Applications of Immunology to Clinical Cancer
Past Attempts and Future Possibilities

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SUMMARY

Reports of attempts to apply immunologic methods to the treatment of human cancer are scattered through the medical literature of many decades and represent in total a considerable effort. Many of these publications report an occasional patient with some evidence of improvement following treatment with vaccines or antiserums. The criteria of response in these reports are usually subjective or undefined. In a few patients objective documentation of tumor regression is reported, but rarely if ever is it possible to conclude that the responses were due to immunologic mechanisms. Such results naturally make us skeptical concerning the validity of the basic concept of an immunologic approach to the management of cancer. This skepticism, however, should be tempered by an equally objective skepticism of the assumption that the tested methods were adequate and truly evaluated the total potentialities of immunologic methods of treatment.

When the possibilities for application of immunology to the control of human cancer are considered on purely theoretical grounds it seems conceivable but not very probable that it can be successfully applied to cancer treatment, and the outlook seems slightly more hopeful for cancer prophylaxis.

The need to consider nonspecific mechanisms of host defense, in addition to specific immune reactions, is stressed.

Scientific research, like literature, has two objectives—the egoistic satisfaction of the investigator and other interested individuals, and the betterment of mankind. The former we call basic research, and the latter applied research. The elucidation of a new component of the complement system might serve, for this gathering, as an example of the former, and for a literary analogy Sandburg's "Fog"—"creeping in on little cat's feet"—should be an uncontroversial example of literature which provides personal enjoyment without affecting mankind. But you will recall that "Fog" was followed by "Chicago"—a poem full of protest, challenge, and pride, with a potential influence on the daily lives of our people. So, too, we have learned that the basic scientist with no declared objective beyond the satisfaction of intellectual curiosity may produce work which profoundly affects the welfare of all people.

As individual scientists we demand no more of our efforts than personal satisfaction; but we recognize that by the synthesis of our achievements the greater objective is occasionally reached, and it is the awareness of this potentiality that motivates the general population (from which we cannot divorce ourselves) to support our studies through such organizations as the American Cancer Society. We cannot, therefore, reasonably conclude this symposium without some consideration of the potential applicability of immunology research to the problem of clinical cancer—although to undertake this analysis is an assignment which would humble and intimidate far better men than me.

As a framework for discussion, Chart 1 is presented as a scheme in which we can categorize the immunologic mechanisms that might be brought to bear against any pathogenic influence. The term defense mechanisms, instead of immune mechanisms, might be preferred by those who limit their definition of immunology to specific immune phenomena and related serum factors. This diagram
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categorizes serum antibody as a specific humoral defense mechanism and complement as a nonspecific humoral factor. The specific cellular category presumably includes the key mechanisms for reactions of the delayed hypersensitivity type. Provision is also made for such nonspecific cellular defense functions as phagocytosis and fibrosis. It will be recognized that this is designed as an all-inclusive concept, an attempt to include all conceivable mechanisms whether now recognized or not. The several components of serum complement are nonspecific humoral factors. Properdin, as conceived by Pillemer and his followers (71), would also fall in this category, as would Kidd's tumor-inhibiting principle of guinea pig serum (36), certain hormones and extracellular enzymes, and intracellular ground substance.

If these are the immunologic mechanisms potentially available for application to clinical cancer we must consider the methods by which their efficacy might be increased. These are suggested in Chart 2, in which a third dimension has been added to indicate that, in addition to active stimulation of the patient's own mechanisms of defense, there is also the possibility of passive transmission of defense factors produced in a different individual.

Active immunization resulting in both immediate and delayed types of hypersensitivity with such vaccines as diphtheria toxoid and B.C.G. are commonplace in clinical practice. Stimulation of nonspecific defense mechanisms by neutral polysaccharides is illustrated in many laboratory studies (39, 40, 67) and probably affects both humoral and cellular mechanisms as judged by coincident changes in serum properdin (39) and in phagocytic and enzymatic activity of the reticuloendothelial system (65, 66, 97). Passive immunization by the administration of serum antibodies produced in humans or animals is an established technic in clinical medicine. Other passive methods of altering host immunity are known only as experimental procedures. Properdin preparations have been administered as an attempt to supply a nonspecific humoral defense factor (77, 84). The transfer of immunity of the delayed hypersensitivity type has been accomplished by the injection of immune cells (6, 34, 54) and cell fractions (42). If such cells grow in the recipients as a tissue graft the term adoptive immunization is applied. This effect is active in the sense of a continuing production of antibody, but is passive in the sense that the antibody is not a product of the recipient's tissues. The eighth block in Chart 2 is blank because it is essentially an unexplored approach, but nonantibody-producing cells can be passively transferred as grafts under certain experimental conditions.

This schematic representation of defense mechanisms may be useful for orientation as we review what attempts have been made to apply immunology to the problem of clinical cancer.

**PAST ATTEMPTS TO TREAT CANCER PATIENTS BY IMMUNOLOGIC METHODS**

**ACTIVE SPECIFIC IMMUNIZATION ATTEMPTS**

Most of the clinical research efforts have been attempts to apply the methods of specific immune therapy to the treatment of patients with advanced cancer. It is self-evident that this has been an empirical (and sometimes unenlightened) approach, because all hope of success through this approach hinges on the existence of antigenic differences between cancer cells and normal tissues which might allow selective or preferential action

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**Chart 1.—Host defense mechanisms**

**Chart 2.—Methods of influencing host defense mechanisms.**
of antibody against the cancer cell. The wry fact is, as amply documented in this symposium, that there is no proof and only scanty data to suggest that cancer cells contain such unique antigens. This state of knowledge dictated the clinical experimental approach, for the only available source of a hypothetical cancer antigen was the cancer tissue itself.

Attempts to treat cancer patients by active immunization were started more than half a century ago. Leyden and Blumenthal in 1902 (44) injected two advanced cancer patients with a filtrate of their own tumor tissue. There was no apparent change in their downhill course, but some enlarged glands appeared to shrink. Bertrand in 1909 (4) published a report of a favorable clinical response to cancer tissue vaccine in one patient. Coca and Gilman in 1909 (see Ref. 18) reported objective regression in one patient with cancer of the neck following autologous cancer vaccine injections and consequent infections, and similar treatment of another patient in whom evaluation was impossible because of definitive surgery. Roswing in 1910 (78) reported treatment of seventeen patients with phenolized autogenous tumor homogenates. No therapeutic effect was noted. In 1911, Betti (5) reported unquestionable objective regression of breast cancer accompanied by most impressive subjective improvement of unstated duration in a 42-year-old woman with coincident hyperthyroidism who was given intramuscular and intratumoral injections of a human tissue autolysate. The source of the autolysate is not recorded. High fever accompanied its use, intensive treatment with arsenicals preceded its use, and amelioration of the hyperthyroidism occurred concurrently. Thus, the factors which effected the tumor regression cannot be dissected. The endocrinologic change is intriguing, since it invites the speculation that a change in endocrinologic balance might have caused the tumor regression, or that the tissue vaccine might have caused an immunologic depression of thyroid function.

Coca and co-workers in 1912 (18) used formalinized or phenolized (and occasionally fresh) autologous cancer tissue to vaccinate (subcutaneously) 39 patients with histologically proved cancer and used similarly prepared homologous tissues for 48 (several received both). Six patients had no recurrence of cancer after the definitive surgery which yielded the tissue from which their vaccine was produced, but it must be presumed that these cases represented successful excision rather than a therapeutic effect of the vaccine. A few patients with advanced cancer experienced brief periods of subjective improvement, but, with few exceptions, the cancer continued to grow progressively. The exceptions were five patients who exhibited objective regression of proved cancer. Each of these had received homologous vaccine only—that is, none of the patients who were inoculated with their own cancer showed any objective improvement. Of the five regressions described by the authors, two consisted of liquefaction necrosis and ulceration of epidermoid carcinomas. Such changes are not uncommon in the natural history of these cancers and are not acceptable as proof of tumor inhibition. The other three regressions were impressive, objective, started within a few days of treatment, and persisted for periods of weeks to months before growth recommenced. However, two of these patients developed high fever and abscesses at sites of vaccine injections, and the authors point out the probability that the regressions were due to the bacterial infection rather than an immunologic response to cancer antigens. The remaining patient (scirrhous breast cancer) had no fever and no abscesses. No cause of the regression other than the vaccine could be suggested, but metastases recurred after 4 months in spite of repeated use of the same and other vaccines. Attempts to demonstrate antibody formation using precipitin and complement-fixation technics with two of the whole tissue homogenates as antigens gave negative results in the three patients whose sera were studied.

Risley (76) used identical methods with completely negative results. Autogenous tumor was used for ten patients and tumor from other patients for ten others. Pinkuss and Klonsinger (73) likewise used phenolized autogenous tumor vaccines to treat seven patients with metastatic or recurrent mammary or uterine cancer. Stammler (90, 105) reported disappearance of uterine cancer after vaccine injections in one patient who was still well 6 months after treatment.

Lunckenbein (47) reported treatment of three patients with extracts of autolyzed cancer tissue. A patient with mammary carcinoma simplex and axillary and supraclavicular nodes had objective regression of primary and metastatic lesions after intravenous and subcutaneous injections of an autogenous tissue preparation. Follow-up was only 10 weeks. A patient with colloidal thyroid cancer metastatic to neck nodes was treated with autologous tissue material immediately after surgery, and no follow-up data are presented. The third had postoperative recurrence of lip carcinoma. Injections (route) of the mammary cancer autolysate were followed by inflammation, necrosis, slough, and granulation of a large submental tumor, but
the general course was downhill, with appearance of metastases in cervical nodes and death in 2 months.

Vaughan (100) reported extensive clinical studies with active and passive immunization methods for cancer treatment. Over 200 patients with a wide variety of histologically confirmed cancers were treated, but his report is restricted to 100 who had at least a 1-year follow-up. He produced vaccines (99) from human cancer tissues by homogenizing in alcohol, drying, and resuspending in saline; or, by extracting with 2 per cent sodium hydroxide in absolute alcohol, discarding the soluble fraction, and extracting the residue in water. The sources and types of tumor tissue are not itemized, nor is it recorded whether single or pooled specimens were used; but it seems clear that these were homologous rather than autologous vaccines. Furthermore, each of the 88 (?) patients who received vaccines apparently received antiserum preparations as well (see below). Of these 88, 31 were treated concurrently by definitive cancer surgery, and 23 were "apparently well" at least 1 year later. Among 38 who had inoperable or recurrent cancer two had "apparent recovery" (presumably meaning no obvious disease 1 year or more after treatment), and four were "markedly improved." Among nineteen patients who had either a small tumor specimen to treat six patients with cancer, and another patient without effect on her disease. One of these patients had growth of a tumor nodule at one of the implant sites.

Kellock and co-workers (35) reported attempts to treat cancer patients by autologous vaccination. They exposed freshly excised and minced cancer tissue to X-ray at a dosage (2 rads) considered sufficient to kill the cells without significantly reducing antigenicity. This vaccine was then implanted in the rectus sheath deep to the rectus muscle within a few hours of excision. From a total of 30 patients treated, twelve are described in some detail. In no case was there any alteration of the course of disease that could not be attributed to antecedent treatment by surgery or X-ray.

Figari (25) used "ultrapeptones" from various tumor specimens to treat six patients with cancer of the skin and was convinced that the procedure was efficacious. He reported that patients showed improvement over a period of 1–3 months but gave no details.

Rubens-Duval (81) wrote extensively on the treatment of cancer patients by local or oral administration of ultra-homoeopathic doses (10^-20 dilutions) of "globulins" extracted from tumor tissue by an alchemic series of extractions by acids, alkalis, organic solvents, and trypsinization. Other methods of treatment including X-ray were used concurrently, and no persuasive evidence is presented of any effect of the "specific protein therapy." A similar report on the use of an enzymatic extract of cancer was published (58) by de Nabias. This, too, was used in conjunction with treatment methods of established efficacy and also presents no evidence of effect attributable to the experimental treatment. Laborde (38) reported that this method had no value in breast cancer patients.

Stone and co-workers (91) repeatedly implanted autologous cancer tissue fragments that had been frozen at −20°C for 5–23 days into three patients with breast cancer. One patient had a surgical cure of her disease, another was followed for only a month at the time of the report, and the third died of unknown cause 2 months after surgery. Another patient received implants of breast cancer from another patient without effect on her disease. One of these patients had growth of a tumor nodule at one of the implant sites.

Witebsky, Paine, and Egan (104, see page 840) used tumor extracts as vaccines for the treatment of about twenty terminally ill patients with various cancers, including thyroid adenocarcinoma. No improvement was reported.

Active immunization of cancer patients is currently being attempted in at least three institutions in this country. These studies were stimulated by the work of the Grahams (88), who demonstrated complement fixation when sera of twelve patients (among 48 who were studied) were incubated with a water-soluble nucleoprotein-rich antigen extracted from autologous cancer tissue. Each of these patients had had recent excision or radiation-induced regression of her gynecologic cancer. Having this evidence that autoantibodies against cancer antigens might occur, they injected 114 patients who had advanced cancer (mostly cervical, uterine, and ovarian carcinomas) with autologous cancer vaccines (29). The preparation of the vaccine was varied, to include whole-cell suspensions, cell extracts, and cell fragments, and these were administered by intradermal or intramuscular routes in a variety of Freund-type adjuvants. Only 29 patients were available for critical evaluation, after elimination of patients who had other forms of therapy or who died soon after vaccination, and among these there was objective evidence of tumor regression in three. One of these had received radiotherapy 2 months before vaccine, another had progression of disease until 3 months after vaccination and then improvement commenced, and the third had surgery just preceding vaccine which left behind residual but not...
massive disease. Thus, none of the patients will satisfy strict criteria for evaluation of a therapeutic effect due to vaccination. Moorman and Roberts of the Pasadena (California) Medical Research Foundation have recently started to use this same autovaccination technic in conjunction with chemotherapy for advanced cancer. They have observed no striking therapeutic response, but their data are still inadequate for evaluation. Finney, Byers, and Wilson (26) gave repeated intramuscular injections of autologous cancer vaccines in Freund’s adjuvant to nine male patients with advanced carcinomas and sarcomas. Their vaccines were whole tissue minces repeatedly frozen and thawed to kill the cells. They reported that, about 2 weeks after initial intramuscular injection of the vaccine, there was inflammation followed by softening and shrinkage of subcutaneous tumors in all (? three) patients who had such lesions. These investigators also studied passive administration of serum (see below).

Cinander and co-workers (17) treated a woman with metastatic choriocarcinoma by sensitizing her to her husband’s leukocytes. The rationale was that this tumor, which usually arises from fetal tissues and hence is biologically a homograft, should contain tissue antigens contributed by the husband and hence foreign to the patient. These same antigens should be present in some or all of the husband’s cells, which could thus serve as a vaccine. The patient improved subjectively and objectively and was without evidence of disease a year after the final vaccination. During the period of recovery the patient was also passively immunized with serum from rabbits immunized against the husband’s sperm.

A considerable literature was published circa 1905 on treatment of cancer by the sera of Doyen or Schmidt. These were animal antisera against microorganisms thought to be the etiologic agent of the cancers. Since the basic premise was fallacious and the reports of therapeutic response are unconvincing, review of these papers is not attempted. References can be found in the U.S. Army Medical Library Index Catalog, series 2, under Cancer—serum therapy.

PASSIVE SPECIFIC IMMUNIZATION ATTEMPTS

Passive immunization for the treatment of cancer in man has been attempted sporadically since before the turn of the century. Héricourt and Richet as early as 1895 (32) were using antiserums produced in dogs and donkeys by injection of human tumor emulsions to treat cancer patients. Over 100 patients were treated, but the data are largely unpublished. According to Vidal (102) the therapeutic results, if any, were subjective and transitory, and eventually the cancer progressed in spite of continued treatment.

Dor (19) in 1901 treated two melanoma patients with a goat antiserum prepared against human melanoma and reported tumor regression, but the follow-up for both patients was only 3 weeks. Borrell (11, see page 117) in 1903 reported that he had patients under treatment with antisera to their own cancer, but no subsequent report has been found.

Vidal, at the Second International Congress for Cancer in 1910, discussed the whole problem of immunological treatment for cancer in an article which could well be reprinted in the present symposium, since it makes embarrassingly clear how little real advance there has been in the intervening 50 years in our concepts or our application of immunologic mechanisms in clinical cancer. Vidal (102) also reported that he had treated about 94 cancer patients with “cytolytic sera” produced by injecting various human cancer tissues into animals. Among 40 evaluable cases, three who received no form of treatment other than the serum showed impressive improvement (cancers of tongue, breast, and rectum); these striking responses were considered to be probable responses to the serum treatment, but concurrent minor surgical procedures could conceivably have caused the improvement; another nineteen showed “considerable amelioration,” and fifteen showed no effect.

Berkeley (3) treated 89 patients with antisera prepared in various animals against human cancer tissues. A single preparation of serum was used for most patients but a few received serum prepared against their own cancer. Thirty-nine patients had been given serum in conjunction with definitive surgery. Only 32 who had recurrent or inoperable cancer at time of serum treatment were evaluable. None of these was cured, but a temporary favorable response was claimed for two-thirds of them, and impressive tumor regressions lasting at least 6 weeks are described for two patients with breast cancer and one with cancer of the mouth.

Odier (64) had “two remarkable results” from serum treatment. Vaughan (100), whose studies of cancer vaccines have been noted above, injected his human cancer tissue preparations into rabbits or sheep and used the resulting antiserums to treat patients. The relationship between the tissues injected into the animals and the patients subsequently treated is not recorded, but it seems cer-
tain that the animals did not receive the tumor of the individuals treated. Four patients were treated with serum from sheep and eight with rabbit serum. It is recorded that "in all cases there was improvement in the condition of the malignant growth" (sic), but no details are given. Six cases of acute nephritis were attributed to the treatment, and it was abandoned as too toxic. In further studies blood was collected from the sheep at the time of the maximum mononuclear leukocytosis which followed (4–5 days after) the injection of the vaccine. An extract was then prepared from the blood cells (99) on the hypothesis that the mononuclear cells were producing the desired "ferments" (antibodies) and would therefore be a richer and more specific source than the serum.

Lucatello and Malon (46) used sheep or rabbit antihuman-leukemic-blood sera to treat three leukemia patients but observed no significant response. Lindstrom (45) in 1927 used "myelotoxic sera" to treat ten patients with myelocytic leukemia. These were produced in rabbits or sheep by the injection of leukocytes from the individuals to be treated. Doses of 10–15 cc. of these sera were administered intramuscularly one or more times. Some were adsorbed with the patient’s erythrocytes to diminish hemolytic activity. Five showed impressive objective improvement as judged by peripheral blood picture and shrinkage of spleen or nodes, but in three, and perhaps all, of these the antileukemic effect might have resulted from x-ray or arsene treatment used concurrently.

Over the past decade, Murray (56) has treated at least 233 cancer patients by use of serum globulin from horses vaccinated with human breast cancer, or with gastric cancer, or with colon cancer plus various other types of tumors. Each horse received many injections of fresh or stored tumor tissue (storage conditions not stated) from many patients over a period of several months. Only 91 patients who received at least 250 cc. of serum were considered acceptable for evaluation. Each was presumably treated with the serum appropriate to the cancer diagnosis (i.e., horse antihuman breast cancer serum for breast cancer patients, etc.). Among 62 patients with metastatic breast cancer eight survived more than 2 years after start of treatment (seven were alive at time of report), and fourteen survived more than 1 year. Forty died within 1 year of treatment. Among 27 patients with advanced colon cancer two survived over 2 years (including one still alive at 3½ years), six were still alive more than 6 months after treatment at time of report, and the other nineteen were dead with survival times not stated. Among 33 patients with unresectable or recurrent gastric cancer three survived more than 1 but less than 2 years after treatment, and 30 died within a year. Subjective improvement was reported for many patients. Partial recalcification of osteolytic bone lesions is reported in "some." One patient had re-epithelialization of ulcerated skin which had previously been treated by x-ray. Three patients with signs consistent with brain metastases showed objective improvement of their neurologic defects. Murray states that his serum treatment produced "improvement in patients beyond what could have been expected in the ordinary course of the disease . . . ." The published data, however, do not seem sufficient to substantiate even that restrained conclusion.

Grace et al. (27) reported shrinkage of skin lesions of leukemia cutis after local infiltration of serum from a rabbit which had been vaccinated with lymph node tissue from the same patient. Normal rabbit serum had no effect, but no normal lymphoid tissue was available for production of an antinormal lymphocyte serum, so the result could not be accepted as evidence of a specifically antineoplastic effect.

Nungester, Bierwaltes, and Knorpp (63) administered an I\textsuperscript{131}-tagged antisera (produced by inoculation of rabbits with the patient’s tumor tissue) to a patient with widespread melanoma. Complete regression of the tumors followed, and the patient remained in good health until he died of myocardial infarction 8 years later. Complete autopsy (exclusive of head) revealed no evidence of tumor. Stimulated by this observation, Vial and Callahan (101) injected rabbits with cell suspensions, cell fractions, or melanin from melanoma patients and labeled the resulting sera with radioactive iodine. An unstated number of patients were given intravenous injections of the antisera prepared against their own tumor, and other melanoma patients were given antisera prepared against melanoma or melanin of different patients. No tumor regression was observed, and only one patient showed any suggestion of selective uptake of the I\textsuperscript{131} in tumor tissue.

Buninauskas et al. (15) used serum treatment for three patients with breast cancer whose surgery had been only simple mastectomy because of suprachlavicular node metastases. Each patient’s tumor was inoculated into three sheep, and gamma globulin from the sheep serum was given intravenously to the same patient. The minor shrinkage in size of metastases following treatment seems insignificant. In vitro studies of tumor cell agglutina-

\footnote{Additional data by personal communication with Dr. W Nungester (University of Michigan Hospitals, Ann Arbor, Michigan), May 16, 1961.}
tion by the sheep antiseraums suggested the possibility that the injected antiserum might have become coated on tumor cells in the one patient so studied.

Brittingham and Chaplin (14) treated a patient with chronic myelocytic leukemia by injections of serum gamma globulin from a normal human who had repeatedly been inoculated intravenously with fresh whole blood from the leukemia patient. Leukagglutinins against the patient’s leukocytes were demonstrable in the serum. While there was no objective improvement attributable to the treatment, the patient claimed subjective improvement and showed no progression of disease during the 4-week treatment period. Since there was rapid progression of disease before and after the treatment period, there may have been a slight antileukemic effect.

A recent publication from the Institute of Experimental Biology of the U.S.S.R. Academy of Medical Sciences (60) indicates that an equine anticancer serum is being produced there for therapeutic purposes, but I am not aware of any report of clinical data.

Moore and co-workers (55) injected large doses of commercial pooled human gamma globulin into thirteen patients with advanced cancer with the hypothesis that if antibodies against spontaneous cancer are ever produced they might be represented in such serum pools. No antitumor effects were observed.

Finney and co-workers (26) prepared gamma globulin from the serum of three patients who had been inoculated with autologous tumor vaccine in Freund’s adjuvants (see above) and who had developed autoantibodies in their sera as judged by the tanned erythrocyte agglutination technic with autologous tumor used as the test antigen. Selected tumor nodules were then treated by inoculation of this autologous gamma globulin into or around the nodules. Subsequent measurements of nodule diameters indicated a significant shrinkage of those so treated, whereas there was no change in controls injected with normal serum, saline, or nothing. The published data are scanty and subject to criticism on two points: some of the globulin injections were intratumoral where mechanical rather than immunologic mechanisms may have caused regression, and the globulin injected as a control medication was from normal pooled human plasma rather than from the same patient before vaccination. This is, however, an ingenious experimental approach to the problem of demonstrating autoantibodies in the face of antigen (tumor) excess and is of particular interest because if antitumor autoantibody does in fact exist the harvesting and concentrating of autoantibody globulin from repeated bleedings followed by massive systemic administration is technically feasible for clinical application.

Cinander et al. (17) observed a complete remission of choriocarcinoma after active and passive sensitization of the patient against her spouse’s tissue. The rationale is discussed above.

Sumner and Forsaker (95) transfused whole blood into two patients with melanomas from a patient who had had a spontaneous regression of melanoma. One of the recipients had a complete regression lasting at least 5 years. This study differs from all of the preceding by the use of whole blood, thus transferring possible leukocyte-fixed antibodies in addition to serum antibody. The opportunities for such a study are as rare as spontaneous cancer regressions, but the observation should encourage similar attempts whenever possible.

Lewison et al. (43) tried an ingenious variation on the passive antibody approach. Stimulated by the studies by Peterson and Campbell (70) on antibody in colostrum, they injected human breast cancer tissue into the udders of pregnant cows (over periods from a few days to a year), then administered the milk obtained for a week after calving to seventeen patients with breast cancer. Four of these patients were treated with milk from cows given injections of their own cancer tissue. Symptomatic improvement was reported as striking in one patient and slight in nine, but none showed objective evidence of tumor regression. This approach avoided the problem of sensitization to heterologous proteins but faces the admitted criticism that antibody probably cannot be absorbed through the gastrointestinal mucosa (30).

**Attempts To Stimulate Nonspecific Host Defenses**

Attempts to treat cancer patients via nonspecific immunologic factors have been few. Two interesting but nonproductive studies by Braunstein (13) and Blumenthal (7) and their co-workers might be mentioned in this category. Since experiments on mice with transplantable tumors showed a mononuclear leukocytosis after oral and parenteral administration of extracts of spleen and other viscera, it was hypothesized that such a response indicated a host defense reaction. Acting on this premise they administered such preparations to cancer patients, but the clinical data are so unimpressive and nonobjective as to merit no further discussion.

More recently, there was considerable clinical interest in the use of sera designed to stimulate the
reticuloendothelial system. These were known as antireticular cytotoxic serum (ACS) or reticuloendothelial immune serum (REIS) and were sera from goats or rabbits which had been given injections of bone marrow and/or spleen of the species being treated. Such sera were shown to fix complement when mixed with homologous spleen or marrow antigens and were impressively cytotoxic at "high" dosage in various tissue culture and in vivo systems. The interested reader will find supporting data and more extensive bibliographies in the following references (1, 8, 9, 48, 75, 92, 93). Interest in ACS as a therapeutic agent for a wide range of disease states was based on the fact that subinhibitory doses of certain toxic substances have a diametric stimulatory effect. Bogomolets, who introduced the concept of the reticuloendothelial system (RES) as a functional unit, followed by the concept of Metchnikoff in reasoning that an antiserum with cytotoxic effects on the RES might, at very low concentrations, stimulate the RES to greater immunologic efficacy. The data supporting the stimulatory effect of ACS are very scanty. Bogomolets' review (9) cites Soviet studies in experimental animals indicative of stimulated growth of connective tissue, increased resistance to rickettsial and bacterial infections, increased phagocytic activity of leukocytes, and elevated complement levels resulting from ACS treatment. The original reports of these studies were not available to the present reviewer, and no other studies of the effect of ACS on immunologic factors have been uncovered. Pomerat (74) observed a statistically significant, but unimpressive and nonreproducible, stimulation of outgrowth of chick embryo heart fibroblasts in plasma clot tissue cultures when rabbit anti-chick-spleen serum was added to the nutrient medium at a dilution of 1:400. (Control cultures received identical amounts of normal rabbit serum.) This serum inhibited outgrowth at dilutions up to 1:20, and had a complement-fixing titer of 1:1200. An impressive and objective study of the effects of ACS on bone healing (93) did demonstrate accelerated healing in approximately 50 rabbits treated with a single intravenous dose of 0.00125 ml of goat anti-rabbit-spleen-marrow serum as compared with an equally large number of controls treated with normal serum or with nothing. Another group, treated with 0.1 ml of the ACS, showed striking inhibition of healing.

Clinical trials of ACS (goat or rabbit antihuman marrow and/or spleen serum) for the treatment of advanced cancer were first performed in Russia. Fed'ushin (24) reported that some patients showed regression of metastases and prolongation of life (original report not yet reviewed). In this country, Davis (20) treated 68 patients. Marked or moderate subjective benefit was reported in 23, but there was no suggestion of prolongation of life, and the only objective change was improvement in the peripheral blood picture in a patient with myeloid leukemia. Skapier (82) reported therapeutic trials of ACS with no other concurrent treatment in seventeen patients with Hodgkin's disease. Two of these had received no previous treatment of any kind. No tumor regression was recorded, but eleven were considered to be partial therapeutic responses as judged by improvement in hemoglobin levels, weight, or erythrocyte sedimentation rates.

Koressios (37) treated 89 cancer patients with serum preparations that were apparently of this type, but the published data do not present a clear picture of what was done or the results obtained.

It seems unfortunate that a hypothesis which has some logical appeal and which excited enough interest to be applied to clinical treatment of scores of patients should have received insufficient basic study to support or refute the working hypothesis. It is also disconcerting to note that, on the basis of relative body weights, the course of treatment used by both Skapier and Davis was 10 times the serum volume (antibody titers unknown) that promoted bone healing in the rabbit studies of Straus and co-workers (94), and one-tenth the dose that impaired healing. In view of the postulated mechanism of action, and since the only available laboratory data indicate a very critical dose-response relationship, one might question whether a suitable dose was chosen for the clinical trials.

The administration of tubercle bacilli (BCG) or various neutral polysaccharides or zymosan (a crude polysaccharide preparation from yeast) in appropriate dosage to experimental animals inhibits growth of transplanted tumors (12, 31, 65, 66, 69). This antitumor effect is paralleled by, and conceivably is the result of, an increased activity of certain nonspecific cellular and humoral host reactions, which may function as defense mechanisms. Crude polysaccharide-rich bacterial filtrates ("Coley's toxins") or purified bacterial polysaccharides ("Shear's polysaccharides") have been used extensively, erratically, and often uncritically, as chemotherapeutic agents for human cancer for 70 years (59). There is no question that lymphomas (but rarely carcinomas) often shrink after such treatment and that therapeutically useful regressions sometimes occur. That these agents are seldom used at present is due to the availability of better therapeutic methods rather than a denial of their effect.
Since these agents produce a variety of reactions, including fever and arteriolar hypotension, the mechanism of tumor inhibition is unresolved, and there is no clinical evidence to indicate that they work via host mechanisms rather than directly against the tumor. However, the studies of Old, Landy, Pillemer, and their several co-workers (39, 40, 65, 66) indicate that these polysaccharides, in appropriately small doses, do stimulate the phagocytic activity of the reticuloendothelial system and increase certain virocidal, bacteriolytic, hemolytic, and zymosan-binding reactions of serum. These serological reactions were intensively investigated by Pillemer, who concluded that they were all due to a nonspecific humoral defense factor for which he coined the term properdin (71). The concept of properdin as a single nonantibody serum globulin is being critically scrutinized and questioned by many investigators, e.g., Nelson (61); but the serologic reactions are real, the term is convenient, and Pillemer’s concept has at least served to reawaken interest in the question of humoral defense mechanisms other than antibody.

In cancer research particularly, where attempts to utilize specific immune mechanisms for therapy have been so uniformly discouraging, Pillemer’s concept of immunologic mechanisms which do not require activation by a specific and unique cancer antigen has great appeal. Interest was further heightened by studies from many laboratories, with a variety of assay technics, reporting that among patients with advanced cancer of all types serum properdin activity was frequently low or undetectable (16, 23, 33, 41, 49, 57, 62, 68, 79, 80, 83, 87). Although subsequent studies indicate that properdin-rich serum fractions have no direct inhibitory effect on cancer cells (88), the relationships of polysaccharides with RES activity and serum properdin titers, and with tumor growth, suggested further clinical studies of polysaccharides.

Zymosan has not, to my knowledge, been used to treat cancer patients, and clinical use seems unlikely because of its particulate nature and the nonuniformity of biological activity in various batches. In Switzerland3 small intravenous doses of a lipopolysaccharide from Brucella abortus equi (Pyrexal—Wander Co.) were used to treat two sarcoma patients. One showed slight regression of skin nodules but died about 2 weeks later. The other had slowly progressive disease apparently unaffected by treatment. Serum properdin levels showed no significant fluctuations in either patient. I have administered single or repeated small doses of this same polysaccharide to fourteen patients, and a polysaccharide from the mouse tumor S-37 (prepared and supplied by Dr. Murray Shear of the U.S. National Institutes of Health) to eight patients, in an attempt to increase serum properdin levels (unpublished). Most of these patients had adenocarcinoma, and none had leukemia or lymphoma. Since this was not planned as a therapeutic trial, patients were not selected who had objectively evaluable disease, nor was treatment by other means withheld. Therefore critical evaluation for antineoplastic effect was not attempted, but there was no obvious abatement in the progressive course of disease in any patient. A majority had no demonstrable serum properdin as judged by Pillemer’s zymosan assay technic (72). In only two patients did properdin levels increase significantly after treatment, and, since fluctuations of similar magnitude have occasionally been observed in untreated persons, it is questionable whether this can be attributed to the polysaccharide treatment. One of two normal control subjects showed a transient rise of borderline significance (from 8 to 12 units) after a single 0.1-μg. dose of pyrexal intravenously. Eichenberger and Isliker (22) demonstrated a 75 per cent increase in mean properdin titer in a group of five normal humans given this same dose of pyrexal.

Dismal though the results have been, as gauged by therapeutic results, the trials of antireticular cytotoxic sera and polysaccharides are of interest because they represent attempts to stimulate the host to defend itself against a pathogenic process which may be antigenically inert.

ADMINISTRATION OF NONSPECIFIC DEFENSE FACTORS

Interesting clinical experiments—equally futile to date but possibly prophetic of future avenues of therapeutic endeavor—have attempted to supply to the cancer patient nonantibody defense factors of which there is a suspected insufficiency. This approach bears the same relationship to the method just discussed, as does passive immunization to vaccination. It differs not only in that it is experimental, but in that it is unproved at best, and perhaps mere fantasy. The administration of serum complement as whole serum or as complement-rich serum fractions would be a valid example of passive administration of nonspecific immunologic factors, but there appears to be no complement deficiency in cancer patients (53, 83, 86), nor is any disease state known in which complement administration would be therapeutic. Human serum fractions of high properdin activity (500–1000 U/ml) were prepared (89) and were administered

3 Personal communication from R. Grimm (Wander Co., Bern, Switzerland), June 30, 1958, and March 9, 1961.
intravenously to five children with neoplastic diseases in doses sufficient to obliterate a properdin deficit and maintain normal levels for several days (unpublished). A child with neuroblastoma survived 16 weeks after her first properdin treatment but had no tumor regression. The others had no immediate change in their hematologic picture, but died of their acute leukemia 3, 4, 9 and 35 days after the first properdin treatment. Certainly these few terminal cases do not constitute a therapeutic trial of this serum fraction, but the problem has not been pursued further because of the difficulty of producing the material, the danger of transmitting viral hepatitis, and laboratory data which indicated that high levels of properdin (plus complement) were not directly or solely capable of inhibiting cancer cells in tissue culture (10, 88) or bacterial infections (77) in mice. Kidd's (36) tumor-inhibiting principle "TIP" of guinea pig serum which inhibits certain transplanted mouse and rat tumors is probably an example of antieaner serums have no effect on cancer, but there is no known parallel in clinical cancer.

Non-specific defenses may be cellular as well as humoral and, theoretically, defective cellular defenses might be remedied by "passively" administering more competent cells as homografts.

Niehals' "fresh cell therapy" might be mentioned as an attempt to introduce new cells into patients with various diseases, but competent physicians have rejected the method as of doubtful efficacy and potentially dangerous (103).

Homografts of bone marrow are being performed currently at several institutions as an experimental method of therapy for leukemia (50-52, 96). It has usually, but not always (52), been used as an adjuvant to total-body radiotherapy, and is generally conceived as being simply a replacement for the patient's marrow that has been deliberately destroyed in an attempt to eradicate the leukemia. Studies of genetic markers and the occurrence of "homologous disease" in some patients and animals following transplantation of marrow or spleen cells (51, 52) provide abundant evidence that in some of the recipients homologous granulocytes and lymphoid cells and erythroblasts propagate and function after transplantation. There is no reason to doubt that other phagocytic and fibroblastic cells from the donor may also propagate and function in such chimeric individuals. At this writing the attempts to use marrow transplants for the treatment of leukemia—regardless of what mechanisms might be operative—seem as fruitless as all the other experimental approaches which have been reviewed. But, just as we should not condemn the specific immune approach because of a long life of repeated failure, we must not abandon this embryonic idea so recently conceived.

**Evaluation of Past Attempts**

It is not difficult to evaluate these attempts to apply immunology to the treatment of cancer in man. One can conclude—with no expectation of controversy, with no fear of prematurity, and without accusation of personal prejudice—that they have contributed nothing to applied immunology, that they have failed to give any indication of a cancer-specific antigen or other Achilles' heel susceptible to immunologic attack, and that there is no proof that they have ever influenced the course of cancer in any patient. This is truly a discouraging evaluation; however, the objective of this conference is not to assess what has been done, but rather to examine these clinical experiments for any scrap of evidence suggesting that immunologic mechanisms might potentially have some effect on cancer. We must beware the temptation to expand these specific conclusions into the general dogma that cancer vaccines or anticaner serums have no effect on cancer, because lack of proof does not constitute negative evidence. Unless we could prove that the occasional clinical responses which were observed were unrelated to the treatment used, these rare responses cannot be considered biologically insignificant even if we doubt that they resulted from the treatment administered, and no matter how insignificant they are by the criteria of statistical analysis. Regardless of how brief, regardless of how infrequent, regardless of how little therapeutic benefit may have ensued, if any one of these reported responses was in fact a result of some immunologic reaction we cannot say that it is inconsequential.

**Future Possibilities**

Fortunately, Dr. Harry Weaver's letter of invitation to this conference stated that its purpose "is not so much to document progress as it is to point up problems, to depict the nature of obstacles to further progress, and to evaluate the validity of present concepts." He gave us a ready-made excuse for our inadequacies by writing that "whereas our answers to these questions today may appear ridiculous in retrospect, it is only in this way that we can visualize the thread of significance in research and vitalize the germ of further progress." It is only by virtue of this affidavit of policy that I dare leave the small but firm posi-
tion of historical review to proceed into the treacherous quicksands of prediction.

I have tried to express my personal estimate of future possibilities for treatment of cancer patients by modifying my diagrammatic representation of immunological mechanisms (Chart 3). In this figure I have left blank the blocks which represent areas in which I can not conceive of any applicability to cancer therapy. Shading by diagonal hatch-marks indicates possibilities which are conceivable but not very probable. Heavier cross-hatching would indicate a greater probability of effective clinical application, and solid black squares would indicate established usefulness. Chart 3 is monotonously shaded, indicating little optimism but not a complete negation. The lack of optimism should need no apology after the foregoing recital of repeated failure to date. Rather, it is the residuum of hope that requires defense.

I will make no attempt here to discuss the scanty pros and overwhelming cons of the question of cancer-specific antigens, since I have previously considered this problem (85) and it has received more expert attention in this symposium. Rather, I will proceed with this discussion neither denying nor assuming that cancer-specific antigens exist, but will try to consider what possibilities may exist for cancer control through immunologic mechanisms in either of these eventualities. On this basis I will hereafter use the term cancer antigen without further apology.

**Possibility of Therapy**

The blocks in the foreground of Chart 3, representing responses of the patient himself to a newly imposed antigenic or nonspecific stimulus, are uniformly and lightly shaded because it seems self-evident that, regardless of the fundamental efficacy of defense mechanisms against cancer, the patient who has now got cancer must be an individual who has failed to respond to the stimulus of his own cancer. Certainly, we must admit that the cancer patient is exposed to maximum amounts of whatever cancer antigens there may be and has failed to produce an effective response against them. On the other hand, one can argue that the failure of cancer to excite nonspecific reactions (e.g., fibrosis) does not mean that such reactions would not restrain the cancer if they could be induced; or that in the patient with cancer a potentially effective antigen had somehow been prevented from acting—perhaps because of a temporary nonreactivity of the host at a critical moment, or failure of the antigen to reach the antibody-producing cells. If we admit any logic in these arguments, then we cannot completely deny the possibility of stimulating host defenses of the cancer patient and should keep an open mind to such conceivable approaches as (a) more effective RES stimulants, (b) slight modification of cancer-specific antigens or haptenes to yield a molecule which serves as a fresh antigenic stimulus, yielding a new type of antibody which would still retain the ability to react with the original tumor antigens in situ, (c) a more effective manner of presenting the cancer-specific antigens to antibody-producing tissues, or (d) ways of increasing the contact of tumor-inhibiting antibodies with the cancer cells.

The upper blocks in the background (Chart 3), representing passively acquired humoral defense factors, are shaded in the same manner. Passive treatment has the advantage that it requires no participation by the host, thus side-stepping the problem of a host that may in any way be immunologically incompetent. It requires, however, both the recognition and the production of yet-to-be-discovered humoral substances and introduces the problems of quantity production, doubtful efficacy in advanced disease, and toxic reactions. The block designating passively acquired nonspecific cellular defense mechanisms is blank, because, although it is quite conceivable that such cells as granulocytes and fibroblasts could be administered (grafted) to suitable recipients, there is no reason to suspect that even if such transplants were successful such cells would have any influence on the cancer. The companion block, representing the passive acquisition of cells capable of producing antibodies, is accorded some consideration because such cells could react against the postulated cancer-specific antigens. These cells might first en-
counter the cancer antigen after their transplantation, as illustrated by studies in mice (2, 91, 98) in which the recovery of mice from leukemia is thought to be enhanced by an immunologic reaction of homologous marrow transplant against the genetically different leukemia cells of the host. Alternatively, the transplanted antibody-forming cells might have had accidental or planned contact with the cancer antigen prior to their transplantation. This situation is now well known as the laboratory phenomenon of "adoptive" immunization. One can also conceive that it might occur transiently during a brief period of cell survival of the antibody-producing cells in the fresh-whole-blood transfusion.

Possibility of Prophylaxis

Everything said up to this point has been concerned with cancer therapy, but even in the infectious diseases our only major medical achievements through immunology are prophylactic. It would certainly be remiss and out of character, if, after letting our imagination rove so freely thus far, we stopped without considering the prospects for cancer prevention by immunologic methods. This is attempted in Chart 4.

To be prophylactic, a therapy must act at the time of exposure to a pathogenic agent or during the early stages of pathogenesis which precede recognizable disease. With few exceptions, we do not know the etiologic agents of human cancer, the time of exposure, or the duration of the incubation period. What evidence we do have from clinical and experimental oncology suggests that exposure may occur over long periods of time, perhaps starting very early in life, and with delayed development of the pathology. In such situations there is no value in prophylactic methods which give only brief protection. This eliminates consideration of all passively administered humoral factors and of cellular factors unless they are self-replicating. This means that only cell grafts can be considered, and even if one adopts the hypothesis that the cell endowment of some individuals may be inadequate for self-defense against oncogenesis, there is certainly no way at present of assessing this competence. For these reasons, I can see no possible application of any type of passive immunity to cancer prevention. This conclusion is indicated by the lack of shading in the four blocks in the background of Chart 4. The blocks in the foreground are different, for actively acquired resistance to disease may be of long duration. Even the heightened nonspecific resistance which follows administration of B.C.G. may persist for many months (67). I am not aware that such a possibility has yet received any experimental investigation in cancer, even in mice where the method of evaluation would seem straightforward. If animal experiments should indicate a diminished incidence or later onset of neoplasms related to a sustained stimulation of nonspecific host factors, it is not inconceivable that similar treatment could be applied to man. Admittedly, the problems of experimental application and evaluation in man would be formidable.

The remaining two blocks of Chart 4 are the only ones which are cross-hatched, indicating that only in the mechanisms of specific immunity do I conceive any real probability of cancer prophylaxis. Here is the point of emphasis of all of the laboratory and clinical experience in immunology.
without viruses as cancer-specific antigens, there is a reasonable basis for hope that true cellular antigens specific for cancer may exist—developing by a finite number of cancer-specific antigens, and as a result of the neoplastic transformation regardless of its cause. If such antigens are unique in every individual there is clearly no possibility for prophylaxis as a principle of true cellular immunity. The possibility for prophylaxis does exist.

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