have been reported with tilorone. The drug appears to be unique in that it enhances humoral immunity while suppressing delayed hypersensitivity responses (70, 71). The mechanism by which it exerts its effects on the immune system is unknown at present.

Cellular Immunity

The interaction of sensitized, thymus-derived, lymphocytes with specific antigen results in the release of cell-free soluble factors, termed lymphokines by Dumonde, that mediate the expressions of cellular immunity (72). The activities that have been defined include chemotactic factor, blastogenic factor, skin-sensitizing factor, lymphotoxin, macrophage migration inhibition factor (MIF), and interferon (73). In addition, there is now evidence for the existence of a macrophage activating factor as discussed above. While these factors appear to have a major role in effecting and regulating the cellular immune response, their selective manipulation by pharmacologic agents is not possible at present. Indeed it is not yet clear whether or not these activities reside in a few or many different molecules.

Transfer factor (TF) is a small (<10,000 mol wt) molecule or molecules, found in circulating human leucocytes which is reported to have the remarkable property of transferring to the lymphocytes of the nonsensitive human recipient the donor's pattern of delayed skin reactivity and, presumably, immunologic memory (74). The exact mechanism by which this occurs is unknown, and study has been hampered by the lack of a suitable animal model and a system for assaying biological activity. Nonhuman primates are currently being given TF in several laboratories and these studies should yield findings that may complement prior work in humans. Assay may now be possible using the in vitro technique described by Ascher et al (75). The concentration of inhibitors and toxic substances during the preparation of water-dialyzed TF seems to have been a major problem.

Clinical applications of TF would most logically be found in infections or neoplastic diseases associated with anergy or states of immunologic deficiency. Attempts at immunological reconstitution with TF have been carried out in patients with the Wiscott-Aldrich syndrome (76, 77), Chediak-Higashi syndrome (78), and Swiss-type agammaglobulinemia (79). Some of these patients have responded with clinical improvement of chronic fungal infections (77, 79), development of delayed hypersensitivity reactions (77, 79), and the production of MIF in vitro (77, 79). Because the deficiencies that result in these diseases are not well defined, inferences as to the mechanism of action of TF must be speculative. Treatment with TF is not without hazard; a patient with severe combined immunodeficiency disease developed a polyclonal gammopathy and a fatal lymphoproliferative disorder following TF administration. Although lymphoproliferative disorders may complicate immunodeficiency diseases, the temporal relationship to TF administration in this

patient was striking and led to the suggestion that the absence of a T-cell regulatory function may have allowed TF to stimulate a state of uncontrolled B-cell proliferation (80).

Because of the worsened prognosis associated with cutaneous anergy in certain infectious diseases, attempts to restore delayed hypersensitivity responses with TF have been made. Transfer factor from lepromin-positive persons has been given to patients with lepromatous leprosy with conversion to skin test positively in 11 of 22 cases (79, 81, 82). Erythema and induration developed in six patients; erythema nodosum occurred in four; and fever and arthralgia occurred in three of nine patients given TF (82). The clinical course was not obviously altered by this single-dose treatment; however, it appears that the immune response in lepromatous leprosy is subject to manipulation, possibly to the benefit of the patient. In chronic mucocutaneous candidiasis treated with TF, nine of ten patients have responded with the development of cutaneous reactivity, and lymphocyte transformation or MIF responses to candidal antigen. Clinical improvement was observed in six of the TF responders (79). The repeated administration of TF was associated with clinical improvement and a return of cutaneous reactivity and in vitro lymphocyte responses in single cases of progressive primary tuberculosis (83), disseminated coccidiomycosis (84), and subacute sclerosing panencephalitis (85).

Because of the ability of TF to restore cutaneous-delayed hypersensitivity in some patients and the association between anergy and worsened prognosis in various forms of malignancy, TF has been explored in the immunotherapy of cancer. Repeated daily or thrice weekly, injections of TF were given to five patients with advanced breast cancer for periods of up to 310 days without untoward effects. Delayed sensitivity to tuberculin and/or streptococcal antigens developed in three patients (79). Patients with melanoma have been treated with frozen and thawed leucocyte extracts (86) and dialyzable transfer factor (87) with enhancement of lymphocyte cytotoxicity.

Anergic patients with lepromatous leprosy, mucocutaneous candidiasis, and malignant disease may have lymphocytes that are responsive to the respective microbial or tumor antigens in vitro (88). The serum of such patients may contain an inhibitor or inhibitors with the properties of a "blocking antibody" or antibody-antigen complex. In these circumstances, TF may restore the delayed hypersensitivity that had been "blocked." Whether this is accomplished through an antigen-specific transfer of immunological memory (74) or through adjuvant effects (89) the phenomenon is of fundamental biological importance and may have therapeutic potential. Because of the variable course of patients with diseases for which TF has been given (with or without specific chemotherapy), controlled studies will be required for confirmation and extension of these findings.

Tetramisole and levamisole, the active levo form, are antihelmintic drugs that have been shown to augment cellular immune responses in several systems. Tetramisole restores cell-mediate responsiveness to brucella vaccine in mice without affecting antibody titers (90). An inhibitory effect of levamisole on malignant metastatic tumors in mice has been reported (91); however, results with other animal tumors have been negative (92). Levamisole has been shown to restore or augment

cutaneous-delayed hypersensitivity reactions in some anergic patients with cancer (93, 94). Serum antibody concentrations, in response to influenza vaccination, were only temporarily increased (95). The effects of levamisole are associated with enhanced macrophage function. The phagocytic activity of murine peritoneal cells, after sensitization by inactivated *Salmonella typhimurium,* could be increased by pretreatment of the animals with levamisole. When levamisole was given to mice without sensitization, the phagocytic activity was not increased; however, levamisole, added to macrophages in vitro, significantly increased phagocytic activity (96). Tetramisole and its optical isomers can transiently increase the clearance of carbon particles in mice without increasing hepatic or splenic weight indicating that enhanced phagocytosis, rather than hyperplasia, was involved (97). The enhanced phagocytosis resulting from levamisole treatment is associated with a lowering of the contact angle between a sessile drop of buffered saline and a macrophage monolayer, a measure of the hydrophilic properties of the macrophage that correlates with phagocytic activity (98).

Antitumor activity has been reported for polysaccharide preparations from various natural sources, such as higher plants, fungi, lichen, bacteria, and yeast. One of the more active polysaccharides of this group is lentinan, a compound with well-defined physical and chemical properties (99), which is active against mouse sarcoma 180 (100). The effectiveness of pretreatment and the ineffectiveness on tumor-cell cultures suggest a host-mediated antitumor action. An action on cellular immune mechanisms is supported by the findings that the antitumor effects cannot be demonstrated in thymectomized mice and are lost after treatment with antilymphocyte serum (101).

INTERFERON AND INTERFERON INDUCERS

The antiviral protein, interferon, is produced by host cells in response to viruses and other intracellular parasites. Interferon production has proved to be a widespread phenomenon in nature occurring in all vertebrates so far examined (102). Fibroblasts, epithelial cells, and macrophages, as well as lymphoid cells will produce interferon in response to a viral infection (102). Many other more complicated microorganisms and their extracts, as well as synthetic materials (particularly polyanions like double-stranded RNA), can induce production of interferon by cells in vivo or in vitro.

There are at least two approaches to using the interferon mechanism to prevent the spread of virus within the infected host: 1. stimulation of endogenous interferon production, and 2. administration of exogenously produced interferon. In contrast to the successful application of interferon-inducers in animals, it is not yet clear that these substances will be of use in man. So far, the potential dangers of side effects of these inducers as well as the difficulty in controlling the time of their application in relationship to development of naturally acquired disease have prevented successful systemic application in man. On the other hand, in local virus-infected areas such as the respiratory tract or cornea there have been some encouraging results in clinical trials. For example, double-stranded RNA has been shown to modify acute

herpes keratitis, in a similar fashion to frequent applications of IUDR, by two groups of investigators (103, 104). In addition, a recent well-controlled, study by Panusarn et al (105) clearly demonstrated that prophylactic installation of a synthetic small molecule interferon-inducer blocked rhinovirus infection in the human respiratory tract. In comparison to untreated infected control volunteers the efficacy of the agent was demonstrated by the prevention of symptoms and the prevention of virus shedding in association with the production of elevated interferon titers in nasal washings. Thus, the therapeutic and prophylactic activity of locally applied interferon inducers has been demonstrated in man under well-controlled trial conditions. Whether such observations can be extended to useful effects in field studies can only be known with further investigation.

The action of a particular interferon is usually limited to cells of the same animal species that were used for production. For this reason, interferon with activity in humans must be obtained from human or primate cell sources. Administration of interferon prepared in human cells (either leucocytes or fibroblasts) is in the early stage of clinical investigation. The material is quite expensive to prepare, and clinical trials can be considered only an attempt to establish minimal effective doses in man. Any widespread extension of such work will require a major improvement in production methodology and efficiency. Present trials are under way with human leucocyte interferon which is made available by a single large blood transfusion center in Finland as a byproduct of blood collected for transfusion (106). These facilities can provide interferon for only a limited number of demonstration experiments; it is difficult to envision extension to other blood transfusion centers because of the quality control, special methods, and training required for optimum production. In addition, the Finnish source is unique in that it depends on a centralized blood bank for the entire country handling large quantities of blood each day—in contrast to the decentralized, locally supervised, smaller volume systems functioning elsewhere in the world. Therefore, two major alternative means for production are being explored. The first and most immediately implementable is the use of diploid or other human cell substrates. The use of such substrates is more acceptable to many authorities than the use of leucocytes, as human cells are currently in use in the production of certain widely employed viral vaccines. The second solution to the problem of availability is less immediate but perhaps more satisfactory for its ultimate widespread clinical applications. Efforts are under way currently to determine the structure of human interferon by groups who would like to have such information for its possible organic synthesis. Although the expense of such synthetic material might be great, the high specific activity of interferon suggests that only minute quantities may be required per person treated.

Results with human leucocyte interferon have been somewhat encouraging as to its safety (107, 108) and efficacy (109) if sufficiently large doses are given. In a randomized placebo-controlled trial, topical application of interferon was successful in protecting normal individuals from rhinoviral infection without any side effects (109). Here, symptoms, virus shedding and seroconversion were prevented in interferon-treated volunteers in contrast to placebo recipients. Potential side effects could limit the dosage employed with systemic administration of interferon. However,

preliminary clinical trials are under way; for example, data have been accumulated on the results of regular intramuscular injection of human leucocyte interferon into patients with a wide variety of late-stage tumors. Strander et al (107) have treated 28 patients with up to 3×10^6 units, 3 times weekly, for 2½ yr with no limiting side effects. Fever was noted in most patients receiving the injections. Late in the course of treatment, antibodies were noted to some of the contaminating inactivated virus used to stimulate leucocytes and to proteins of the chick cells in which it was grown, but not to the interferon itself. The wide variety of patients studied and the uncontrolled nature of this trial precludes any conclusions regarding efficacy, but does indicate that this dosage is virtually free of toxicity.

Currently, human leucocyte interferon is also being given intramuscularly to patients with malignancy (108) and acute varicella-zoster infections in doses of $10-25 \times 10^6$ units per 12 hr for a period of up to 6 days. Initial studies were oriented toward examining possible toxicity and establishing pharmacokinetic data (108). When the intravenous and intramuscular routes were compared directly, a higher peak interferon response was seen after slow intravenous infusion. However, serum levels greater than 25 units were obtained more rapidly and were sustained longer following intramuscular administration. There was no evidence of local inactivation following intramuscular administration.

In contrast with early work in small animals on the pharmacokinetics of homologous interferon which indicated a serum half-life of approximately 5 min after rapid intravenous injection, the results in man indicated a half-life of approximately 4 hr after sustained infusion. This difference appeared to be due to more complete tissue saturation in man, with slow release from tissue sites maintaining blood levels after the infusion was stopped.

The rise of varicella-zoster complement–fixing antibodies and the increase in vesicle fluid interferon and cells were unimpaired in the interferon-treated patients. A fever of 38–40°C was seen in association with interferon administration; however, no treatment-limiting toxicity was observed. The average level of circulating interferon obtained by either the intramuscular or intravenous route is higher than reported levels in naturally occurring human viral disease and is approximately 17 times the minimal inhibitory concentration for varicella-zoster virus in human cell cultures (109). In keeping with its high molecular weight, systemically administered interferon appeared to penetrate very poorly into cerebrospinal fluid or skin vesicles, and it appeared in the urine of only 1 of the 9 patients carefully studied. Interestingly enough, that individual had a rare form of renal disease (amyloidosis) which caused him to excrete plasma proteins in the urine as well.

These observations on the route and safety of the material plus the ability to maintain high circulating antiviral levels with intramuscular injection form the basis for further clinical studies of systemically administered interferon in man. Randomized placebo-controlled trials are currently under way to evaluate the activity of human leucocyte interferon on the clinical course of early localized zoster and varicella in patients with malignancy. The risk to the patient of these infections is significant. The symptoms persist for a relatively long period of time allowing for the treatment of patients early in the course of the disease when there is still a risk

of complications caused by continuing viral replication. Interferon levels from the infection are low at all sites early in the disease, and rise with healing only in the vesicles, not in the serum. Thus, the circulating interferon titers produced by intramuscular injection of exogeneous interferon may prevent virus from spreading to distant sites, such as the viscera, or prevent cutaneous involvement beyond the primary dermatome in localized zoster.

Undoubtedly, further trials will be conducted using exogenous interferon against various viral infections and malignancies in the future as more interferon becomes available. Whether the very encouraging results (102) obtained in animal studies can be extended to man for practical applications will only be known from work yet to be performed. High doses of interferon appear to have effects on rapidly dividing cells including both tumor cells and normal hematopoietic and lymphoid cells. Whether dosage regimens can achieve enough selectivity of action to produce antitumor or antiviral effects in man without serious side effects will only be known with further investigations.

It is important to keep in mind a recent study on the use of cytosine arabinoside to treat disseminated zoster. The drug actually prolonged disease in patients (who had preexisting immunodeficiency) in association with further immunosuppressive effects on viral immunity (110). Such results dictate caution in the application of "antiviral" drugs and clearly indicate the need for monitoring host defenses, as well as other parameters of efficacy and toxicity, in well-matched groups of treated and concurrent control patients.

IMMUNOTHERAPY OF CANCER

The use of a variety of natural and synthetic substances that enhance immunological and nonspecific host defenses has been accompanied with varying degrees of success during attempts to prevent and treat malignant disease. Agents judged to have some activity in clinical or experimental situations include BCG (111, 112), *C. parvum* (113), endotoxin (LPS) (114), vaccinia virus (115), "transfer" serum (116), and polyribonucleotides (117). Of these agents, BCG has received the most study at both the experimental and clinical levels, and an extensive review of this work is available (118). BCG is a live attenuated derivative of the bovine tubercle bacillus which acts as a stimulator of the RES and can confer resistance upon mice to bacterial (119, 120) or viral (121) infection. Prior administration of BCG to laboratory animals has been shown to prevent the growth (122) or cause regression (123) of transplanted tumors that either arose spontaneously or had been induced by chemical carcinogens or by viruses; however, stimulation or tumor growth has occasionally been seen (118). As a result of the preventive effects of BCG on the development of new tumors in animals, the incidence of death from leukemia in children receiving BCG vaccination has been determined. Two groups have reported a decrease in leukemia deaths in BCG-immunized children while other studies failed to demonstrate a significant effect (118).

Treatment of established tumors in experimental animals has proved more difficult than prevention. Success has been dependent upon small tumor size and an

intact immunological response of the host. In view of these requirements other forms of cancer treatment such as chemotherapy (124), surgery (125), and endocrine therapy (118), which are effective in reducing tumor load, have all been shown to increase the effectiveness of BCG immunotherapy in experimental models.

BCG immunotherapy in man can be divided into two types of treatment, intralesional or systemic, which may depend upon different mechanisms of action. Local injections of BCG or PPD have resulted in improvement of several different types of cutaneous neoplasms such as basal and squamous cell carcinomas, mycosis fungoides, lymphangiosarcoma, reticulum cell sarcoma, and Kaposi's sarcoma; the most striking effects have been seen in malignant melanoma (112, 118, 126). In patients selected for immunocompetence and limited disease, intralesional injection of BCG can eliminate cutaneous metastases in about 20% of patients with melanoma (118). Visceral metastases are not affected, and the effect on the life span of these patients is unknown.

Chemotherapy may reduce the number of malignant cells by several orders of magnitude but is often not successful in the complete elimination of these cells which then become responsible for a clinical relapse. Immunotherapy may have a particular role in the complete elimination of reduced numbers of abnormal cells—as shown in experimental mouse leukemia (127). The addition of immunotherapy to the chemotherapy of acute lymphocytic leukemia in humans has shown favorable results (128) or has been without demonstrable effect (129, 130). The use of irradiated allogeneic tumor cells, *Corynebacterium granulosum, C. parvum,* or double-stranded RNA appears to have no benefit as compared with BCG (118). The use of BCG immunotherapy in acute myelogenous leukemia has given promising results in terms of length of remission (131, 132); however, a definite effect on the survival of patients with acute or chronic myelogenous leukemia has not been shown.

Although BCG immunization of a normal host is a safe procedure (133), complications have occurred. The most frequent have been localized abscesses and regional lymphadenitis (134); but pulmonary tuberculosis (135), erythema nodosum (136), granulomatous hepatitis (137), lupus vulgaris (138), osteoarthritis (139), osteomyelitis (140), and pancytopenia (136) have all been reported. BCG immunotherapy in anergic patients with cancer might be expected to result in more frequent and more severe complications, particularly if large doses of viable bacteria or repeated injections are used (141–144). The occurrence of chills, fever, malaise, and hepatic dysfunction due to disseminated BCG infection have been reported in these patients (145, 146). The strains of BCG which have been used in these studies, (Tice and Glaxo), are susceptible to isoniazid, *p*-aminosalicylic acid, and ethambutol; isoniazid has been effective in patients with progressive infection (147). Recently, a lipid-free, water-soluble, BCG fraction with adjuvant and antitumor activity has been described (148). The further development of noninfectious, pharmacologically active immunostimulants appears to be both feasible and worthwhile.

The mechanism of action of BCG is complex, and further definition of normal host defenses will probably result from attempts to isolate the pathways by which BCG effects occur. The presence of a chronic granulomatous response at the site

of BCG injection and in regional lymph nodes, as well as chronic inflammatory response at the tumor site, appear to correlate with a favorable outcome (118). Tumor cells may be killed as a result of an immune response directed at either the BCG antigens or the tumor antigens themselves (118). Evidence has accumulated for nonspecific stimulation of T-derived cells (involved in lymphocyte cytotoxicity), by a macrophage-produced lymphocyte-activating factor when BCG is given systemically to mice (149). Although the effects of BCG are thought to be mediated primarily by the immune response, other mechanisms may also be involved. The intravenous injection of PPD into immune mice can result in the presence of circulating interferon (150) which may be related to the resistance to viral infections and malignant disease present in BCG-treated animals. An enzyme involved in the metabolism of polycyclic hydrocarbon carcinogens is present in lymphocytes and macrophages (151, 152), and it is possible that BCG could influence the metabolism of these carcinogens. These mechanisms may be important if the persistence of viral or chemical carcinogens is necessary for neoplastic transformation.

Further observations at the clinical level are necessary to define the indications for immunotherapy. The beneficial local effects in selected melanoma patients are well established; however, it is not known whether BCG can prolong the life of these patients. Although there are reports of the beneficial effects of BCG in the prevention and treatment of acute leukemia, confirmation by controlled studies is lacking at this time. At the fundamental level, the host defense mechanisms that best correlate with favorable results in experimental tumors must be defined in order to develop pharmacologic agents that can selectively enhance these defenses.

NUTRITIONAL FACTORS

Metal Metabolism

The inverse relationship between elevated iron concentration and the LD_{50} of challenge organisms provides evidence of a role for iron in the outcome of experimental infections (153–155). Persons with hyperferremia associated with the destruction of iron-containing liver cells (as in viral hepatitis or in louse-borne relapsing fever) or due to hemolysis (as in malaria, bartonellosis, and sickle cell disease) are particularly susceptible to salmonellosis (156). Neonatal infections may be related to both an iron overload and a lack of transferrin (157, 158). Lack of transferrin may be, in part, responsible for the increased incidence of bacterial infections in children with kwashiorkor (157). Presumably, these states of altered iron homeostasis result in greater or lesser availability of iron to the invading bacterial or fungal pathogen which must compete with the iron binding proteins of the host by the production of microbial "siderophores" with high association constants for iron (157). Recently, an association has been described between the virulence of different strains of tubercle bacilli and the quantity and stability of mycobactin, a factor responsible for the transport of iron to the bacterial cell (159). However, the relationship of iron metabolism to the host-parasite interaction may be more complex as suggested by the suppression by iron of bactericidal proteins in leucocyte lysosomes (160), the detoxification by iron of bacterial factors of virulence (such as endotoxin, diphtheria

toxin, and lecithinase), and the stimulatory or inhibitory effects of iron on bacterial secondary metabolism (161).

From the above considerations, one would not expect administration of iron to benefit patients with infection. Indeed, the injection of iron sorbitol citrate (a form in which iron can reach the glomerular filtrate) was shown to exacerbate chronic pyelonephritis in humans (162) and experimental pyelonephritis in rats and mice (163). On the other hand, treatment with desferrioxamine would lower the level of iron saturation and might favorably influence the host. Evidence has been obtained for a beneficial effect of desferrioxamine in the treatment of experimental infections caused by *Listeria monocytogenes* in mice (153, 154, 164). Iron-unsaturated lactoferrin impaired the replication of *Candida albicans,* a property that was lost after saturation of the protein with iron. However, the distribution and concentration of lactoferrin in leucocytes and parotid fluids of patients with chronic mucocutaneous candidiasis was not different from controls (165). A decrease in serum iron concentration occurs during a number of infections (166–168) and results from a decreased assimilation from the gastrointestinal tract and increased storage in the liver. Although it is reasonable to suppose that this decrease in iron concentration may be beneficial to the infected host, direct evidence of a protective role in man is lacking.

Other inorganic ions may be related to the outcome of infection. The level of zinc, required for both microbial and human metabolism, is also decreased in the plasma of man and animals during infection or after administration of microbial products (169). Patients with elevated plasma zinc levels from intravenous feeding and those with urinary excretion of zinc associated with diabetes are prone to candidal infections which may be related to the requirements of the yeast for this metal (157). In addition, the growth of tumor cells requires zinc, and its deficiency in rats has been shown to inhibit the growth of experimental tumors (170).

Finally, the plasma inorganic phosphate concentration falls to levels suboptimal for bacterial growth during Gram-negative sepsis (157); however, hypophosphatemia is also detrimental to leucocyte chemotaxis with an associated high rate of sepsis as occurs in parenteral hyperalimentation (171). At the present time it is unclear whether these mechanisms are related to microbial virulence in vivo, and indications for intervention with these aspects of metabolism in the infected host have not been defined.

Vitamins

In addition to its known nutritional properties, Vitamin A in large doses has been shown to labilize lysosomal membranes (172), act as an immunologic adjuvant (173–176), and induce nonspecific resistance in mice to challenge with *L. monocytogenes, P. aeruginosa,* and *C. albicans* (177). These activities were differentiated from endotoxin effects by lack of pyrogenicity and the inability to increase colloidal carbon clearance or to cause gelation of limulus amebocyte lysate (177). Serum levels of Vitamin A were found to be low (7 of 12) or low normal (5 of 12) in patients with mucocutaneous candidiasis, and a relationship between hypovitaminosis A, abnormal keratinization, and susceptibility of epithelial surfaces to fungal infection was postulated (178). Hypovitaminosis A is related to an increased susceptibility to

systemic candidal infection in rats (179) and to dermatophytosis in Guernsey cattle (180). Although Vitamin A in excess is toxic, the replacement of a relative deficiency in certain patients may be beneficial.

The advocacy of large doses of Vitamin C for prevention and treatment of the common cold has attracted much attention and there are now three controlled trials that indicate beneficial effects (181–183); however, other studies have been negative (184, 185). A rational basis for such effects is not presently known, and indeed, it is impossible to determine whether or not the viral infection itself could have been interfered with or whether the reported Vitamin C effects could have been due to the suppression of symptoms.

Nutritional Status

The interaction between malnutrition and infection is generally, but not always, detrimental to the malnourished host (186). New opportunities for therapeutic intervention could involve the replacement of occult nutritional deficiencies, the removal of essential nutritional factors from invasive microbial or neoplastic cells, or the enhancement of host defenses by the pharmacologic action of certain nutritional factors. The realization of these opportunities will depend upon an improved understanding of the exact mechanisms by which host defenses are influenced by the different nutritional factors.

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