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Immunotherapy of Cancer

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Clinical studies have indicated a close relationship between immunological competency and the growth of human cancer. Studies in animal systems have indicated that immunological mechanisms are important in host-tumor existence. A number of agents are in the process of being evaluated for their immunotherapeutic potential in patients with cancer. These include levamisole, bacille Calmette Guérin (BCG) and Corynebacterium parvum. While the precise role of these agents in the treatment of human malignancy remains to be clearly defined, they do appear to be useful as adjuncts to other forms of cancer therapy such as radiation therapy, chemotherapy and surgical operation.

THE CONCEPT that both human and animal neoplasms contain antigens against which the host is capable of reacting immunologically is not new. The advances in the immunology of infectious diseases at the turn of the century prompted an intense investigation into the applicability of immunizations to protect against cancer. However, the lack of highly inbred strains of animals prevented the investigation of tumor specific antigens. Without these inbred animals it was impossible to distinguish between histocompatability antigens carried in the tumor cells from tumor specific antigens.

The concept of tumor immunology was largely ignored until 1943 when Gross was able to immunize mice against a sarcoma induced by methylcholanthrene arising within an inbred strain of mice.[1] It remained for Phren and Main in 1957 to definitively show the presence of tumor specific transplantation antigens in carcinogen induced murine tumors.[2] These studies established tumor immunology as a legitimate concept and since 1957 there has been a virtual explosion of work in animal and human tumor immunology.

Tumor antigens have been identified by a variety of different techniques in animal neoplasms induced by both viruses and chemical agents. In view of this evidence in animal neoplasms it was logical to assume that human neoplasms also contained cancer specific antigens similar to those found in animal tumors. This rationale is supported by some rather impressive clinical observations which indicate the importance of host-tumor defense mechanisms:

- It is clear that under certain conditions spontaneous regression of malignancy does occur. Reports of spontaneous remission of proven malignant tumors have been well substantiated.

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ABBREVIATIONS USED IN TEXT
BCG = bacille Calmette Guérin
RNA = ribonucleic acid

Hypernephroma, melanoma, neuroblastoma and choriocarcinoma are the major types of tumors that most frequently regress spontaneously.[3]

- Cancer cells have been found repeatedly in the systemic venous blood in a high percentage of patients during surgical operation. The presence or absence of these tumor cells in the blood, or in wound washings at the time of operation or following operation, does not correlate with survival. This observation suggests that host factors are important in preventing the growth of these circulating tumor cells.
- The development of rapidly progressive, recurrent disease 10 to 20 years after control of the primary tumor is also indicative of the presence of host-tumor defense mechanisms.
- Another compelling clinical observation is that patients with depressed immune responses have an increased risk of developing cancer.[4] Children with congenital immune deficiency syndromes have a very high incidence of cancer at any early age. In addition, malignant conditions are apt to occur in transplant recipients who are receiving immunosuppressive agents. These observations, along with the observations that cancer patients with a general depression in their immune reactivity, have more rapidly growing neoplasms and a poorer prognosis following surgical therapy strongly[5] indicates that immunological mechanisms are important in the host-tumor relationship.

There has been considerable laboratory evidence to corroborate these clinical observations. These studies have clearly shown the presence of tumor associated antigens in human tumors and indicate that the host responds immunologically to these antigens. A variety of *in vitro* techniques have been employed to identify these antigens including immunofluorescence, colony inhibition, complement fixation, immunodiffusion, lymphocyte mediated cytotoxicity, lymphocyte blastogenesis and various radioimmunoassays. These *in vitro* assays not infrequently show evidence of tumor immunity in patients with progressive disease. The explanation for this may be the presence of circulating soluble substances that appear to interfere or block certain immunological reactions directed toward tumor antigens.[6] These studies, as well as studies in animal systems, have suggested the importance of the immune response in human cancer, and it seems reasonable that these principles of tumor immunology be applied to the treatment of cancer.

Immunotherapy

Immunotherapy can take one of several forms. These can be grouped under the following classifications:

- *Nonspecific immunotherapy* involves stimulation of the immune system with adjuvants that have the ability to nonspecifically enhance a patient's capacity to respond to his own tumor.
- *Active specific immunotherapy* involves the active stimulation of the immune system with tumor vaccines in an attempt to induce specific autoimmunity against cancer specific antigens. These vaccines can take the form of whole tumor cells or fractions of tumor cells.
- *Passive or adoptive immunotherapy* involves the administration of antisera, immune lymphoid cells or subcellular fractions from another host which has been specifically immunized against cancer specific antigens.

By far the most extensive experience with these different modalities has been with active nonspecific and specific immunotherapy. Adoptive or passive immunotherapy has been employed, but to a much lesser extent.

Passive or Adoptive Immunotherapy

Theoretically, passive or adoptive immunotherapy can be employed with the use of humoral antibodies, or cell mediated immune factors. Therefore, this form of immunotherapy may employ the administration of serum or lymphoid cells, or fractions of lymphoid cells. Since the accumulated evidence suggests that tumor immune destruction is mediated by cellular immune mechanisms, most of the attention directed toward adoptive immunotherapy has centered around cellular immune mechanisms. Some clinical studies have been done using humoral antibodies, but results of these have largely been inconclusive. Indeed, certain observations suggest that humoral antibody may have an adverse effect on the tumor-host relationship. Passive immunotherapy with antisera produced in a foreign species has been attempted. However, such antitumor sera have proved very toxic in the recipient because they contain antibodies against the normal

tissues of the recipient. Other sources, such as from patients who have been cured of the malignancies and in whom there are high titers of cytotoxic antibody, also may be used. A comprehensive review of the serotherapy of malignant disease has recently been published.[7]

The possibility of adoptive immunotherapy using lymphoid cells or fractions of lymphoid cells has stimulated a great deal of interest. In animal studies with highly inbred laboratory animals, experiments have indicated that transferred lymphocytes that persist in the host have a significant immunotherapeutic effect.[8] However, this is difficult to apply in a clinic because of the difficulty of rejection of the transferred lymphocytes due to HL-A differences between the donor and the recipient. Several studies have been done attempting to induce remissions in cancer patients with lymphocytes from patients in whom there previously has been a complete clinical remission.[9] These studies have not been universally successful and certain logistical problems at present make this a very difficult form of immunotherapy.

The use of extracts from lymphoid cells is particularly attractive because these extracts do not contain HL-A specificities, and therefore are not rejected by the recipient. The most promising subcellular materials are immune ribonucleic acid (RNA)[10,11] and transfer factor.[12,13] Immune RNA is information RNA that is extracted from the lymphoid organs of specifically sensitized donors. This immune RNA is capable of transferring immunologic capabilities to unsensitized lymphocytes. Human tumors are used to immunize a heterologous species, such as sheep, and RNA extracted from the sheeps' lymph nodes is used for immunotherapy. Immune RNA generated in this fashion is capable of transferring to normal lymphocytes the ability to kill tumor cells *in vitro*. In essence, the immune RNA instructs the lymphocytes to destroy the tumor cells. There are a number of trials that are now evaluating the effect of immune RNA in a variety of human neoplasms.[11] At present it is clear that immune RNA is well tolerated in humans; however, the results of these clinical trials are too preliminary for conclusions to be drawn from them.

Transfer factor is another agent capable of transferring immunity from one human to another.[12,13] Transfer factor extracted from leukocytes from one human is capable of transferring immunity to skin grafts and tuberculosis and a variety of other antigens. Transfer factor is very easy to prepare, it is well tolerated and it contains no HL-A antigens against which the recipient can react. Transfer factor has been used in a variety of clinical trials including studies with malignant melanoma, breast cancer, renal cell carcinoma and sarcoma.[13] Some impressive results have been reported using this method of immunotherapy and sufficient information has been generated to set up prospective trials evaluating transfer factor. At present several institutions in the United States are evaluating transfer factor as an adjuvant to surgical operation in patients with sarcomas. These studies are not of sufficient duration to draw any concrete conclusions.

Active Nonspecific and Specific Immunotherapy

There are a variety of agents capable of a nonspecific immune stimulation. These agents enhance antibody formation to a variety of different antigens, they nonspecifically enhance cellular immunity and they may stimulate macrophage function. Many of these nonspecific stimulators have been evaluated but BCG, Corynebacterium parvum and levamisole have received the greatest attention and the most thorough evaluation. For these reasons, this discussion will focus on these nonspecific immunostimulators.

BCG

Old[14] and Halpern[15] showed several years ago that BCG has significant antitumor activity against a wide variety of animal neoplasms. In animals a single injection of BCG results in enhanced humoral immunity, increase in macrophage function, increased resistance to bacterial infection, accelerated homograft rejection and increased resistance to tumor challenge.[16] Results of animal and clinical studies have indicated that local tumor control is most readily achieved by the direct intralesional injection of BCG into cutaneous tumor nodules. Also, the direct local injection of BCG into the neoplasm is the best way to stimulate systemic tumor immunity. This antitumor effect of BCG is abrogated by pretreatment with antilymphocyte serum, and with cortisone. In addition, it is ineffective in thymectomized mice. This evidence and experience in human studies indicate, therefore, that an immunocompetent host is necessary for BCG to be effective.[17]

The second most effective immunizing technique is with BCG mixed with tumor cells and in-

jected intradermally in an area that drains to the regional lymph nodes that drain the tumor being treated. BCG is least effective when given at sites distant from the tumor.[18] Animal and clinical studies have indicated that BCG immunotherapy is effective against a limited amount of disease. For instance, in certain animal leukemia models BCG appears to be effective against only 10^5 cells. Since this form of immunotherapy is known to be effective against a limited amount of disease, it is generally felt that it should be used in combination with a major cytoreductive modality such as surgical operation, radiation therapy or chemotherapy.

Clinical experience with BCG as an intralesional immunotherapeutic agent in patients with cutaneous melanoma[17] indicates that BCG is most effective in patients who are immunocompetent as determined by their ability to express delayed cutaneous hypersensitivity to antigens such as tuberculin purified protein derivative and 2-4-dinitrochlorobenzene. When injected directly into intradermal melanoma deposits, BCG vaccine has induced complete remission of the injected tumor in 90 percent of immunocompetent patients. In addition, in 15 to 20 percent of these patients there also was regression of uninjected melanoma nodules indicating that in some cases the effect was not limited to the injection site. It should be emphasized that intralesional administration of BCG is most effective against intradermal deposits. Subcutaneous and visceral metastases do not respond as well. Patients who are anergic respond poorly to intralesional injection, and uninjected nodules in these patients never regress. Intralesional BCG has also been effective in the treatment of intradermal local recurrences of breast carcinoma following a mastectomy.[19] It should be pointed out that this kind of local nonspecific immunotherapy has also been accomplished with the use of 2-4-dinitrochlorobenzene in the treatment of squamous and basal cell carcinomas of the skin.[20] After sensitization to this chemical, the patients' neoplasms are treated with topical applications of 2-4-dinitrochlorobenzene cream. More than 90 percent of superficial basal cell, or squamous cell, carcinomas have been destroyed by the local delayed cutaneous hypersensitivity reaction induced.[21]

Nonspecific immune stimulation with BCG has also been used as an adjuvant to chemotherapy in an attempt to induce a systemic effect. BCG vaccine was first used in this fashion by Mathe as an adjunct to chemotherapy for the treatment of leukemia.[22] Combining chemotherapy and immunotherapy increased the remission rate and prolonged the duration of response in these patients. Although two subsequent trials failed to confirm these observations, there were considerable differences in Mathe's trials and the subsequent two trials. Since that time a number of investigators evaluating a combination of chemotherapy and immunotherapy with BCG have indicated that administration of BCG alone, or in combination with allogeneic vaccine, is capable of increasing the duration of remission and the incidence of remission in patients with leukemia.[23]

BCG immunotherapy as an adjunct to surgical operation has been evaluated in several human neoplasms. However, the most extensive and thorough investigation of this surgical adjuvant modality has been evaluated in patients with malignant melanoma.[17,24] Following operation, patients with malignant melanoma, who have regional lymph node metastases, have a high incidence of recurrence, and it is felt that if immunotherapy is initiated immediately after surgical operation the microscopic residual disease can be more effectively controlled. A recent report by Eilber and co-workers has indicated that BCG immunotherapy used as an adjunct to surgical procedures in patients with malignant melanoma is capable of prolonging the disease-free interval in these patients.[24] In this study, excision of the primary malignant melanoma and regional lymph node dissection were done and patients were given BCG immunotherapy with or without allogeneic tumor cell vaccine. The interval from initial treatment to recurrence was plotted by the life table analysis method (Figure 1).

In patients treated with adjuvant immunotherapy there was a lower incidence of recurrence than in those treated by operation alone at all points on the graph. Immunotherapy also delayed recurrence (Figure 2). In 50 percent of the patients treated by operation alone, recurrence developed within four months. The median time to recurrence in the group treated by operation and immunotherapy was ten months. These findings suggest that BCG immunotherapy used as a surgical adjuvant when there is minimal residual disease is capable of increasing the disease-free interval.

BCG is currently undergoing extensive evaluation as a surgical adjuvant. One intriguing recent report by McKneally and associates describes the

use of intrapleural BCG administered as a surgical adjunct following pulmonary resection for lung cancer. The preliminary analysis of survival data indicates a definite beneficial effect of the intrapleural BCG.[25]

BCG comes in a variety of different preparations and strains. There is evidence that some strains are not as effective in immunostimulation as others. In addition, the dose of BCG seems to be important as well as the technique of preservation of the BCG organisms. All of these variables tend to explain some of the discrepancies in clinical trials when different strains of BCG are used in different doses and different techniques of preservation are used.

Both local and systemic complications of BCG immunotherapy have been described. The most severe toxicity of BCG occurs in patients who are receiving intralesional BCG, and who have extensive disease. Intratumor injection of BCG frequently results in fever, chills and localized abscesses with sinuses that drain for a relatively long time. Regional lymphadenitis is a frequent observation, and occasionally systemic infection has occurred associated with granulomatous hepatitis. Fatal anaphylactoid reactions have been described in patients receiving multiple injections of large doses of BCG intralesionally. Smaller doses of BCG should be used in patients with positive tuberculin tests. Pretreatment with antihistamines and aspirin before and several days after the intralesional injections decreases toxicity. Persistent symptoms have been treated successfully with isoniazid.

In contrast to intratumor injection with BCG vaccine, the administration of BCG by the intradermal multiple tine puncture technique is usually quite well tolerated with a low incidence of hepatic dysfunction, and only mild fever and malaise lasting for a short period of time following vaccination. Anaphylactoid reactions, or pronounced toxicity, has not been associated with this method of administration. The complications of BCG immunotherapy have been reviewed recently by Sparks.[26]

Some of the difficulties inherent in the use of live BCG organisms have stimulated interest in

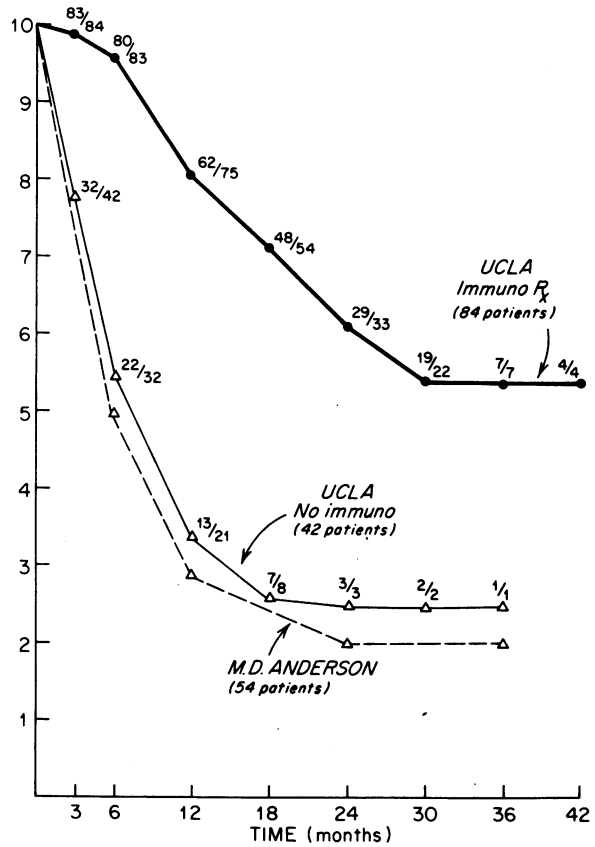

Figure 1.—Recurrence rate of Stage II melanoma, showing the percentage of patients free of recurrent melanoma after regional node dissection or node dissection with postoperative immunotherapy.

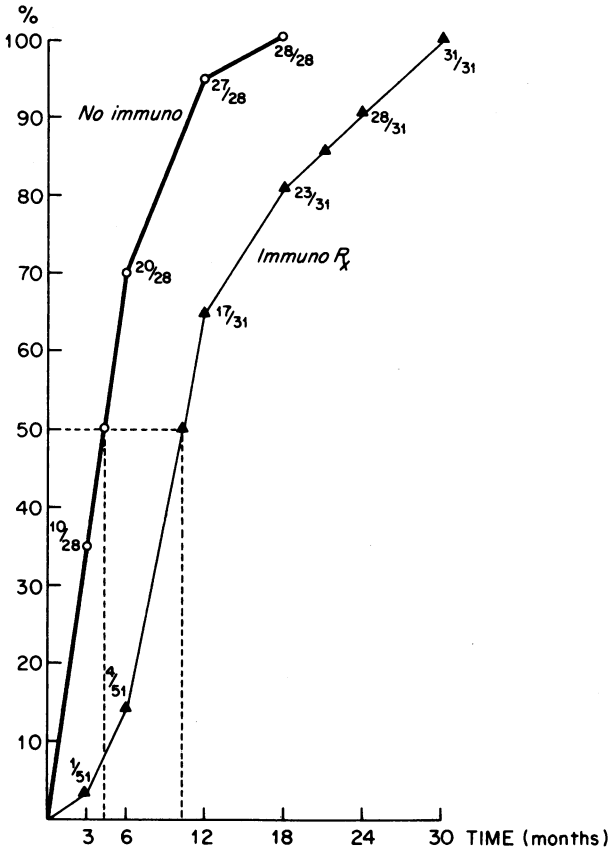

Figure 2.—Time to recurrence of melanoma after a disease free interval. Difference between disease free interval in those treated by surgical operation alone and those treated by operation and immunotherapy.

certain extracts of BCG. These extracts, such as methanol extractable residue (MER) and the Ribi vaccine, are currently being evaluated in clinical trials.[16]

Corynebacterium parvum

Corynebacterium parvum is a Gram-positive anaerobic organism. In contrast to BCG, C. parvum is not used as a viable organism, but is formalin killed. Also in contrast to BCG, it can be administered intravenously whereas BCG is not given by the intravenous route in man. C. parvum is a potent macrophage stimulator when given intravenously, and when given by this route it is a strong T cell suppressor.[27] However, when given by the intradermal or the tumor intralesional route, it is a T cell stimulator. This stresses, again, the importance of the route of injection in determining the mechanism of action of these immunostimulating agents.

C. parvum, like BCG, is very effective when given intralesionally, and it is also effective when mixed with tumor cells and injected into a region that is drained by the same lymph nodes that drain the tumor. It is effective as a surgical adjuvant in animals; however, the clinical experience with C. parvum as a surgical adjunct is not as extensive as with BCG and surgical adjunct trials in man are just beginning. While there has been relatively little clinical experience with the use of C. parvum as an adjunct to surgical operation or with its use as an intralesional agent, C. parvum has been evaluated extensively as an adjuvant to chemotherapy.

Israel and Halpern have reported on the use of C. parvum in combination with chemotherapy for a variety of solid tumors.[28,29] Patients who had the best prognosis were those who were immunocompetent before therapy, and who received C. parvum in addition to chemotherapy. The patients who received C. parvum in addition to chemotherapy had a longer duration of remission, a higher incidence of remission and maintained their immunological competence better than those patients who received chemotherapy alone. These trials were conducted in patients with disseminated carcinoma of the lung, advanced lymphosarcomas, breast carcinoma and advanced melanoma. The data indicated that the patients receiving C. parvum in addition to chemotherapy had a prolonged survival. Patients with good cell mediated immune function, as judged by reactivity to a variety of skin test antigens, responded better to C. parvum treatment than those with poor cell mediated immune function. Interestingly, the patients who received C. parvum had a better hemopoietic tolerance to the chemotherapy than patients not receiving C. parvum.

The initial Phase I trials indicated that C. parvum could have significant toxicity. Given by the subcutaneous route, pain, swelling, fever and chills frequently developed. Intravenously given C. parvum in doses in excess of 5 mg per sq meter has been associated with cyanosis, bronchospasm, and significant chills and fever. However, in doses between 2.5 and 3.5 mg per sq meter intravenously given C. parvum has been very well tolerated. The agent should be administered slowly intravenously for the first two to three injections. It is usually given weekly, and after three to four injections it generally can be injected more rapidly. The optimum dose and route of injection for induction of tumor immunity have not yet been defined.

The extensive experience with C. parvum and BCG in animal investigations and the clinical experience to date allow certain conclusions regarding the general principles of immunotherapy with these agents:

- It is clear that this form of immunotherapy is effective only against a limited amount of disease. Patients with large tumor burdens, in general, do not benefit from the administration of these immunotherapeutic agents alone. In view of the above, it is clear that immunotherapy should be used in combination with a major ablative modality such as chemotherapy, surgical operation or radiation therapy.
- Immunotherapy is most effective when the immunotherapeutic agent comes in close contact with the tumor. This may take the form of direct intralesional injection, or the injection of a nonspecific agent in combination with whole tumor cells or subcellular products.
- Immunotherapy generally does not induce regression of established tumors when given systemically.
- Experience has indicated that the immunotherapeutic agent should be administered, if possible, in the region which is drained by the same lymph node group that drains the neoplasm being treated.

Levamisole

Levamisole is an entirely different agent from C. parvum and BCG. Levamisole is a chemical

compound that can be taken orally in the form of a tablet. This agent was originally developed as an antihelmentic, and has been used extensively in Western Europe and other parts of the world to treat intestinal infestations in man. Its effect on the immune system was discovered by accident. There has been some reluctance to describe levamisole as a nonspecific immunostimulating agent because it appears to have little or minimal effect on an intact normal immune system. The most dramatic effects have been in patients with depressed immune function. When used as an immunotherapeutic agent in animal systems, levamisole has little effect on established animal tumors. In addition, it is not as effective as BCG or C. parvum as a prophylactic agent in animal tumor models. However, levamisole has been shown to effectively augment chemotherapy in animal neoplasms. It increases the duration of response, and the cure rate of chemotherapy, and also prevents the immunosuppression of chemotherapy in these animal models.[30-32]

The oral administration of levamisole has been shown to enhance phagocytosis and *in vitro* lymphocyte function in animals. In man, levamisole has been shown to have an effect on T cell function and macrophage function. It augments macrophage chemotaxis in man, and the elaboration of macrophage immobilization factor. In certain patients with depressed T cell levels, levamisole administration returns the T cells to normal levels. This has been observed in patients with Hodgkin's disease, systemic lupus, rheumatoid arthritis and herpes simplex.[33] In patients with the latter three diseases, the increase in T cell levels following the administration of levamisole is frequently associated with improvement in symptoms. The ability of levamisole to augment depressed delayed cutaneous hypersensitivity reactions in man is controversial; however, the weight of evidence suggests that it does.

This unique agent is currently undergoing extensive clinical trials. Two of these are of sufficient duration to merit discussion. Rojas has reported an impressive increase in the disease-free interval and survival in patients with locally unresectable breast cancer treated by radiation therapy and administration of levamisole.[34] In this study, patients with locally unresectable carcinoma, with no clinical evidence of distant metastases, were treated with radiation therapy with or without concomittant levamisole administration. After 30 months there was a statistically significant difference in the survival of patients receiving levamisole. Of the patients receiving levamisole, 90 percent were alive, whereas only 35 percent of the control group were alive. In addition, at 24 months the recurrence rate was statistically significantly different in favor of the levamisole treated group.

Levamisole has also been used as a surgical adjuvant following pulmonary resection for lung cancer.[35] In this study, which was done in Western Europe, patients undergoing pulmonary resection for lung cancer were treated either with placebo or levamisole. The placebo or levamisole was begun five days before surgical operation. Levamisole therapy was continued intermittently following operation until recurrence. Within certain disease categories there was an impressive difference between the patients treated with levamisole compared with controls. Small primary lesions with negative lymph nodes were not affected by the levamisole treatment. However, in those patients who had large primary lesions, vascular invasion or regional lymph node metastases, the survival of the patients receiving levamisole was significantly better than the control group. There was an interesting pattern of recurrences within the two groups. Patients receiving levamisole had a low incidence of distant recurrence, and when recurrence occurred it was usually local. Certainly these impressive clinical results in breast cancer and lung cancer require confirmation. There are trials currently underway in this country, and elsewhere, evaluating levamisole as an adjunct to surgical operation and other cancer cytoreductive therapy.

Levamisole, in the doses used in clinical trials, has had relatively few side effects. With 150 mg per day administered for three days every two weeks, the side effects have been quite acceptable. Occasionally patients complain of mild nausea, but this is relatively uncommon. No changes in hematological measurements have been reported. In higher doses levamisole has caused more consistent nausea, and occasional neurological symptoms such as hyperreactivity, increase in insomnia and agitation.[27]

Conclusions

A large number of data has been accumulated both in animal studies and in clinical studies indicating that the immune response is an important component of the host-tumor interaction. Several

clinical trials, although preliminary, indicate that immunotherapy may be a useful adjunct to the other forms of cancer therapy, such as radiation therapy, surgical operation and chemotherapy. However, it is becoming clear that immunotherapy, as it is currently used, has limited effectiveness. Immunotherapy alone is not effective against large amounts of tumor, and it is certainly most effective when all gross disease has been eliminated and only microscopic residual disease remains.

Our knowledge regarding the clinical application of immunotherapy is quite limited and many unknown factors remain to be defined. For example, the optimum dose of the immunopotentiators has not yet been defined, the duration of treatment necessary for maximum effect is unknown and much of the basic mechanisms of action has yet to be defined. For these reasons, and also because immunotherapy may actually accelerate tumor growth in certain conditions, the use of immunotherapy should be limited to those who are experienced in its use and who are conducting properly stratified, randomized trials in order to determine its most effective application in humans.

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