IMMUNOTHERAPY OF CANCER:

PREMISES, PROMISES... PROMISES

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Therapeutic approaches to the control and cure of Cancer are unsatisfactory at best, and deleterious at worst. Except for unusual neoplasms (generally those in which the rate of spontaneous and unexplained remissions is relatively high) the ability to treat effectively the patient with malignant disease is a dismal business. The recent spectacular advances in modern medicine have conditioned physicians (and the public) to expect miracles as routine matters of course, and it is by these standards that the therapy of cancer is usually judged. Consequently, the restless search for new, novel and different approaches to the therapeutic dilemma goes on, giving renewed hope through wonder drugs, faddist diets, and transcendental meditation. While it would be ludicrous to associate the nascent discipline of Immunotherapy with these suspect approaches, it is worth making the point that in this uncritical environment, wherein the hope for success outstrips reasonable standards of judgment, any new treatment modality that purports to be of aid in clinical malignant disease will acquire a devoted following of believers before it has "proven itself," or, as the case may be, "disproven itself."

As an idea whose time had come, immunologic surveillance virtually exploded on the immunologic scene sixteen years ago (1,2). At a time when it was becoming fashionable to doubt whether the elaborate immunologic apparatus was devised simply to protect against invading pathogenic organisms, the proposal that the "true" purpose of the immune response was to protect against the emergence of aberrant clones of somatic cells (i.e., neoplastic) was welcomed enthusiastically. Lines of interested participants formed quickly to get on the bandwagon which shortly became a moral imperative. Since it became fashionable to believe that the fundamental role of the immune system was to protect against cancer, surely it should be possible to enlist this very system in therapeutic efforts to control, and even cure, malignant disease. Now, a decade and a half later, we are beset with the legacy of this brash endeavor. Can we make anything out of it? Does immunity have anything to do with cancer? Can the immune response be manipulated to tip the balance against the tumor and in favor of the patient? The purpose of this review is to attempt to delineate the underlying principles upon which rational immunotherapy can be based, to describe the various approaches to immunotherapy that have been proposed and practiced at the clinical level, and to attempt to assess the current level of success. Several recent reviews of immunotherapy have appeared and are listed in the bibliography (3,4,5,6,7,8,9).

I. Principles of Tumor Immunology

During the early portion of the 20th century, experimental studies in laboratory animals established that malignant tissue could be grafted from one animal to another and that the laws of transplantation applied. When tumor graft donor and recipient were genetically dissimilar, the grafted tissue elicited an immunologic response which brought about its destruction. Alloantigens of transplantation type are expressed on tumor cells just as they are on normal cells, and these antigens evoke specifically sensitized lymphocytes and antibodies which are instrumental in the rejection process. It was not until 1943, however, that the signal observation was made: in a xenogeneic system, tumor cells were shown to express unique antigens not found on histologically similar normal tissue (10). Moreover, work with methylcholanthrene induced tumors in mice showed that the neoantigens expressed on the surface of the neoplastic cells elicited an immune response within the host of origin, a response which provided the host with the capability of destroying the tumor (11). Since then, the basic principles of

tumor immunology have been formulated.

PRINCIPLES OF TUMOR IMMUNOLOGY

1. Tumor cells possess neoantigens (tumor specific transplantation antigens-TSTAs)

2. Hosts possess antigen reactive cells specific for tumor specific transplantation antigens.

3. TSTAs elicit immune responses which lead to tumor destruction.

A. Tumor cells possess neoantigens (tumor specific transplantation antigens-TSTAs).

Following the observation that tumors express unique antigens not found on normal tissues, it was discovered that several categories of antigens could be found on the surface of neoplastic cells (12).. Virally induced tumors very often contained tumor specific transplantation antigens dictated by the virus. Some virus directed antigens were identical with the antigens expressed on the viral envelope; others were different antigens inserted into the tumor cell membrane but not found in the viral envelope. Virally induced antigens tend to be similar for individual tumors induced by the same virus. When tumors are induced by carcinogenic agents such as 3 methylcholanthrene and then analyzed for the tumor specific antigens, each tumor expresses a unique antigenic determinant bearing little or no cross reactivity with other tumors induced by the same carcinogen. There is evidence that tumors of common histologic type express tumor specific transplantation antigens that cross react with each other, but not with antigens found on tumors of different histologic type. The fourth category of antigens which are expressed predominantly on neoplastic cells are so-called fetal antigens, that is, the molecular products of genes which were active during fetal development but have long since been inactivated in the differentiated host. Their re-emergence on tumor cells bespeaks the dedifferentiation characteristic of neoplastic growth. The unique antigens that are expressed on the surface of tumor cells may also be shed into the tumor's environment, either in the form of cell membrane fragments or as secretory products (13).

B. Hosts possess antigen reactive cells specific for tumor specific transplantation antigens.

The clonal selection hypothesis upon which modern immunologic dogma is based, states that adult individuals are replete with clones of antigen reactive cells capable of recognizing a variety of antigens which the host regards as non-self (2). By implication, cells potentially able to recognize self-determinants are specifically deleted from the normal complement. Since tumor specific transplantation antigens represent neoantigens and in a sense non-self, it would be expected that clones of antigen reactive cells capable of recognizing these neoantigens might be present in adult individuals. In fact, the theory of immunologic surveillance, first proposed by Dr. L. Thomas and elaborated by Dr. Burnett (1,2), specifically proposes the existence of antigen reactive cells capable of recognizing TSTAs, recirculating and peripatetic cells effecting a surveillance system which operates throughout development and adult life to recognize and destroy the randomly mutating malignant degenerates of normal tissues.

C. TSTAs elicit immune reponses which lead to tumor destruction.

It is now well established that the exposure of antigen reactive cells to their specific antigen, in this case tumor specific transplantation antigens,

leads to their clonal expansion and differentiation into effectors. As a consequence, antibody of IgM and IgG type as well as specifically sensitized (killer) lymphocytes are produced. It is the interaction of these specific immunologic mediators with tumor cells bearing TSTAs that sets in motion inflammatory and destructive host mechanisms which lead to eradication of the tumor. It is important in this context to consider that the destruction of tumors at the cellular level need not necessarily occur by immunologic means, or exclusively by immunologic means.

II. Mediators of tumor destruction

1. Non-Immune
   Activated Macrophages
   Complement Activation
   Innocent Bystander Injury - Delayed Hypersensitivity

2. Immune - Directed at TSTAs
   T Lymphocyte effectors (killers)
   Cytotoxic Antibody
   "Armed" Cells - B, K, Macrophages

A. Non-Immune

Destruction of tumors, as is the case with the destruction of normal tissues and grafts, involves events occurring at the molecular and cellular level. The adult individual is equipped with several non-immunologically related modalities which can be brought to bear on neoplastic tissues. Activated macrophages can be induced to attack, kill, and degrade cells in their immediate environment (14, 15). Neoplastic cells are particularly susceptible to this capability of macrophages, irrespective of the mechanism by which the "angry" macrophages were activated. It has been proposed that the activated state of macrophages in the spleen accounts for the extremely low incidence of non-hematologic metastases that develop in this organ.

Activation of the complement system, especially via the alternative pathway, may be enlisted in the host's non-specific response to a tumor tissue (16). The activation may be on the basis of cell surface determinants, viral particles, or release of other intracellular materials which activate the properdin system. As a consequence of this activation, the tumor cells have affixed to their surface the third component of complement which renders them more susceptible to phagocytosis. In addition, the alternative pathway once initiated may proceed to completion of the lytic phase thus destroying tumor cells directly.

Simple observation of an intense delayed hypersensitivity reaction elicited in the skin of an individual highly sensitive to PPD reveals that there is innocent bystander destruction of the superficial epidermis. This observation relates to the general phenomenon of innocent bystander injury which occurs adjacent to tissue sites in which cell-mediated immunologic reactions occur (17). Just like epidermal cells, neoplastic cells are susceptible, perhaps even more so, to the mysterious destructive forces unleashed in these inflammatory reactions and as a consequence are destroyed.

B. Immune-directed at TSTAs

There are also highly specific immunologic effectors produced by an individual responding to a tumor bearing TSTAs. The most prominent effectors demonstrable in vitro are specifically sensitized T lymphocytes (18, 19). These thymus-influenced cells, bearing recognition structures able to interact directly with TSTAs on malignant cells, are able directly to kill tumor target cells. That killer and target must be identical at antigenic determinants dictated by the major histocompatibility complex is a curious and enigmatic recent discovery (20). Specifically sensitized T cells can also be assayed by adoptive transfer wherein they are able to procure the rejection or destruction of malignant cells. The precise relationship between the in vitro phenomenon of cell-mediated cytotoxicity and the in vivo destruction of tumors is not altogether clear; the implication is that effector T cells destroy target tumor cells upon contact, but this has not been formally proved.

Certain antibodies specific for tumor specific transplantation antigens are able to fix complement, especially those of the IgM variety, and these turn out to be potent inhibitors of tumor growth; in fact, they are able to destroy dissociated tumor cells (21). As a consequence, malignancies of the hematologic variety, especially in the leukemic phase, are particularly susceptible to the action of cytotoxic antibodies.

It has been demonstrated that anti TSTA antibodies, that is, immunoglobulin molecules reactive with tumor specific transplantation antigens, can affix via the Fc portion to the surface of certain cells: B lymphocytes, macrophages, and even polymorphonuclear leukocytes. The attachment is through a receptor on each of these cells for the Fc component of the immunoglobulin molecule. It has been shown in vitro that such "armed" cells are able to effect destruction of target cells (tumor cells) (22). Whether this in vitro phenomenon is merely a test tube artifact or an expression of a real capacity that the host possesses in vivo is unresolved; if true, it represents yet another immunologically specific mechanism for procuring tumor destruction.

In the aggregate, the panoply of non-specific and immune modalities that can be brought to bear upon neoplastic tissues in the adult human being is impressive. Yet in the face of this formidable defense, malignant tumors emerge and are directly responsible for the demise of a great number of human beings. How can this be? What are the factors that allow for the successful emergence of tumors in the face of such a stalwart system of surveillance and immune responsiveness?

III. Immunologic Factors in Successful Tumor Growth

In the relationship, that special relationship, that exists between host and tumor, successes of the latter are directly attributable to defects in the ability of the host to respond to the tumor, or to unique properties that the tumor itself has assumed.

IMMUNOLOGIC FACTORS IN SUCCESSFUL TUMOR GROWTH

1. Tumor related
   Fail to express TSTAs
   Release of Immunosuppressive Factors
   Release of large amounts of TSTAs
   Excessive tumor load

2. Host related
Immunodeficiency: generalized or restricted to TSTA ARCs
Low genetic resistance: Ir gene endowment
Enhancement: antibodies, blocking factors
Suppressor T cell activity
Immunostimulation
Normal immune response is inadequate

A. Tumor related factors: the tumor is responsible for its own success.

To the extent that an immune response is instrumental in preventing the emergence or the unlimited proliferation of neoplastic cells due to the recognition of tumor specific transplantation antigens, a tumor which fails for one or another reason to express such antigens would be at a relative advantage. Our current state of knowledge does not allow us to make a definitive statement about the proportion of tumors which fail to express TSTAs: the best evidence suggests that the majority of human tumors do. Some, however, express TSTAs that are extraordinarily weak (23). Alternatively, certain tumors release into their environment relatively large amounts of TSTAs and these may be immunosuppressive in their own right. It has been suggested that specific inactivation of thymus derived lymphocytes potentially reactive with TSTAs is caused by the release of large amounts of these antigens in the vicinity of the tumor (24). In fact, TSTAs are widely disseminated throughout the host, in the form of membrane bound fragments, or as whole cells freely circulating in the peripheral blood and lymph (25).

Tumor cells are capable of synthesizing and secreting into their environment other factors that are not antigenic in their own right but which have pharmacologic properties that permit them to suppress the response of the host (26). Some tumors accomplish this by releasing virus or virus products while others synthesize and release active macromolecules such as alpha feto protein which are directly immunosuppressive (27).

The presence of excessive tumor load within a patient is often associated with generalized immunodeficiency; the relationship between excessive tumor load and immunologic responsiveness of the host is well documented although the pathogenic mehanism is obscure.

B. Host related factors - The host contributes to the success of the tumor.

The sophisticated armamentarium with which the host confronts neoplastic tissue may be marred or deficient in which case neoplastic cells are able to emerge. Several specific host problems have been identified.

1. Immunodeficiency.

Diffuse immunodeficiency whether resulting from developmental or acquired defects or from prolonged immunosuppressive therapy has been associated with an increased incidence of malignant neoplasms. While a broad spectrum of histologic types has been seen, there tends to be a proclivity for the emergence of lymphoreticular neoplasms (28).

Immunodeficiency, however, does not have to be diffuse to be related to tumor development; it may be restricted to the tumor specific transplantation antigens confronting the patient. The exact mechanisms by which such restricted

immunodeficiency can arise have not been worked out, but several hypotheses have been advanced. If the tumor specific transplantation antigens are of fetal origin, one might expect that these antigens would have procured clonal deletion of the appropriate antigen reactive cells during fetal life, thus leaving the adult host bereft of antigen reactive cells with these particular specificities. It has also been suggested that the vertical transmission of oncogenic viruses can similarly lead to the clonal deletion of antigen reactive T cells and set the stage for successful tumor development.

2. Low genetic resistance: Ir gene endowment.

Studies in inbred strains of mice have revealed that susceptibility to oncogenic viruses is related at least in part to the complement of Ir locus alleles in the major histocompatibility complex (29). Little is known of the role such genes play in the development of human neoplasms, but the extent to which there is a genetic predilection for the development of tumors within a family may be related to the presence of high or low responder Ir genes.

3. Enhancement

Up to this point, the ability of the immunologic apparatus to recognize TSTAs has been taken as a good omen, and equated with protection against the tumor. It is clear, however, that under some circumstances TSTAs elicit the formation of special categories of immunoglobulins which are poor complement fixers and which end up preventing rather than promoting the destruction of tumors (30). The mechanisms responsible for this fascinating paradox are only dimly understood but will be presented in more detail below.

4. Suppressor T cell activity.

Ideas about the activities of a subpopulation of T lymphocytes which suprresses immune responsiveness are only now being formed. That these suppressor T cells are able to diminish the capacity of a host to respond immunologically to a tumor seems clear, but the extent to which this is an important component of a host's susceptibility to a tumor remains to be worked out (31).

5. Immunostimulation.

In the face of a great deal of evidence which implicates the immunologic apparatus in the protective armor by which a host defends against neoplasia, Prehn et al. (32) and Fidler et al. (33) have now demonstrated that, at least locally, in the region in which lymphocytes first encounter a newly emergent clone of neoplastic cells, the specific reactivity of those lymphocytes promote the accelerated growth of those neoplastic cells, rather than leading to their destruction. Whether this is an artifact of experimental designs or represents another unexpected paradox of immunologic reactivity remains to be determined.

6. Normal responsiveness is inadequate.

To complete the list of host factors which allow the emergence of a successful tumor, one has to include the possibility that the conventional immunologic response when confronted by a rapidly growing tumor is simply inadequate to the challenge and is defeated before the tumor has been contained.

C. Pathogenesis of enhancement

Enhancing factors: non-complement fixing antibody
soluble antigen-antibody complexes

1. Afferent blockade: interfere with TSTA recognition by antigen recognizing cells

2. Central blockade: interfere with clonal expansion and differentiation of killer lymphocytes

3. Efferent blockades: prevent interaction between killer cells and TSTA bearing tumor cells by binding to tumor cell and/or killer cell

The existence of enhancement has complicated our understanding about that special relationship between host and tumor. In enhancement, the immunologic response cannot be taken for granted: in this situation, a highly specific response protects rather than promotes the destruction of neoplastic tissue (34). Two kinds of enhancing factors have been described: a) antibodies, which fix complement poorly and thus do not initiate complement mediated destruction of neoplastic cells, and b) soluble antigen-antibody complexes in which the antigenic components are TSTAs (35). The precise role either or both of these factors play in the enhancement phenomenon is unclear. It is possible to identify, however, the several stages within the immunologic reflex arc at which enhancing factors thwart the effective development of the completed response. In the afferent limb, these enhancing factors interfere with the recognition by antigen reactive cells of TSTAs. In the central processing mechanism, enhancing factors interfere with the TSTA dependent clonal expansion and differentiation of lymphocytes into killer cells. Moreover, the presence of enhancing factors in the periphery allows them to compete with the TSTAs on neoplastic cells for the attention of killer cells. Thus, when enhancement occurs in the clinical setting of neoplastic disease, simplistic ideas about stimulating the immune response must be modified to insure that what is obtained is tumor destruction rather than tumor enhancement - no easy task.

IV. Principles of Immunotherapy

A. Goals.

Destroy all tumor cells
Suppress tumor cell growth
Prolong clinical remissions
Prevent metastases
Reduce tumor mass(es)

In discussing the principles of immunotherapy it is important to keep in mind what the goals are of such a therapeutic regimen. Obviously, the ultimate and complete destruction of all neoplastic cells is the paramount goal. Short of obtaining that, one would aim for the suppression of tumor cell growth. An expression of this therapeutic effect would be the prolongation of clinical remissions and the prevention of the appearance of metastatic disease. More often than not, the immunotherapist must be satisfied with evidence that the approach has achieved at least a reduction in the tumor cell mass.

B. Strategies.

Establish histologic diagnosis
Establish in vitro tumor cell cultures
Reduce tumor mass to minimum ($<10^8$ cells)
(surgery, chemotherapy)
Immunize to TSTAs; stimulate non-specific host defense mechanisms
Promote T cell function; suppress B cells

The strategies employed in conducting an immunotherapeutic approach to malignant disease require a precise histologic diagnosis of the tumor. This implies that certain kinds of tumors are more responsive to one form of immunotherapy than another. Next it is important to attempt to establish a line of cultured cells from the patient's tumor for use later in the preparation of tumor specific transplantation antigens and as targets to monitor the presence of killer lymphocytes.

Prior to the institution of immunotherapy, it is important to reduce the tumor mass to a minimum (hopefully, less than 10^8 cells) by whatever means are at hand: radical surgery, chemotherapy, irradiation. It has now been well shown that immunotherapy can achieve little in the face of an overwhelming tumor mass.

Immunotherapy itself has two not-exclusive approaches: first, to immunize the patient to TSTAs and second, to stimulate non-specific host defense mechanisms. Because of the implications that the phenomenon of enhancement engenders, one must attempt to promote T cell-mediated function while at the same time suppressing the humoral immune response.

V. Approaches to Immunotherapy

Immunoreconstitution
- Passive
- Active
- Adoptive

Immunostimulation
- Non-Specific
  - Local, Systemic
- Specific
  - TSTAs, Modified TSTAs
- Selective

Approaches to immunotherapy are based on two assumptions: 1) the patient with cancer is immunodeficient and reconstitution of that deficiency will equip him with the immunologic machinery that will bring about the destruction of the tumor. The deficiency may be generalized and apply to diverse antigens or it may be restricted, with clonal deletion only of cells recognizing tumor specific transplantation antigens. 2)The patient's immunologic apparatus is normal but for one or another non-immunologic reason has not recognized and mounted a specific response against the malignant tissue. As a consequence, an immunostimulant, whether specific or non-specific, should awaken within the patient a destructive attack aimed at his tumor.

A. Immunoreconstitution

Passive

Anti-Tumor Sera: Allgenic, xenogenic
Biologic factors: Thymosin, Transfer Factor

Active

Stem cell reconstitution: Bone marrow transplant
Lymphocytotherapy: allogeneic, non-activated
autologous, grown in vitro
autologous, PHA activated

Adoptive

Lymphocytotherapy:
allogeneic cancer patient as donor
cross transplantation amoun cancer patients
autologous, activated with TSTAs in vitro
Biologic factor:
Immune RNA

Passive reconstitution includes those methods which provide the patient with effector molecules or with inducing agents which promote maturation of immunocompetence. It is passive in the sense that the agents are unable to replicate themselves; their physiologic effects last only until they are degraded and inactivated. Active immunoreconstitution usually concerns the provision of lymphohematopoetic cells in a clinical setting wherein stem cell deficiency exists. Adoptive reconstitution arms the patient with fully differentiated effector cells (lymphocytes) which are adoptively transferred to carry out their specific immunologic mission within the recipient: to recognize TSTAs and effect tumor destruction.

1. Passive immunoreconstitution.

Anti-tumor sera.

Attempts to bring about tumor destruction by the use of specific antisera were made as early as the turn of the century. Since then this approach has been made many times, generally with no success. The antisera containing anti TSTA antibodies that have been employed have been raised in other humans without disease, in cancer patients, and even in other species (36). In animal systems, it is essential that the anti-tumor antibody be highly cytotoxic, which generally means that it must be of IgM type. Because of its molecular size, this immunoglobulin tends to stay within the vascular tree and rarely reaches into the interstities of solid tissues and organs in significant concentrations. Perhaps for this reason, animals have been freed of malignant disease with the use of specific antisera, but only when the malignancy is of the hematopoetic system, very often in the leukemic phase (36). To date, no successful use of antisera directed at leukemic or solid tumors has been reported in man.

More recently, Bansal et al. (37) have reported that in addition to blocking factors in the presence of serum of patients and animals bearing tumors, there also appear to be unblocking factors which are thought to be antibodies directed at the enhancing factors. These unblocking factors abrogate enhancement and permit destruction of the tumor presumably by effector lymphocytes. No adequate clinical trials of unblocking factors have been employed to date, but the idea is an intriguing one with interesting possibilities.

Biologic Factors

Another form of passive reconstitution employs biologically active factors which induce differentiation of uncommitted immunocompetent lymphocytes. Thymosin, an extract of the thymus gland, has been used to induce immunologic maturation in patients congenitally lacking thymic function (38). It has been proposed that some patients with malignancy are in a similar albeit acquired state of immunodeficiency and might benefit from the effect of a maturation factor such as thymosin. To this date, no significant reports have appeared in the literature of the use of this agent in malignant disease.

Transfer factor (39), a small molecular weight biologic factor extractable from lymphocytes, has been advocated in the immunotherapy of malignant disease (40). Still clouded in mystery and controversy, transfer factor has resisted biochemical definition. It has recently been championed by well respected members of the immunologic community (41, 42) who have lent credence to the idea that transfer factor has biologic activity. There is no general agreement, however, about the nature of that activity: it is either a non-specific adjuvant, and/or it has the capacity of conferring immunologic specificity to particular antigens. Confusion has arisen because extracts containing transfer factor have often been contaminated by trace amounts of antigen. Work with patients who are deficient in T cell-mediated immunity has shown the transfer factor is able to induce dramatic relief from chronic debilitating cutaneous fungal infections (43). Its use in the treatment of human cancer has been anecdotal and success has been limited to the demonstration that in vitro cell-mediated immunity to tumor associated antigens may be improved (40). Problems of isolation, purification and standardization remain for transfer factor and this has limited its applicability to immunotherapy for cancer.

2. Active immunoreconstitution.

Stem cell reconstitution

The ability to reconstitue stem cell defects by means of bone marrow transplantation is now a well established one. Its applicability to patients with immunodeficiency is appreciated and its usefulness in the treatment of aplastic anemia seems assured (44). The initial enthusiasm to use bone marrow transplantation in the therapeutic regimen for acute leukemia swept across the hematologic scene approximately eight years ago. After considerable experience. it would now appear that clinical benefit of attempting bone marrow transplantation in this setting is very slim. Only isolated cases of cure or prolonged remission have been seen. With regard to immunotherapy, the major role for active immunoreconstitution through bone marrow transplantation may be as a preventative maneuver in patients with primary immunodeficiency whose defect renders them highly susceptible to lymphoreticular neoplasms.

Lymphocyte therapy

In 1963 Woodruff and Nolan (45) reported that the intravenous inoculation of allogeneic spleen cells into patients with advanced malignancy led to improvement in the clinical course. This surprising announcement spawned a series of more or less thoughtful clinical trials in which lymphocytes from various sources were infused into patients with cancer hoping for therapeutic benefit. Perhaps the most bizarre was the clinical experimental design in which cross transfusions were set up between two patients with similar histologic malignancies (46).

The idea was that as each patient became immunized to the histocompatibility antigens on his cross transfusion mate's cells, he would be induced to become immunized also to the relatively weak tumor specific transplantation antigens. While success was reported in the free press, it became the burden of the scientific and clinical journals to report that no therapeutic benefit seemed to accrue on other than an anecdotal basis. Alexander was able to show that such cross transfusion experiments could cure malignancies in animals (47), but the report of two deaths from patients with HLA incompatibilities rendered this approach very unattractive (48). Another attempt to provide the patient with cancer with sufficient numbers (whatever that means) of immunocompetent lymphocytes has been to grow up large numbers of autologous lymphocytes in tissue culture (49). Moore et al. were able in a few patients to give up to 300 grams of autologous lymphocytes grown in vitro, but these workers claimed little specific effect (50). Others have attempted to activate autologous lymphocytes in vitro with mitogens such as phytohemagglutinin, but again clinical results have been uneven and unrepeatable.

3. Adoptive immunoreconstitution

Mention has already been made of adoptive lymphocytotherapy in which lymphocytes have been harvested from allogeneic donors. Some investigators have attempted to immunize cancer patients with allogeneic tumor cells and then to harvest lymphocytes from immunized donors to transfuse them back into the original tumor donor. Cross transplantation experiments listed above are a variation on this theme. Probably because of the lack of HLA matching, the ability of the transferred cells to survive in the incompatible recipient is so limited that it is not surprising that little or no clinical benefit was achieved. Perhaps a more reasonable variation on this theme has been to harvest lymphocytes from patients with cancer and to expose these cells in vitro to the tumor specific transplantation antigens of the patient's own tumor (49). Specifically sensitized cells can be generated in this fashion and the intent of the experiments would be to reinfuse cells sensitized in this manner back into the patient and thus provide him with an adequate population of killer lymphocytes able to bring about the destruction of his tumor. A report of such therapy was made as a letter to the editor of Lancet in 1968 with the notation that the clinical course of the patient will be followed. No further report of this work has appeared since then, and thus the same dismal assumption must be made: failure to achieve any clinical benefit.

Under the category of adoptive immunoreconstitution must be considered the use of subcellular fractions such as the enigmatic biologic factor, immune RNA. Only a few laboratories throughout the world have reported that RNA from specifically sensitized populations of lymphocytes can confer on naive lymphocytes (and hosts) immunologic specificity and reactivity (51, 52,53). Pilch has promoted this idea and done a considerable amount of experimental work in animals purporting to show that tumors could be rejected by animals receiving appropriate immune RNA (54). Despite the presence of this idea in the immunologic literature for more than a decade now, it has not caught on. Whether this indicates that it is an unattractive idea to immunologists or that investigators have attempted and failed to repeat the results is unclear. Except for a few clinical trials which are uncontrolled and sporadic, immune RNA has not been adequately tested for its efficacy in the clinical arena (9).

In summary, immunoreconstitution, while having great promise, has not claimed or achieved impressive clinical success. To date, passive administration of anti-tumor antibodies and reconstitution with activated or normal lymphocytes have each failed in various clinical trials. To be fair, clinical evaluation is now

in progress for thymosin, transfer factor, and immune RNA, but unless we are lucky, the prospects do not appear to be bright.

B. Immunostimulation

Non-Specific
- Generalized:
  - Systemic - BCG, C. Parvum, adjuvants
  - Local - BCG, DNCB
- Selective:
  - T cell specific - Levamisole
  - B cell specific - Anti-plasma cell serum, Cytoxan

Specific
- Whole tumor cell vaccines
  - Living cells: autologous, allogeneic
  - Inactivated cells: irradiation, Mitomycin C
  - Modified cells: iodoacetate; Neuraminidase; viral
- TSTA containing vaccines
  - Crude cell extracts
  - Purified TSTAs

If left to his druthers, the immunologic purist would like to prepare a highly specific form of tumor specific antigen and administer it to a patient in just the right way so that an appropriate and selective cell-mediated immunologic response would be obtained which would then destroy the tumor cells. At a practical level, this idealized regimen is rarely possible, even in the prophylaxis of infectious diseases, much less in cancer immunology. More often than not, we must be satisfied with very non-specific approaches, because they are more readily available and much less demanding.

1. Non-specific immunostimulation

In a sense, adjuvants have made modern immunology possible. By using these agents, immunologists have been able to study the immune response to a variety of antigens that would otherwise have been impossible. Conceptually, however, adjuvants have had to take second place in immunologic considerations. They lack specificity and in that sense are immunologically irrelevant. As a consequence, we know less about their mode of action than we should, especially since the most effective (sic) means of immunotherapy now available fit into this category. For our purposes, adjuvants are considered as agents which, when combined with antigen permit or induce the immunologic apparatus to respond to the latter in a highly specific way. Adjuvants of whatever diversity have two unifying qualities which seem to be important in their effectiveness: they act as depot for the release of small amounts of antigen over long periods of time, and they activate macrophages. It is probably this latter effect which is most relevant in the context of immunotherapy of cancer. The most common adjuvant in immunologic research is that devised by Freund, consisting of an inert oil, an emulsifying agent, and killed tubercle bacilli. This particular adjuvant when mixed with antigen regularly induces a high degree of delayed hypersensitivity and antibody formation. For reasons which are still obscure, certain adjuvants activate macrophages and promote the development of cell-mediated immunity, while others promote humoral immunity.

The most widely used immunotherapeutic agent in clinical cancer immunotherapy is BCG, Bacillus Calmet-Guerin (mycobacterium bovis). This agent was developed at the turn of the century as a potential means of immunizing large populations against tuberculosis. It is known that in animal systems, BCG is a very potent adjuvant, activating macrophages and promoting T cell immunity. Because of its widespread clinical use over the past eight years, a conference devoted to BCG was sponsored by the National Cancer Institute in 1973 and a monograph has resulted (55). The use of BCG in malignancy stems from the work of Zbar, Ribi, and Rapp who demonstrated that BCG inoculated into hepatomas of guinea pigs would induce regression of the tumor (56). It is generally agreed that the means by which BCG procures its anti-tumor effect is predominately through the activation of macrophages (57). However, it has been suggested that there may be cross reacting antigens expressed on BCG and certain tumors (58, 59); nonetheless, it is likely that the major effect of BCG is through non-specific means. Because this agent is not without hazard (its toxicity will be dealt with later), extracts of BCG have been prepared - methanol extractable residue is an example (60). Clinical trials are now underway.

Another adjuvant that has been used to treat human malignancy is Corynebacterium Parvum (61,62). Generally administered as a suspension of killed organisms, it has been shown to be a T cell stimulant in man although animal studies suggest that it acts by expanding the B cell component of the immune response. While other adjuvants have been advocated (inducers of interferon such as poly-C or poly-U), significant clinical trials have only been carried out with BCG and C Parvum.

The previous discussion has concerned itself with the systemic administration of adjuvants. Yet the best evidence that adjuvants act in an anti-tumor manner comes from the local installation of these agents directly into the tumor mass (63). This has been accomplished in animal systems as mentioned before in hepatoma and is now under study as a means of treating a variety of local tumor lesions, especially malignant melanoma (64).

Perhaps the most interesting application of non-specific immunostimulation in the control of malignant disease has been with dinitrochlorobenzene, DNCB, which has been used in the local treatment of cutaneous tumors (65). The innocent bystander effect which non-specifically destroys cells which happen to be at the site of an acute inflammatory reaction initiated by cell-mediated immunity has been employed successfully in the treatment of malignant melanoma, squamous cell carcinoma and mycosis fungoixes (65). What is particularly intriguing about these trials of immunotherapy is that not infrequently lesions of the skin distant from the site of application of DNCB have regressed. This extraordinary finding remains unexplained but is of considerable great interest.

It was mentioned previously that many times the host response to a tumor is self-effacing such that T dependent tumor destructive forces are balanced by tumor protective B cell effectors. If it would be possible to stimulate selectively the T cell system, or inhibit selectively the B cell system, then the balance between host and tumor could be tipped in the host's favor. Recently, levamisole, an effective anti-helminthic agent, has been studied for its ability to stimulate T cell-mediated immunity selectively (66). An experimental study reported in 1972 claimed inhibition and cure of solid malignant tumors including metastases in mice (67). Two years later, the same compound was found to be inactive in four other animal tumor systems (68). Currently, this drug is under intensive

investigation for its ability to stimulate only T cell-mediated immunity and the results although inconclusive are promising.

Selective inhibition of B cell-mediated processes

Cyclophosphamide, if administered prior to exposure to antigen, has been shown in experimental animals to inhibit antibody formation and at the same time allow T cell immunity to develop unmolested (69). Another possible therapeutic approach would be to employ xenogeneic anti-plasma cell serum which would inactivate the cells responsible for producing enhancing antibodies. No clinical data exists on the possible efficacy of either of these approaches.

2. Specific immunostimulation.

The possibility of producing a vaccine which would contain tumor specific transplantation antigens that could be administered to a patient, stimulating his immunologic apparatus to mount a specific attack against the tumor, is an attractive idea. Because of difficulties in preparing purified TSTA extracts, most investigators have opted for using vaccines containing whole tumor cells. Obviously, it would be disastrous to inoculate a patient directly with a suspension of viable autologous tumor cells. In order to allow immunostimulation without risk of producing new tumors, there are two approaches: first, to expose tumor cells to x-irradiation or to a mitotic inhibitor such as mitomycin C which renders them incapable of subsequent division, but preserves the TSTAs. A somewhat different approach is based on the assumption that tumors of common histologic type express cross reacting antigens. By using as vaccines allogeneic tumors of histologic type similar to that of the patient, one might enable the patient's immune response to perceive the TSTAs on the allogeneic cells as foreign and thus awaken the host's response to similar antigens on his own tumor cells. Clinical experiments with these methods have been scanty and those that have been controlled have shown sporadic responses (9, 70, 71), but overall evaluation is not possible.

An alternative approach is to modify the patient's own tumor cells. The assumption is made that the patient's tumor specific transplantation antigens are insufficiently strong to elicit a primary response and that chemically modifying the antigenic determinants on the tumor cell surface the determinants would be rendered more antigenic. Dr. Morton Prager of this institution has carried out an interesting series of experiments using iodoacetate modified lymphoma cells in mice with a measure of success (72); others have attempted to strip the sialic acid coat from tumor cells with neuraminidase, thereby hoping to unmask supposedly hidden TSTAs (73). Both approaches are reasonable and in their experimental development phase.

Because of the difficulty of preparing purified antigens from insoluble membrane fragments and of identifying these antigens in an immunologically specific way, work toward producing TSTA containing vaccines has moved very slowly (74). Since isolation of such well defined and easily identified antigens as the major transplantation antigens of mice and men is only in its infancy, it is not surprising that progress with TSTAs has been slow. This approach may represent a reasonable one for the more distant future.

Finally, attempts have been made to combine specific and non-specific immunostimulation into a single regimen. Killed or inactivated tumor cells admixed with BCG or other adjuvants have been administered to patients with various

malignant diseases. While enthusiastic reports have claimed success, it is yet too early to tell whether the clinical benefit is greater or not with this combined therapy.

VI. Current Status of Immunotherapy of Cancer

Immunotherapy is now being studied for effectiveness in a wide variety of malignant neoplasms. The National Cancer Institute through its International Registry of Tumor Immunotherapy has identified and enumerated every protocol now under study (75).

CURRENT IMMUNOTHERAPY PROTOCOLS

Tumor	Number of Centers
Malignant Melanoma	68
Hematologic	39
Lung	35
Breast	20
Gastro-Intestinal	19
Pelvic	15
Sarcomas	12
Head and Neck	10
Multiple Tumors	35
Miscellaneous	7

Interest in immunotherapy of malignant melanoma clearly stands out. Sixty-eight individual protocols, covering nine different therapeutic regimens, are involved. The next most common group of disorders attracting immunotherapeutic attempts are the hematologic malignancies, especially the acute leukemias. Except for these two major disease groups, only the immunotherapy of carcinoma of the lung and the breast are undergoing systematic evaluation of even comparable intensity. Only in malignant melanoma and acute leukemia is there sufficient experience to appraise.

It is not easy, however, to assess the efficacy of immunotherapy in these conditions. Spontaneous remissions are not rare in melanoma and chemotherapeutic approaches have already achieved a level of success that would have been unthinkable a decade ago. In fact, the rapid improvement in prognosis for patients with either of these two diseases treated by non-immunotherapeutic measures has been remarkable. This makes it difficult to sort out the effects unique to the institution of immunotherapy. At the very least, it makes retrospective studies meaningless. And not all that much time has elapsed since the beginning of widespread interest in immunotherapy so that a collection of five year survivals can be displayed.

At the recent International Conference on the Immunobiology and Immunotherapy of Cancer held under the auspices of the New York Academy of Sciences last November, 1975 (76), strong pleas were made for in vitro methods of monitoring the effects of immunotherapy in cancer patients. It was highly recommended that studies be carried out determining changes in levels of T lymphocytes in the peripheral blood, and in the ability of lymphoid cells from treated patients to elaborate macrophage migration inhibitory factor in vitro and to carry out in vitro cell-mediated cytotoxicity using the patient's own tumor cells as targets.

In this manner it was felt that some reasonable index of the effectiveness of immunotherapy could be determined. While this cannot be denied, the dilemma remains. The correlation between the development of these kinds of in vitro responsiveness with the patient's response to his tumor in vivo is totally obscure.

All this is by way of saying that the ultimate measure of success must be determined by the clinical course of patients with cancer. Has the tumor mass regressed in size or disappeared? Have the number and incidence of metastases been reduced? Has the interval between remission and re-emergence of disease been prolonged? And has the patient's survival been extended?

Of all the immunotherapeutic approaches listed above only BCG either with or without inactivated autologous tumor cells or injected locally into tumor lesions has been employed sufficiently often in enough studies of reasonable experimental design of a prospective nature to make it possible to form some kind of judgment about effectiveness. And then, only BCG therapy in acute granulocytic leukemia and malignant melanoma warrants consideration based on sufficient experience.

For analyzing the effectiveness of BCG it is worth stating that not all preparations are alike. Five different strains have been utilized: the Pasteur, the Phipps, Tice, Montreal, and Glaxo strains (77). These strains are all adminstered as viable organisms and are used to promote cell-mediated immunity, the Pasteur strain having the greatest effect in this regard. The extent to which the BCG vaccine is contaminated with dead organisms limits its effectiveness; the dose of organisms that are administered makes a great deal of difference as to the amount of immunostimulation achieved. It has been injected into tumors directly and into body cavities containing malignant cells; it may be placed intradermally by injection, puncture, or scarification; it is even administered intravenously, by mouth, or via aerosol. The administration is regularly attended by local severe inflammatory reactions often with the development of sinus tracts from which it is possible to isolate living organisms. When inoculated systemically or at multiple sites, it causes fever, chills, and granulomatons disease of liver and lung.

Only three clinical examples have been chosen to consider with effectiveness of BCG: intralesional administration in malignant melanoma, and systemic treatment for disseminated melanoma and in acute granulocytic leukemia.

A. Intralesional BCG.

It was initially reported by Morton and co-workers that the Glaxo strain of BCG when injected into cutaneous nodules of malignant melanoma caused impressive regression of the injected nodules (78). While occasional non-injected nodules regressed as well, in general, only injected tumors shrunk. This interesting observation has been corroborated by an independent study wherein five of fifteen patients treated with intralesional BCG had significant clinical responses. One patient had a complete remission which involved both injected and uninjected lesions (79). It is clear that the smaller the tumor the more likely remission can be induced.

Because of the ease of measuring cutaneous lesions, the evidence that BCG causes regression of inoculated tumors is incontrovertible. This effort has now been extended to other forms of cutaneous neoplasms as well (17). BCG has even been successfully employed intrapleurally in patients with malignant

pleural efusions secondary to bronchogenic carcinoma (80). Numerous clinical trials throughout the country are now underway to put this form of therapy to a broad based test of efficacy. The situation appears to be moderately bright in that the likelihood is great that BCG when injected intralesionally will be found to be therapeutically useful. Whether this benefit is commensurate with the morbidity of BCG therapy is another matter. In addition to the side effects mentioned above, two deaths have been reported in patients receiving intralesional BCG in which this agent appeared to be instrumental (81).

B. Systemic BCG in malignant melanoma

It is very difficult to get a handle on current use of BCG in this disease (82). Over the past two and a half years alone, seventy-two articles have appeared in clinical literature presenting data bearing on the use of BCG in the treatment of malignant melanoma. The problems are manifold. The extent of disease at the time of the institution of BCG varies from study to study. In some studies BCG is given only when there appears to be total surgical exision of the original lesion such that immunoprophylaxis is attempted; in other studies BCG is given in the presence of disseminated disease in an effort to blunt the tumor's advance. Two recent articles will be cited: the first from M.D. Anderson Hospital (83) in which a combined dimethyl triazeno imidazole carboxamide (DTIC) and BCG regimen was employed in eighty-nine patients who were diagnosed as having unresectable disseminated metastatic melanoma (stage 4). Chemotherapy administered with DTIC and the Pasteur strain of BCG was administered at the same time via scarification. Parameters of immunocompetence were assayed at the beginning and throughout the course of the DTIC-BCG program. Of the eighty-nine patients, 27% (24) achieved a partial or complete remission. In fact, only five obtained complete remission. At the end of a twenty month interval only 6 of the 24 patients had had any clinical benefit and remained alive. There were no controls included in this study; instead the patient population was matched retrospectively with controls from previous treatment groups.

The second study is that of Eilberg, Thornton et al. (84) and is perhaps the most carefully carried out series reported to date. 126 patients were studied with malignant melanoma, stage 2 disease (that is, microscopic evidence of tumor in the regional nodes draining the original tumor site) . Forty-two patients were treated with surgical intervention alone. Eighty-four patients were treated with surgery following which the Tice strain of BCG was administered by the Tine technique intradermally into both axillae and groins. Patients with head and neck melanomas also received BCG into the region of their draining cervical nodes. Doses were applied every week for three to twelve month intervals. At the end of one year BCG was administered every two weeks, following which it was continued every month for those patients still alive. At the end of two years the incidence of metastases appearing at distant sites was one-half in the BCG group compared to the patients treated with surgery alone. Among the latter patients, 50% had experienced a recurrence at four months. The patient group treated with BCG also had a median remission time of six additional months before reaching the 50% level. Thus, in this reasonably well controlled study of a prospective nature, BCG given in a prophylactic sense following removal of the original tumor and draining nodes was able to achieve significantly prolonged remission and survival. Though no mortalities were reported in this group from therapy, a fair amount of morbidity was seen with the immunotherapeutic regimen outlined above. To summarize, it would appear that non-specific immunostimulation with BCG has a definitive role to play in the therapy of malignant melanoma and might be expected to be most efficacious in selected patients with stage 1 or 2

disease. It is employed as a prophylactic to prevent or retard the appearance of metastases. Incidentally, the idea of using BCG prophylactically stems from a controversial report which claimed that the incidence of leukemia in children inoculated at or shortly after birth with BCG was much less than in untreated control populations (85).

C. BCG therapy in acute granulocytic leukemia

Acute granulocytic leukemia ought to represent a very stringent test of the efficacy of any new therapeutic approach. It is easy to diagnose, the rate of spontaneous remission is virtually zero, and in the untreated state the disease generally runs its course over a short interval, the median survival time from diagnosis being approximately three months. Moreover, until very recently, no effective chemotherapeutic drugs had been discovered. Against such a sinister background, any new agent such as BCG if effective ought to be readily obvious within a reasonably short period of time.

However, the waters were muddied early on by a non-controlled study of the use of BCG in patients with acute lymphoblastic leukemia (86). It was claimed that the vaccine prolonged remissions obtained by chemotherapy. Subsequently, three different studies attempting the same clinical trial came to the opposite conclusion: BCG did not influence the course of acute lymphocytic disease (87,88, 89). Over the past four years three major centers have undertaken the study of BCG in acute granulocytic leukemia and in each instance the BCG therapy was instituted only after remission had been obtained by chemotherapy; the BCG was then used as an adjunct to the regular chemotherapeutic consolidation and maintenance regimens. Two of the studies actually employed controls who received identical treatment without BCG. One study, however, was unable to contain itself and so was forced to treat all patients with BCG; it then used as its controls a retrospective analysis. In the studies reported by Powles et al. (90), 107 patients with acute granulocytic leukemia were allocated randomly into two groups to decide remission treatment. The patients were then given an induction chemotherapeutic regimen and forty-five attained complete remission. Patients in remission then received maintenance chemotherapy and one group also received immunotherapy with Glaxo BCG which was injected intradermally along with irradiated tumor cells. Of the nineteen patients which received only chemotherapy during their remission, seven remained alive at the time of report with a median survival of approximately 303 days and only five were still in their first remission (median remission length 188 days). Of the twenty-three patients that also received immunotherapy during their remission, sixteen were alive at the time of the report with a median survival time of 545 days and the eight that were in their first remission had a median remission length of 312 days. The difference in survival between the two groups was highly significant. In a similar study carried out by Vogler and Cheng (91), forty-one patients with AGL were randomly allocated to groups receiving either the Tice strain BCG plus methotrexate or methotrexate alone. The median duration of remission from the onset of remission was forty weeks in the BCG/methotrexate group and twenty-six weeks in the group treated with methotrexate alone. Following consolidation the BCG/methotrexate patients had a median duration of remission of twenty weeks compared to only 9.7 weeks for the methotrexate alone group. The third study which chose to use as its control a retrospective group of patients also came to the same conclusion (92): BCG prolongs remission in acute granulocytic leukemia in which the remission is obtained by chemotherapeutic means.

VII. Future Prospects and Promise

The next five years will be critical in determining the ultimate place of immunotherapy in malignant disease. Most, if not all, of the treatment modalities outlined previously are now being incorporated into immunotherapeutic regimens and clinical trials are underway. Almost all have been tried in the recent past and produce less than spectacular success. With an eye toward realism, it seems unlikely that any one or any combination of possible approaches will accomplish the major breakthrough that is hoped for. But as stated earlier at the beginning of this presentation, our expectations for immunotherapy may be unrealistic and outside the bounds of what can possibly be accomplished. One could interpret the mediocre benefits now recognized from the clinical use of immunotherapy as revealing the relatively unimportant role the immune response plays in host defense against cancer. After fifteeen years, critical evidence in favor of the concept of immunologic surveillance has not been obtained. Conceptually, it may be inappropriate to expect that a system which is unique by virtue of its capacity to exhibit exquisite specificity could be brought to bear therapeutically on a disease process in which the unique TSTAs are weak antigens, and apparently trivially involved in the malignant process. The lesion in malignancy after all is not that neoantigens are placed on the surface of the cells and thereby offend the host. To our knowledge, these antigens are irrelevant to the malignant process. More likely, the pathogenesis of malignancy is operative at the molecular level of genes and their interaction, an arena where immune mechanisms might not be expected to play a prominent role.

At the risk of committing heresy, it seems nonetheless reasonable to take a skeptical view of immunotherapy of cancer. If the immunologic response can be brought to bear meaningfully and effectively upon the tumor-host relationship so that the host is spared, then let it be proven by appropriate clinical trials. Without unrealistic expectations, it may be easier to tolerate the gradual realization that immunotherapy may be only peripherally important in the control of malignancy.

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