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IMMUNOTHERAPY OF CANCER

Report of a WHO Scientific Group

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WHO SCIENTIFIC GROUP ON THE IMMUNOTHERAPY OF CANCER

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IMMUNOTHERAPY OF CANCER

Report of a WHO Scientific Group

A WHO Scientific Group on the Immunotherapy of Cancer met in Geneva from 30 May to 4 June 1966. The meeting was opened by Dr A. N. Klimov, Director, Biology and Pharmacology, who welcomed the members on behalf of the Director-General.

Professor M. F. A. Woodruff was elected Chairman, Professor G. Mathé Vice-Chairman, and Dr D. Metcalf and Dr C.M. Southam Rapporteurs.

1. INTRODUCTION

This report is concerned with the role of immunological factors in resistance to cancer and with the possibility of preventing or treating cancer by immunological methods. Every attempt has been made to keep in mind the relation of the data presented to human cancer and, for this reason, the discussion of animal experiments has been restricted whenever possible to work with autochthonous or syngeneic tumours, since these are the most appropriate experimental models for cancer in man. In each area of discussion, the available data concerning human cancer are discussed together with the data from animals, so that differences and similarities can be evaluated and so that the deficiencies and the special problems of human cancer investigation will be evident.

The Group has been very conscious of the need to keep constantly in mind the possibility of applying immunological methods to the treatment or prevention of human cancer. In the last analysis the effectiveness of any particular form of immunological therapy in the treatment of human cancer can be assessed only by clinical trial, but individual members of the Group differed as to when and what clinical studies should be initiated. There was, however, agreement that when a particular procedure has been shown to be effective against animal tumours, and its safety has been established in so far as this can be done by tests in animals, that procedure should be tried clinically as soon as possible.

On the other hand, it would be a tragedy for the individual patient and a deterrent to medical progress if ill-conceived clinical trials were undertaken which did not permit the value of the new procedure to be properly assessed, or which prevented or delayed the application of therapeutic methods of known efficacy.

2. ANTIGENICITY OF EXPERIMENTAL ANIMAL TUMOURS

The fact that neoplastic cells are recognized as "non-self" by the host organism seems to be the rule rather than the exception for many different experimental tumour systems. In several systems in which the immunogenic reactivity of a given tumour in the host animal has not been demonstrated, some condition of the host such as tolerance may be responsible, not the absence of tumour-specific antigens.

A variety of tests have been employed to demonstrate different types of tumour-specific antigen. These tests include complement fixation, immunofluorescence, cytolysis, agar-gel precipitation, immunoelectrophoresis, and transplantation. Identification of the various types of cell constituent as antigenic may depend entirely on the type of test employed. An antigen may provisionally be considered as specific for tumour tissue if the normal tissues of the autochthonous or syngeneic host do not exhibit activity in the same type of test. It is recognized, however, that a tumour-specific antigen may be present in normal tissue in such a low concentration that it cannot be detected by the currently available tests.

Evidence has been obtained that antigens present in the C57BL/6 strain of mouse leukaemia (TL) and in mouse hepatoma (alpha globulin) are repressed in the corresponding normal tissue of their hosts but may be present either in embryonic tissue of the same species (e.g., the alpha globulin of the hepatoma) or in normal tissue (thymus) of other strains of the same species (TL antigen in A mice).

It has been also shown that animals infected at birth with certain RNA tumour viruses such as those causing Gross and Moloney leukaemia, avian lymphomatosis, or mammary tumour may not recognize antigens present in infected tumour cells. This immunological unresponsiveness may be attributed to specific immunological tolerance of the host, since non-infected animals recognize the antigenic specificity of the same tumour tissue.

Although at present it is impossible to correlate the appearance of any type of tumour-specific antigen with the development of neoplastic characteristics in the cells, the presence of tumour-specific transplantation antigens (TSTA) may be more significant from this point of view than that of the other tumour-specific antigens.

TSTA can be detected in animal tissue by a variety of tests. These include :

- (1) resistance of the animals to challenge with tumour tissue either after surgical removal of a syngeneic tumour or after immunization by implants of either living or irradiated cells;
- (2) destruction of tumour cells, either *in vivo* or *in vitro*, by lymphoid cells obtained from animals immunized against isogeneic tumours;
- (3) the presence of specific antigens at the cell membrane as demonstrated by immunofluorescence; and
- (4) in the case of tumours induced by DNA tumour viruses, resistance to tumour implantation in animals infected with isologous virus as adults or prevention of tumour development in newborn animals injected with isologous virus and implanted 30-70 days later with cells transformed by the same virus.

TSTA by its nature must be localized on the cell surface and it can usually be demonstrated more readily in tumours that appear soon after exposure of animals to a chemical or viral carcinogen rather than in those which appear later. It seems likely that antigens located deep within the cell are not effective in eliciting significant anti-tumour immune responses. In some studies on polyoma-induced hamster tumours, TSTA seemed to be lost or repressed in the course of several transplants of the tumour either *in vitro* or *in vivo*.

In spite of its similarity to a weak histocompatibility antigen, the nature of TSTA is completely unknown, attempts to obtain a cellular fraction possessing TSTA activity having failed. So far only living or physiologically intact cells have shown the presence of TSTA. Lysis of cells by a cytolytic virus may on occasion produce a cell extract possessing some TSTA activity. Tumour systems in which TSTA has been demonstrated in syngeneic or autochthonous hosts include tumours induced by chemical carcinogens, physical agents, and DNA and RNA oncogenic viruses.

Tumours induced by physical means such as the subcutaneous introduction of plastic films or exposure to ultra-violet light show the presence of TSTA, although it may be exceptionally weak. The presence of TSTA in tumours induced by ionizing radiations or in spontaneous tumours of unknown etiology has not yet been clearly shown.

The TSTA of tumours induced chemically and physically differ from those of virus-induced tumours in each being unique to the tumour concerned. Thus, two sarcomas induced on opposite sides of the same animal by the same carcinogen have TSTA which as a general rule do not cross react. There are indications that the number of possible TSTA

is not unlimited; cross reactions have been seen when large series of tumours have been tested.

Recent experiments suggest that non-oncogenic viruses may induce the formation in tumours of new antigens that, being recognized by the host, will lead to the specific destruction of the tumour tissue.

The possibility must be borne in mind that contaminating viruses may produce cross-reacting antigens in tumours that are not of viral origin.

3. ANTIGENICITY OF HUMAN TUMOURS

The study of human cancer antigens is hampered by the lack of adequate methods of investigation. The method of transplantation in syngeneic recipients which, in experimental animal tumours, has provided clear evidence that immunological mechanisms are capable of impeding tumour growth, is not available for the study of human tumours, with the exception of autotransplantation (and, theoretically, isotransplantation in identical twins). The development of the serological techniques used so successfully for the antigenic analysis of animal tumours of viral origin depended on knowledge about, and the availability of, the oncogenic virus. In the absence of such identified etiological agents in human cancers, serological analysis depends on a comparison of normal and neoplastic cells for differences in antigenic composition that cannot be related to any factor other than the neoplastic state. This is difficult because of the great number of antigenic differences between different individuals and different normal tissues, as well as of extraneous antigens against unrecognized microbial contaminants. Furthermore, when tumour-specific antigens are demonstrated by such analytical methods, there is no guarantee that these antigens are capable of eliciting immune responses capable of *inhibiting* tumour cell growth.

Quantitative autotransplantation in patients with incurable cancer has shown that large numbers of tumour cells (to the order of 10^6) are required for transplantation of cancer with any regularity, suggesting that some kind of resistance to cancer cell implantation and growth does exist. There is no direct evidence that this "resistance" is due to specific immunological mechanisms, but the demonstration of a direct correlation between "resistance" as measured by autotransplantation studies and the immunological competence of the patients as measured by homograft rejection suggests this possibility.

Autotransplantation before and after attempted immunization with autologous tumour preparations has never been reported. This would now be technically feasible (although not necessarily desirable) with modern methods of freeze-preservation of tumour cells.

The study of pairs of identical twins, one of whom has cancer, offers the only syngeneic system in man for investigating tumour-specific antigens. Transplantation studies of cancer in identical (syngeneic) twins have never been reported, partly because of the rarity of suitable subjects but largely because of the danger of transplantation for the healthy twin. The application of the *in vitro* lymphocyte transformation technique, using lymphocytes from the normal twin and tumour tissue from the twin with cancer, may prove useful in detecting cancer-specific antigens.

The use of serological methods to seek cancer-specific antigens becomes increasingly difficult as the antigenic difference between the immunizing tissue and the antibody producer increases. In the patient himself (or an identical twin) the antigens of cancer cells and normal cells of the type from which the cancer arose should be identical except for the presumptive cancer-specific antigen; hence an antigen-antibody reaction between a patient's serum and his own tumour cells would indicate the presence of either a cancer-specific antigen or extraneous antigens specifically located in tumour tissue. Some careful studies of this problem have given negative results and others have been inconclusive, but a few, including a recent study of Burkitt's (African) lymphoma using immunofluorescence, have shown auto-antibody reactions with cancer cells.

Studies of the antigenic capacity of human cancer that have utilized antisera prepared in animals are extremely difficult to interpret, because the number of human antigens foreign to the recipient animal is very large. The differences detected by such antisera must be considered as probably due to differences in normal antigenic composition between different persons. Only if this explanation can be excluded is it possible to consider an antibody to be specific for a cancer antigen. Comparison of cancer cells with normal cells of the same person, of the same cell type as that from which the cancer arose, provides the best chance of detecting a truly cancer-specific antigen. The use of such methods as adsorption and of tolerance during preparation of the antiserum to reduce the complexity of the reaction and of highly discriminating and sensitive techniques for detection of the antigen-antibody reactions has given results in a few recent studies that give to antigens the appearance of being cancer-specific. However, antigens present in carcinoma of the human gastrointestinal tract were either absent or repressed in normal adult tissue but present in normal embryonic tissue. The question whether such antigens are targets for the immune response of the host remains open.

Antisera obtained from humans during rejection of cancer cell homografts are somewhat less complex than those produced against human cells by experimental animals, and have revealed antigens in established lines of cancer cells that have not been detected in normal cell lines. However, because of the great diversity of normal antigens in different individuals, even these antisera also contain antibodies against numerous

normal cell components, so the basic difficulties discussed above apply to these sera just as much as to heterologous antisera.

Recommendation for further study

The Group recommended that the application of the *in vitro* transformation technique, using lymphocytes from normal twins and tumour tissue from twins with cancer, should be thoroughly investigated as a method of detecting cancer-specific antigens.

4. BIOLOGY OF THE IMMUNE RESPONSE

Immune responses are initiated only in lymphoid organs and, specifically, only in the spleen and lymph nodes and not in the thymus or bone marrow. To initiate a primary immune response, antigen probably needs to travel to the lymph nodes or spleen, although in a secondary response it is conceivable that antigen may react locally with "memory" cells (small lymphocytes) which may then travel to lymphoid organs before undergoing proliferation. Where part at least of the antigen reaches the circulation, the spleen appears to be the major site of the immune response, producing 90% or more of the circulating antibody but a rather smaller fraction of immunologically reactive cells.

The immune response produces its effects by the lymphocytes and possibly the monocyte-macrophage cells and by circulating immunoglobulins produced by plasma cells and possibly lymphoid cells. The effector cells formed during an active immune response appear to enter the circulation with priority over other recirculating lymphoid cells, and on reaching the site of antigen release they accumulate preferentially in this region. This selection of cells may involve processes akin to chemotaxis, the cells following an antigen gradient, or it may depend on a passive process of cell immobilization in sites of high antigen concentration.

It is important to appreciate how highly dynamic the lymphoid organs are.

(1) Lymphocytes are highly motile cells. In mixed *in vitro* cultures of lymphocytes with other cells, the lymphocytes not only move inquisitively among these cells but they also commonly enter their cytoplasm and nucleus.

(2) Lymphocytes circulate from one lymphoid organ to another by the bloodstream. Thus antigenically competent lymphoid cells generated in one local lymph node become distributed throughout the body.

(3) The stem-cell population in all lymphoid organs is continuously being replaced by new cells entering from the bloodstream. The initial development and the continued functional activity of these organs are dependent on this continuing cell immigration. In mice, the source of the new stem cells in adult life is almost certainly the bone marrow, the spleen serving as a temporary reservoir for such cells.

One practical consequence of lymphoid organ repopulation is that lymphoid organ grafts invariably become repopulated by host cells. In cancer patients exhibiting subnormal immune responses based on defects in the lymphoid cell population, lymphoid organ grafts are unlikely to prove beneficial. Further injections of spleen or lymph node cells would probably be of only limited value unless they resulted in the rapid destruction of tumour cells.

The functional capacity of the lymphoid organ is dependent on an effective system of antigen-trapping and antigen-processing macrophage or reticulum cells. The exact mechanism involved is uncertain, but the cells may process trapped antigen, which is then made available in suitable form to antigenically competent cells. The latter respond by a dual clonal proliferative response resulting in the generation of both lymphoid and plasma cells. Alternatively, macrophage cells may produce messenger RNA which enters precursor cells, a proliferative response resulting.

The capacity of lymphoid organs to respond to many (if not all) antigens is dependent on the presence of the thymus and, possibly, several other thymic analogues (e.g., the appendix and tonsil). In chickens, the bursa of Fabricius also exhibits a thymus-like function. Removal of the thymus in early life leads to a state of depressed immune responsiveness to soluble, viral, bacterial, and cellular antigens and to early death of the animal from infection. Thymectomy in adult life has a similar effect, but the depressed responsiveness is delayed in onset until late in life. The influence of the thymus arises in part from its production of lymphoid cells, which travel to lymphoid organs and there enter into active immune responses, but possibly the major role of the thymus is to produce a humoral factor which enables competent immunologically active lymphoid and plasma cell precursors to proliferate at a rapid rate following antigenic stimulation. In the absence of the thymus, cells seeded to the lymphoid organs direct from the bone marrow are unable effectively to initiate immune responses. The biochemical nature of the thymic humoral factor is unknown, but the purification of this factor may well prove to be a potent therapeutic weapon, increasing immunological activity in pre-neoplastic and neoplastic states where the immune responses are depressed.

Since neonatal thymectomy does not depress all immune responses, it is possible either that some immune responses are under the control of

thymic analogues e.g., the bursa of Fabricius, the appendix, or the tonsil, or that the capacity to exhibit some immune responses has been conferred by the thymus before birth.

To sum up, an effective immune response is dependent on the following factors and events :

- (1) the antigen must reach the lymphoid organs;
- (2) an effective system of trapping and processing of antigen by macrophage or reticulum cells must be present;
- (3) lymphoid and plasma-cell precursor cells from the bone marrow must be in adequate supply;
- (4) the thymus must produce cells and thymic humoral factor; and
- (5) in some cases there must be an effective seeding out and concentration of lymphoid cells at the site of the tumour.

The exact mechanisms involved in tumour cell destruction by an immune response appear to vary according to the type of tumour. For leukaemic cells, the primary effector mechanism may well be cell destruction by cytotoxic antibodies. For sarcoma and carcinoma cells, cell destruction may be effected by direct cell contact with immune lymphoid cells. In this case antibody may be protective, coating the cells and preventing adequate cell contact between them and the immune lymphocytes.

Special factors affecting immune responses to tumour antigens

1. *Tolerance.* When an animal in an immunologically immature state is exposed to an antigen, it exhibits tolerance rather than an active immune response. Normally this immature state exists in early foetal life, but it is conceivable that a similar state may be produced in adult life by the release of massive amounts of antigen or by certain immunosuppressive procedures such as whole-body irradiation and cytotoxic drugs, possibly combined with thymectomy.

Tolerance to tumour antigens is known to occur with vertically transmitted tumour viruses, e.g., the Gross and Bittner viruses. Here the foetus is exposed to viral and possibly viral-induced host-cell antigens before the time at which "self" and "non-self" antigen structures are defined. Thereafter the animal accepts the tumour antigens as "self" and cannot be actively immunized against them in subsequent life.

Persistence of tolerance depends on a continuing source of antigen—a requirement fulfilled by the self-replicating nature of tumour viruses. Breakdown of tolerance is to some extent dependent on the thymus, being slowed or prevented by thymectomy but accelerated by thymus grafts and thymic humoral factor, as well as by whole-body irradiation.

If viruses are ultimately shown to be involved in the etiology of human leukaemia and if in at least some cases their mode of transmission is found to be vertical, it may prove impossible to develop an effective active immunization for the prevention or control of these infections unless practical methods of breaking tolerance can be developed. However, in this special situation the deliberate breaking of tolerance may carry a risk of precipitating tumour development.

2. *Aging.* This subject has not been studied as exhaustively as is needed, but the initial data from mouse experiments indicate that in old age immune responses become depressed in some strains of mice. In view of the known susceptibility of the aged to infections, it seems on general grounds probable that in humans a similar decline in immune responsiveness occurs with advancing age.

The reason for this decline in immune responsiveness has not been fully established in mice. Lymphoid organs in old mice certainly contain fewer cells capable of being stimulated by antigens (e.g., sheep red cells), and those cells which are present, when stimulated by the appropriate antigen, proliferate more slowly and to a lesser degree than in young adult animals. One or more of the following four possible factors may be responsible for this decline in responsiveness :

(1) a failure in the supply of bone marrow precursor cells (this may be compared with the finite number of ova in the ovary);

(2) age-related defects in the lymphoid cells themselves, limiting their capacity for antigen-stimulated proliferation;

(3) a decline in the efficiency of the antigen-trapping and -processing system—for which some evidence has now been obtained; and

(4) a decline in thymus function.

The last possibility seems now unlikely to be the sole cause, for thymus replacement studies in aged mice have proved ineffective in restoring immune responsiveness.

The incidence of many common types of cancer increases with age, and the achievement of a general improvement of the immune response in the aged may be of great practical importance, particularly in persons known to be specially at risk of developing cancer.

3. *Immunosuppression by chemical carcinogens.* Many chemical carcinogens are immunosuppressive agents, possibly because of their toxicity for immunologically reactive cells. It has been suggested that an essential part of the ability of such chemicals to induce neoplasms is in fact their ability to suppress immune responses that would normally eliminate the altered pre-neoplastic cells.

In the special instance of the pre-lymphoid-leukaemia state in mice, it has been suggested that infection of the immunologically reactive cells themselves (the lymphoid cells) by the leukaemia virus results in a significant depression of the immunologically reactive capacity of these cells. Other data, however, do not support this suggestion. It has also been suggested that under conditions of continued and intense hyperplasia, e.g., while initiating and maintaining chronic graft-versus-host disease, the reactive cells of the immunologically reactive system may be driven to a state of hyperreactivity ultimately ending in neoplasia. This may be a special instance in which immunological hyperreactivity rather than hypoactivity results in a heightened risk of tumour development.

Influence of immune responses in carcinogenesis

Immune mechanisms appear surprisingly effective in inhibiting the induction of tumours by viruses and chemical carcinogens. This influence can be deduced from the shortened latent period and increased incidence of tumour development following procedures (particularly neonatal thymectomy) designed to depress immune responses. Neonatal thymectomy causes an increased incidence of tumours with polyoma, Gross, Graffi, adenovirus-12 and (combined with bursectomy) chicken leukaemia viruses. Thus neonatal thymectomy is a very useful tool in producing suitable host animals for testing the possible carcinogenicity of viruses isolated from man (e.g., for testing adenovirus pathogenicity in the mouse). Neonatal thymectomy also increases the incidence of tumours induced by benzo-pyrene and dibenzanthracene. Thymus grafting on the other hand, has some protective effect on tumour development. Finally, irradiation is a powerful immunosuppressant. This may play a part in irradiation carcinogenesis.

It seems reasonable to infer that normal animals and humans are continually being protected to some extent from cancer development by immune responses initiated by foreign antigens present in the emerging tumour cells. However, no systematic study has been made on animals of the incidence of spontaneous cancer following immunosuppressive procedures in earlier life. Studies of this kind are important if the experimental conclusions are to be applied with confidence to man. It may also be worth while to study the incidence of cancer in humans subjected earlier in life to immunosuppressive procedures such as thymectomy and cortisone therapy.

Tolerance breakdown and carcinogenesis

In two cases, thymectomy has been shown to have an effect which is the reverse of stimulating to tumour development. It *reduces* the incid-

ence of lymphocytic leukaemia in animals infected with the Gross leukaemia virus and of mammary tumours in mice infected with the Bittner virus. It is curious that both cases involve vertically transmitted tumour viruses to which the young infected animals exhibit specific tolerance. Certain observations suggest the possibility that in the special case of vertically transmitted viruses tumour development may occur only *following* the breakdown of tolerance. In lymphocytic leukaemia in AKR mice the following data are relevant :

(1) There is a very long latent period before leukaemia develops (tolerance tends to break down with the passage of time).

(2) Thymectomy prevents the development of, and thymus grafts restore the susceptibility to, leukaemia (thymectomy slows or prevents the breakdown of tolerance, thymus grafts precipitate the breakdown).

(3) The first leukaemic cells always appear in the thymus but the young AKR thymus is morphologically normal, despite the fact that the cells carry the foreign virus-induced (G) antigen.

(4) In the immediate preleukaemic period, lymphoid follicles and germinal centres develop in the thymus medulla and massive destruction of lymphocytes occurs in the thymus cortex. However, neonatal thymus grafts in such animals are morphologically normal.

(5) In AKR F₁ hybrid mice, injected AKR spleen cells from mice late in the preleukaemic period seed in the thymus and there is an accelerated development of lymphocytic leukaemia derived from F₁ host cells.

These findings suggest the possibility that lymphocytic leukaemia develops following local intrathymic breakdown of tolerance to the G antigen and that leukaemic cells arise not by direct viral modification of infected cells but as a result of a local cell-mediated immune response damaging virus-infected cells.

These observations are only suggestive and need to be pursued further, but they raise the disturbing possibility that, in the special case of *vertically* transmitted viruses, immunological attempts to break tolerance in the hope that tumour virus infection during the latent period will be eliminated may actually precipitate the onset of tumour development. This may be of relevance for the acute leukaemias of childhood, where it is possible that vertically transmitted viruses are concerned.

Recommendations for further study

1. The ability to effect a general improvement of immune responses in the aged may be a procedure of great practical importance, particularly in persons known to be at special risk of cancer development. Consequently, mechanisms underlying the decline in immune competence in

aging animals should be analysed. Preliminary data indicate that there may be more than one manner in which such a decline occurs.

2. The search for viruses as causative agents of human cancer should be intensified. Thymectomy depresses the immune response. Thus neonatal thymectomy is a very useful tool for producing suitable host animals for testing the possible carcinogenicity of viruses isolated from man (e.g., for testing adenovirus pathogenicity in the mouse).

3. Methods of reinforcing immune responses should be investigated—e.g., the use of lymphoid cell or organ transplantation, thymic humoral factor, and adjuvant techniques for producing very powerful antigens such as macrophage-processed antigen.

4. The immune competence of animals during carcinogenesis should be studied, as well as the modifying effects on the incidence of cancer of replacement therapy in the pre-neoplastic period with antibody or immunologically active cells and organs.

5. IMMUNOLOGICAL MECHANISMS IN THE REJECTION OF ANIMAL ANTIGENIC TUMOURS

5.1 Humoral Immunity

A discussion of tumour-specific circulating antibodies with a view to evaluating their biological activity must be concerned with two problems: (1) the nature of the tumour antigens and their anatomical location on the cells; and (2), as it has been recently shown that different immunoglobulins differ in their biological activity, the class of immunoglobulins specific for the tumour antigens. Both of these problems have a direct bearing upon the type of effect antitumour antibodies may have on tumour growth or tumour rejection.

As regards the nature and anatomical location of tumour antigens, antibodies against internal cytoplasmic or nuclear components of malignant cells are not expected to exert any effect on the development and growth of cancer. A typical example is the antibodies against the CF antigens of tumours induced by DNA viruses, which are part of the viral capsid or may represent enzymes or precursors involved in the synthesis of viral components. These antibodies have no demonstrable effect on tumour development.

By contrast, antibodies directed against tumour antigens on the cell membrane may exert biological effects which may vary according to the experimental conditions. Thus the lysis of isolated cells in the presence of complement may be observed, provided that sufficient antigen is present on the cell membrane and complement-binding antibody is used. How-

ever, the damaging effect of physiological serum concentrations of these antibodies on organized tissue is much more questionable.

The class of immunoglobulin to which tumour antibodies belong may also influence their effect on the tumour cells. Although too few studies of this question have been made as yet, it has been established that guinea-pig and mouse 7S γ_1 and γ_2 antibodies differ in their ability to lyse cells in the presence of complement, γ_2 being the only lytic antibodies. Guinea-pig γ_1 antibodies were shown to be able to inhibit lysis of cells by γ_2 antibodies with the same immunological specificity, provided adequate amounts were used. The existence of antibodies capable of combining with specific cellular surface antigens without fixing complement or causing cell lysis raises the problem whether these antibodies may protect cells from damage by lytic antibodies and by the action of sensitized lymphoid cells with, as a result, enhancement of the growth of the tumour. The presence of such antibodies in various experimental animals and in man should be investigated. Available studies indicate that the γ_1 class of 7S immunoglobulins, which has been described in mice and guinea pigs, does not appear to be present in rats, rabbits, or man. IgA immunoglobulin, which seem to be common to most mammalian species investigated, does not bind complement either but is not generally produced in large amounts. Its significance also deserves to be investigated.

5.2 Cellular immunity

The destruction of antigenic tissue by specifically sensitized cells may be caused by several mechanisms. One is the delayed hypersensitivity reaction, whereby sensitized lymphoid cells react with antigen and release a soluble factor which causes the accumulation and aggregation of macrophages. The cellular infiltrate in delayed reactions has been shown to consist predominantly of unsensitized mononuclear cells which have come from the blood and have originated from rapidly dividing cells in the bone marrow. The presence of this infiltrate is essential for the tissue damage in delayed reactions. Thus it has been shown that the transfer of delayed hypersensitivity with sensitized lymphoid cells can be prevented by various treatments of the recipient animals that interfere with the accumulation of the non-specific cells at the site of the reaction. X-irradiation and treatment with cortisone, heparin, or dicoumerol prevent such a transfer. A mechanism must exist whereby the reaction of specifically sensitized cells with antigen causes the accumulation of infiltrating mononuclear cells. Such a mechanism has been described in recent *in vitro* studies demonstrating the release of a factor from sensitized lymphocytes that causes unsensitized macrophages to stick together.

Another mechanism by which sensitized cells can cause the destruction of target cells is through injury brought about by the direct contact

of those two types of cells. Studies of the mechanism of cellular immunity in tissue transplantation have been greatly facilitated by the demonstration that immune lymphoid cells are capable of killing histo-incompatible target cells *in vitro*. The cytotoxic action of the immune lymphoid cells is thought to be basically different from the action of humoral iso-antibodies; it does not require the participation of complement, and death of the target cells is observed as judged by morphological criteria, after 24-48 hours or earlier. Death is due to loss of the ability to divide, whereas humoral antibodies in the presence of a complement kill within one hour. Prior treatment of some neoplastic target cells with humoral iso-antibodies has been shown to inhibit the cytotoxic effect of immune lymphoid cells partly or completely, presumably by competition for the same antigenic determinants. It was subsequently shown in selected systems that normal allogeneic lymphoid cells were as effective in killing target tumour cells *in vitro* as were immune cells, provided that they were aggregated with target cells by phytohaemagglutinin or heterologous antibody. Immunization apparently provides the lymphoid cell with a specific receptor enabling it to attach to the target cells. The death of the target cell, however, may not be due to an immunological reaction but may be related to a non-immunological recognition of antigenic and/or structural incompatibility. The process is referred to as *allogeneic inhibition* or *syngeneic preference*, and was first detected by the observation that certain tumours grew less readily in F₁ hybrids than in the parental strain.

5.3 Mechanisms of escape

The most obvious feature of malignant tumours is that, as a general rule, unless they can be excised completely or destroyed by radiotherapy at an early stage they grow, spread, and kill the host, especially when metastasis occurs. How can this happen if indeed the tumour possesses specific antigens which are absent from the normal tissues of the host? Various answers to this question have been suggested.

Tumours may conceivably escape destruction as the result of a combination of various immunological mechanisms, among which may be mentioned :

- (1) non-specific depression of immunological responsiveness in the host;
- (2) loss of specific antigens;
- (3) induction of specific immunological tolerance to the tumour antigens in the host;
- (4) serological enhancement; and

(5) imbalance between the growth potential of the tumour and the immunological mechanisms.

5.4 Serological enhancement of tumour growth

Immunological enhancement of tumours may be defined as the inhibition of tumour rejection by specific antibody. This paradoxical situation may be manifested as a transient, or more severely as a marked, acceleration of the growth of the tumour graft till the death of the host. Enhancement can be observed after both active and passive immunization. It is now generally agreed that specific antibodies against the graft, whether synthesized by the host or administered to it, are responsible for this phenomenon. Active enhancement is best obtained after immunization with killed tissue or saline tissue extract; immunization with live tissue induces a stronger and more persistent rejection stage. In the course of immunization, a stage of rejection is observed first, and this in time is succeeded by enhancement, especially following immunization with dead tissue. The dual response of rejection and enhancement may reflect the cellular and humoral aspects of the immune response. The antibody nature of enhancement is best demonstrated by the effect of passively administered antibodies. Enhancement is obtained with specific antisera injected shortly before tumour grafting and in some host-tumour combinations for as long as ten days after tumour grafting. Only very minute amounts of the appropriate sera are needed.

Enhancement phenomena are not limited to the growth of allogeneic tumours. Antibodies with enhancing activity have also been found in animals bearing syngeneic chemical-carcinogen-induced tumours and in animals immunized against these tumours or against virus-induced tumours. An important point is that sera from animals resistant both to carcinogen- and virus-induced tumours may nevertheless be shown to possess enhancing activity when passively transferred to another syngeneic recipient. The enhancing activity depends on the dose of serum used: higher doses are more effective in enhancement, while smaller doses can produce resistance.

Addition of specific iso-antiserum to reaction mixtures of immune lymphoid cells and target cells led to an almost total inhibition of the cytotoxic action of the lymphoid cells in some systems, while the presence of antiserum in control suspensions containing normal spleen cells had no effect on the target cells. Animals exposed to oncogenic viruses develop enhancing antibody in their blood. The presence of this antibody has been demonstrated in experiments in which tumours induced by the same virus were injected into animals mixed with serum obtained from virus-exposed animals and normal animals. The presence of enhancing antibody caused an accelerated growth of the transplanted tumour tissue.

The mechanism of enhancement probably involves two processes :

1. The enhancing antibody may interfere with the rejection of the graft by combining with the antigen and thus inhibiting the immune response. The suppression of specific antibody synthesis or of the development of sensitized lymphocytes by passively administered antibodies has been demonstrated in numerous systems—protein antigens, sheep red cells, and transplantation antigens. Recent studies have further shown that such effects may depend primarily on the affinities of the antibodies administered. Higher-affinity antibodies synthesized late in immunization are much more effective in inhibiting antibody synthesis than low-affinity early antibodies. Antibody types that have been shown to be able to suppress antibody synthesis are 19S, 7S, pepsin-digested 5S (the F(ab')₂ fragment) but not monovalent digested antibodies.

2. The antibody may combine with the graft antigens and protect the graft against the damaging effects of specifically sensitized cells, thereby permitting the survival of the tumour which as an organized tissue is relatively insensitive to cytotoxic antibody. Enhancement can be affected by the individual conditions of the graft, a solid graft being much more susceptible to this type of enhancement.

It is also possible that enhancement may depend upon the type of immunoglobulin synthesized, but the characterization of enhancing antibodies has not yet been definitely achieved. The recent characterization of five classes of mouse immunoglobulins should enable rapid advances to be made in the near future. A recent study of this problem involved fractionation of mouse antisera by zone electrophoresis and assay of the various fractions for cytotoxic and enhancing activity. The slowly migrating fractions containing most of the γ_2 immunoglobulins had the bulk of the cytotoxic activity and no enhancing activity. Enhancing activity was found primarily in the rapidly migrating fractions containing γ_1 anaphylactic immunoglobulins. Other studies with carcinogen-induced tumours have shown that mouse 7S antibodies are more active than 19S antibodies in producing enhancement, while 19S antibodies are more efficient in transferring resistance.

Recommendation for further study

To enable immunoglobulins with cytotoxic properties to be separated from those with enhancing or protective properties, it is recommended that further attempts be made to characterize the functional activity of the various immunoglobulins in *several* animal species, since the classes of immunoglobulin in different species cannot be considered to be identical or to have similar properties.

6. IMMUNE RESPONSE TO HUMAN TUMOURS

Although there is no proof that all human tumours possess specific antigens, there are circumstantial grounds for believing that this is true of at least some tumours.

(1) Some human tumours (e.g., carcinoma of the bronchus and some bladder carcinomas) are likely to be of chemical origin and others may be of viral origin. It would be altogether extraordinary if tumours of such origin were devoid of specific antigens in man.

(2) The occasional spontaneous regression of human tumours and the reappearance of tumours after a period of latency that may last for years point to the existence of mechanisms that may inhibit the growth of human tumours. In some, endocrine mechanisms appear to be responsible. When there is no evidence of this, a possibility to be considered is that the mechanisms considered are immunological in nature.

(3) The hyperplastic reaction often seen in lymph nodes related to tumours without metastases is similar to that seen in nodes responding to an immunological stimulus.

(4) Lymphoid infiltration of other human tumours has been reported as being associated with a favourable prognosis.

(5) The incidence of most tumours is greatest at an age when immunological responsiveness has begun to fall off, and of some tumours greater than normal in patients with adult-type hypogammaglobulinaemia.

Knowledge of cellular and humoral mechanisms in man and of the heterogeneity of human immunoglobulins suggests that the same immune mechanisms as influence the origin and growth of tumours in experimental animals could do so in man, in respect of both the inhibition and the enhancement of the tumours.

The only available data concerning enhancement are observations on six patients with chorionepithelioma, which is a unique example of a human allogeneic tumour. Four of these patients had antibodies in their sera that were able to agglutinate the leukocytes of their respective husbands; two with high and two with low titres. The sera of the remaining two patients did not contain antibodies capable of agglutinating their husbands' leukocytes. The behaviour of skin grafts from the respective husbands was correlated with the presence, but not the titre, of these antibodies. The patients without serum antibodies rejected a graft of their husband's skin without vascularizing it. Three other patients kept their respective grafts longer than normally would be expected in non-immunized recipients,

and one longer than is normally observed in a previously immunized recipient. The behaviour of the grafts was also correlated with the prognosis. Only those patients who rejected their husband's skin graft in normal fashion eventually survived.

Although these observations are not conclusive, they offer suggestive evidence for the phenomenon of enhancement in human neoplasms. Since chorionepitheliomas are allogeneic tumours (of foetal origin), they present an unusually appropriate situation for study of both enhancement and immune rejection of a human cancer.

7. IMMUNE STATUS OF TUMOUR-BEARING ANIMALS AND MAN

Few studies have been made on the immune status of animals bearing primary or transplanted tumours. In the special cases of mice with lymphoid leukaemia and reticulum cell sarcomas, severe depression has been observed in the immune response to several antigens. In studies on animals with tumours of other types, the depression of the immune response appears to be less severe.

Many cancer patients have an impairment of the immune response. This is of immediate clinical interest because it may result in increased susceptibility to infections, and it may be of even greater fundamental significance because of the possibility that it influences the progression of the cancer *per se*.

A distinction should be made between primary neoplasms of the reticulo-endothelial system (RES) such as leukaemia, lymphoma, and multiple myeloma, in which there is a primary neoplastic abnormality of tissues directly involved in the immune responses, and all other forms of cancer (epidermoid carcinomas, adenocarcinomas, and non-RES sarcomas) in which the immunologically reactive tissues are not themselves neoplastic. One must also attempt to distinguish the effects of cancer therapy, which may be immunosuppressive, and the consequences of general debility from the effects attributable to cancer *per se*.

The ability of the cancer patient to produce circulating antibody in response to a primary antigenic stimulus is depressed in many patients with RES neoplasms, but is not demonstrably affected in other types of cancer. However, there is as yet little information on the development of different types of immunoglobulins, so the possibility of a significant abnormality in the biological quality of the antibody produced cannot be excluded.

Serum complement, as measured by total haemolytic activity and by titration of the second component of complement ($C'2$), is usually

normal in cancer patients, regardless of the type of disease or of treatment, although very low levels of C'_2 have been observed in a few moribund lymphoma patients. Serum properdin activity has been studied in many laboratories by a variety of methods and has consistently been found to be depressed or undetectable in many cancer patients.

Cell-associated types of immune response appear to be significantly depressed in many types of cancer. Delayed hypersensitivity responses to tuberculin and other microbial antigens and to chemical allergens such as dinitrofluorobenzene are frequently depressed, not only in Hodgkin's disease and other lymphomas but in all types of cancer. The most striking defect is delayed rejection of homografts. Delayed rejection of skin homografts is most obvious in patients with lymphoma receiving chemotherapy, but there appears also to be delayed rejection unrelated to treatment in patients with carcinoma. Impaired rejection has also been demonstrated by the use of subcutaneous homografts of tissue-cultured cancer cell lines, which enable results to be reproduced in a way that cannot be achieved with other cell sources. Many patients with advanced cancer of non-lymphomatous type (but also with RES cancers) who are not receiving treatment that happens to be immunosuppressive show a definite delay in rejection of such homografts. Studies in patients of similar age and comparable debility, but with diseases other than cancer, indicate that this impairment of cell-associated types of immune response (delayed hypersensitivity and homograft rejection) is not attributable to either age or debility *per se*. There is, however, no reason to assume that it is specific for cancer; a similar deficiency is known to occur in patients with uraemia. Furthermore, although the defect occurs without any iatrogenic cause, there is no doubt that immunosuppressive medication and irradiation contribute to the defect in many cancer patients.

The intradermal lymphocyte transfer test is a special type of homograft in which, in addition to the host-versus-graft reaction which occurs with all types of homograft, a reaction of the grafted cells against the host is possible. Studies to date using blood lymphocytes from healthy donors show no convincing differences between the response of healthy persons that of patients with cancer (including Hodgkin's disease and other lymphomas); however, when the test is performed using lymphocytes from patients with Hodgkin's disease, there is less reaction in either normal recipients or patients than when lymphocytes from normal donors are used. This suggests an abnormality of the lymphocytes of the Hodgkin's patients. It has also been reported that skin grafts to cancer patients from donors with cancer are less rapidly rejected than skin homografts from healthy donors.

Still another indication of inadequacy in the responsiveness of cells involved in host immune reactions is impaired mobilization of macrophages in response to skin abrasion in the Rebeck test.

These abnormalities in the immune response have been observed only in patients with advanced disease—usually with massive tumours or widespread metastases and suffering from considerable cachexia—but, even so, these patients do not all have abnormal responses. The frequency and degree of the abnormalities seem to correlate directly with the extent and aggressiveness of the cancer. This suggests that the immunological deficiencies are effects of cancer. However, the methods of studying these immune responses in patients are not very sensitive and the range of normal variation is wide, so that only major deviations can be detected. It cannot be excluded that other more subtle or less complete abnormalities may occur much earlier in these patients and influence the early development of the cancer itself.

8. IMMUNOTHERAPY

Throughout this report an attempt has been made to draw parallels between animal and human cancer and urge that clinical trials should be devised on the basis of appropriate experimental data. It should be recognized, however, that cancer in man often differs considerably in its biological behaviour and its response to therapeutic measures from the usual experimental models of cancer in rodents. This makes a continuing search for additional and more appropriate models necessary and may justify clinical therapeutic trials for which a firm basis of animal studies has not yet been achieved.

8.1 Non-specific stimulation

8.1.1. *General stimulation of defence mechanisms in animals*

The possibility that immune capacity and general resistance can be enhanced by immunologically non-specific means so as to inhibit the growth of tumours has been investigated in several laboratories. Treatment of mice and rats with appropriate doses of BCG, *Corynebacterium parvum*, and zymosan increases resistance to bacterial and viral infections and the frequency of rejection of allogeneic tumours. Such mice also show an increased immune response to various antigens and a hyperactive and hyperplastic reticuloendothelial system.

The most interesting results were obtained with BCG and *C. parvum*, which delayed the appearance of viral leukaemia and chemically-induced sarcomas in mice and retarded the development of syngeneic tumour transplants in some studies.

The exact mechanism by which the increase in natural resistance caused by BCG or other agents acting similarly affects tumour development is not clear. While many of the effects that lead to enhanced resist-

ance to bacterial infection may be involved, it seems probable that the alteration of tumour growth is a result of a more vigorous or accelerated capacity to reject tissues containing antigens not present in the host. It may also be possible that an animal treated with the bacterial products effects a more vigorous rejection of the tumour grafts because of factors that are not immunologically specific. Such a factor could be the increased number of hyperactive macrophage and leukocyte elements involved in the inflammatory reaction triggered by the specifically sensitized lymphoid cells.

Recommendation for further study

Further attempts should be made to characterize biochemically the factors in mycobacteria and *C. parvum* that stimulate increased resistance in animals to infections and tumours.

8.1.2 *General stimulation of defence mechanisms in humans*

Clinical trials of BCG as a stimulant of defence mechanisms have been initiated but cannot yet be evaluated. BCG treatment of patients with acute lymphoblastic leukaemia (in which remission had been obtained by intensive chemotherapy) has coincided with long persistence of this remission, despite the absence of treatment with pharmacological agents.

Complete Freund's adjuvants mixed with autologous tumour preparations have been injected in cancer patients, resulting in severe localized necrosis at the site of injection, but the results have yet to be evaluated. It should be pointed out that the injection of complete Freund's adjuvant produces prolonged severe local granulomatous reactions.

8.2 Active Immunization

8.2.1. *Animals*

The observation that an inoculum of small numbers of tumour cells containing tumour-specific antigens may sometimes develop into a tumour while one containing a larger number of cells is rejected suggests that, at least in certain instances, a tumour may not induce the maximum possible immune response during its initial growth. This provides a rationale for the use of autologous implants of tumour tissue.

Viable tumour cells may grow on injection as autografts, and before they can be used to stimulate the host response they must either be exposed to treatment to render them incapable of division but not to cause lysis or they must subsequently be removed. Exposure to high doses of X-rays meets these requirements for some tumour cells, though some leukaemic cells break up and lose their antigenicity (i.e., fail to induce immunity) when irradiated with doses sufficient to prevent growth. There are indica-

tions that treatment of cells with certain chemicals (iodoacetate formaldehyde) may preserve their antigenicity. Decrease in the activity of normal transplantation antigens has been reported after exposure to X-rays, and it therefore seems possible that the immunizing capacity of tumour tissue may be reduced both by irradiation and by chemical treatment, particularly as weak antigens are probably involved.

Irradiated autologous tumour tissue produced no detectable effect on the growth rate of chemically induced primary sarcoma in mice and rats. The failure of an irradiated autograft to influence the growth of an established primary tumour in the absence of other treatment is not unexpected; even if the host was not optimally immunized against the tumour, the induction of the maximum response that can be induced by immunization would, on the basis of the experience gained from transplantation experiments, lead to the rejection of only a small fraction of the total tumour mass. Immunization with an autograft is only likely to show an effect once the tumour mass has been greatly reduced by other means. The response of some primary tumours to local irradiation with X-rays was increased by implanting autochthonous tumour biopsy material that had been rendered non-viable by exposure to X-rays. Although there is no direct proof that the irradiated autograft increased the host response mediated by the tumour-specific antigens, this seems to provide the most plausible explanation for its radiosensitizing action.

The papilloma induced in rabbits by the Shope virus is of great immunological interest because spontaneous and complete regressions occur in an appreciable proportion of the animals, in striking contrast to chemically induced tumours in rodents, which do not regress spontaneously. Autologous grafts consisting of intact papilloma cells—but not of homogenates, of cells disrupted by freezing, or of non-tumour tissue—increased the rate of complete regression from 20% to about 60%. The addition as an adjuvant of *Bordetella pertussis* cells did not improve the effectiveness of the procedure.

For active immunization, it is of great importance to choose appropriate methods of immunization. Comparison of different procedures producing immunity against subsequent challenge with syngeneic tumour cells suggests that the most effective method is ligation of the vascular supply of a growing syngeneic tumour, followed by the implantation of allogeneic tumour (when the tumour antigen is common) by means of X-irradiated cells. The least effective is the injection of cell homogenates. Another important factor is the dose of antigen. Too small and too large a dose may both be ineffective, particularly in the case of X-irradiated cells. In addition, a state of specifically decreased immunological reactivity that manifests itself by an increased growth rate of tumour cells can be produced by immunization with large amounts of X-irradiated cells. Either tolerance or enhancement or both may play a part in these pheno-

mena. There may be a risk, particularly when active immunization is carried out in tumour-bearing animals, of decreasing the immune response to the tumour through overloading the host with added antigen. In general, the effectiveness of active immunization is dependent on the balance between the number of antigenic tumour cells present in the organism and the level of specific immunity that can be stimulated. The appearance of less antigenic or non-antigenic tumour cell variants in the tumour cell population, as well as the masking of antigens responsible for immunity with antibodies, may alter the effectiveness of active immunization.

Adult animals infected with certain oncogenic viruses such as polyoma, SV40, Rous sarcoma, mouse leukaemia, and adenovirus become resistant to subsequent challenge with tumours induced by the same viruses. This immunity is probably related to the development of TSTA in the infected animals. The production of infectious virus by the cell may not be the necessary prerequisite for synthesis of the transplantation antigen.

Active immunization can also interfere with an already initiated process of malignant transformation in newborn animals infected with SV40 or adenovirus 12. Hamsters can be immunized during the latent period either with large doses of the same virus or with live or irradiated syngeneic cells previously infected or transformed with the same virus but free of the virus. Of special interest for possible future application is the protection of hamsters infected with SV 40 by *human* cells transformed by SV40.

The effectiveness of immunization requires the absence from the tumour cell population of variants insusceptible to immunological influences. In the original tumour cell population the proportion of cell variants will be much smaller than in large tumours.

The prevention of virus-induced tumours is unsuccessful if animals retain their susceptibility to the oncogenic action of virus during adult life, as is the case for some RNA viruses such as those of leukaemia in mice and of Rous sarcoma in chickens. The introduction of antigens responsible for immunity in adult life interferes with the oncogenic activity of the particular virus. As some transplantation antigens appear in virus-infected cells before the completion of the malignant transformation, it would be of interest to study whether such transformation can be interrupted by suitable chemical inhibitors at the stage when the transplantation antigens are already synthesized but the cancer cells have not yet begun to proliferate.

8.2.2 *Humans*

It seems clear from both experimental and clinical evidence that the efficacy of the immune response to cancer is rarely if ever sufficient to reverse the course of advanced cancer, but that it may be sufficient

to slow the rate of cell proliferation and prevent the implantation of relatively small numbers of disseminated cells. Certainly the numerous attempts that have been made in the past seventy years to treat patients with advanced cancer by administration of autologous or homologous cancer preparations (or antisera) give no reason for hope that further use of these same methods without modifications would be of value. The clinical situation in which it seems likely that immunological methods might be therapeutically effective is when there is a minimum of residual disease. Situations that might be considered as "minimal residual cancer" would include, for example, patients with acute leukaemia in whom complete haematological remission has been obtained by chemotherapy and patients whose cancer has been apparently completely removed by surgery but in whom the statistical probability of recurrence is high. An example of the latter is epidermoid lung cancer, of which the recurrence rate after all demonstrable tumour tissue has been resected is about 75%.

It would seem at the present time that any therapeutic procedure for human cancer that is based on the concept of cancer-specific antigens should utilize the patient's own cancer cells as the source of antigen, since studies in experimental animals indicate that, except for virus-induced tumours, cancer antigens are unique to the individual's cancer. There are no adequate data from studies of human cancer to indicate the best method of preparing cancer tissue for experimental use as immunizing antigen. The only data on cancer-specific antigens are from studies of experimental animal tumours, while the only data on human cell antigens are from the homotransplantation or heterotransplantation of cancer cells, the evidence that such data relate to cancer antigens rather than to normal cell antigens not being conclusive.

The following possibilities are being or have been explored :

(1) The transplantation of live cancer cells followed by excision of the resulting tumour nodule probably gives the best results in the immunization of syngeneic recipients of mouse tumours. For human cancer this method seems inappropriate, for it is illogical if residual cancer is known to exist elsewhere and, if used in a patient with no known residual cancer, it creates a risk of dissemination that is not justified for a procedure of unproved value.

(2) A possible extension of this concept is the destruction of naturally occurring primary or metastatic tumours *in situ*, when for valid clinical reasons they cannot be excised completely. This is often clinically feasible by such methods as irradiation, cautery, or cryosurgery, but the only evidence that it may have an immunological effect is circumstantial.

(3) Living but non-propagating cells can be produced by irradiation with X-rays, gamma rays, or ultra-violet rays. Animal tumour cells

treated in this way have been quite immunogenic. This method prevents antigen loss through autolysis, presumably eliminates the risk of auto-transplantation of the cancer, and may cause slight modification of the chemical configuration of antigens.

(4) Frozen whole cells are good antigens in heterologous recipients but lose much of their ability to produce homograft immunity (unless they are so treated as to preserve their viability, in which case they have the same advantages and disadvantages as living cells). Since the cancer-specific antigens may be among those lost in freeze storage, there is a risk that the very antigens an attempt is being made to study will be lost.

(5) Cells disrupted by homogenization or alternate freezing and thawing retain immunogenic homotransplantation antigens and will react with iso-antibodies *in vitro* if used within a few hours, but their *in vitro* reactivity is lost after a few hours of storage at 4° or at -70°C as crude extract.

(6) Cell fractions and extracts would be subject to autolytic inactivation unless enzyme activity is inhibited.

(7) Deliberate attempts to modify antigens to make them more immunogenic without loss of cancer specificity are currently popular. Methods under study in various laboratories include exposure to ultra-violet rays and X-rays, hapten addition, and the forming of complexes with foreign proteins.

(8) The method of antigen administration might be altered to achieve a heightened response by the use of adjuvants, non-specific stimulants, thymic humoral factor, or more effective routes and schedules of administration. The only basis on which a choice of such procedures could be made would be studies of syngeneic animal tumours.

Since the immune response to specific antigens is frequently depressed in patients with advanced cancer, the probability that cancer patients will respond adequately to active immunization procedures is diminished. This implies that attempts to treat cancer by active immunization are unlikely to succeed unless they are tried in patients who have not yet entered the stage of disease where they have gross impairment of the response.

Although there is no direct evidence to support it, the theory that the patient with cancer is tolerant towards antigens of his own cancer cells is an attractive one. One possible method of breaking this hypothetical tolerance might be to immunize with autogenous cancer antigens that have been altered so as to be recognized by the recipient as foreign, but so subtly altered that the resulting immune response is to the antigen of his growing cancer cells. That such an immune response can be achieved by subtle alteration of the structure of autologous antigens has been

demonstrated in model systems by conjugation of simple haptens with protein molecules and perhaps by ultra-violet irradiation. The studies to date with modified tumour-cell antigens have not been adequate to prove that this method is applicable to cell antigens, but it seems to be an approach that offers the possibility of active immunization if a state akin to immune tolerance does in fact exist in the patient with cancer.

The possibilities of overcoming tolerance to cancer antigens by cell transfer of immunity are discussed in section 8.4.

The untoward auto-immune reactions that might result from injection of autologous cancer preparations are insignificant in comparison with the actual effects of progressive cancer. However, for the patient with early cancer or the patient who may in fact be cured of his cancer, the risks of auto-immune complications cannot be so confidently dismissed. Active immunization of mice against syngeneic tumour tissue has not been shown to produce such complications.

The entire concept of cancer prevention by immunological methods hinges on the fundamental questions of whether host defence mechanisms against cancer really exist and, if so whether their efficacy can be increased. These questions cannot be answered for human cancer, but to a limited extent can be answered affirmatively for experimental animal cancer, and the assumption of the biological similarity of man and animals remains basic to medical research.

If at any time in the future the etiological factors in human cancer become known and prophylactic studies become feasible, clearly such studies of cancer immunoprophylaxis would require a long observation period. It is evident from clinical observation that cancer in some individuals has a preclinical course lasting five or more years. If oncogenesis is considered to start in early life then immunization in adult life may be too late. Thus studies of cancer prophylaxis in humans would have to be planned for periods of at least 10 to 20 years, and preferably for the whole span of human life. Such a project would require a well-established organization to assure continuation of the project beyond the lifetime of the initial investigators.

The most hopeful possibility for cancer immunoprophylaxis is identification of the common causative agents which are antigenic and from which immunizing vaccines can be prepared. Viruses offer the best hope for this approach, and this should encourage the continued search for human oncogenic viruses and the further investigation of known viruses to study the possibility that they might have an oncogenic effect in man. We can no longer ignore the possibility that oncogenic viruses of other species may also affect man or that "non-oncogenic viruses" may increase the oncogenicity of other agents (chemical, viral, hormonal, etc.). In this latter situation, the prevention of infection by the "non-oncogenic" virus would lessen the incidence of cancer. The possibility

that ordinary viruses may play such a role in oncogenesis might justify a long-term study of the effect on cancer incidence of immunization against a broad range of common human viruses by killed virus vaccines.

The other hypothetical source of human cancer-specific antigens that might be used in immunoprophylaxis is human cancer cells. It is conceivable, by analogy with experimental animal tumours, that various human cancers might contain common TSTA, especially if the cancers were caused by viruses.

8.3 Serum transfer

8.3.1 *Animals*

Attempts to use antisera specifically against tumours have been based on two modes of action of the sera : their direct cytotoxic action and their transport of a cytotoxic agent.

Specific cytotoxic sera are regularly obtained from syngeneic animals immunized with lymphoid tumours. In the case of other tumours they can be obtained only by the most effective immunizing procedures and only in certain instances. It seems unlikely that they could greatly reduce the growth of established tumours. They might on the contrary have an enhancing effect.

Homologous antisera were shown to be of value in the treatment of syngeneic mouse leukaemia, provided that the number of cells present was relatively small. In order to obtain specific homologous or heterologous sera, the immune reaction against normal individual antigens needs to be eliminated. Making use of the phenomenon of tolerance is a promising way of achieving this. The preparation of purified antibodies with cytotoxic activity and study of the best way of using them may be of importance.

The difficulties encountered in the passive immunotherapy of cancer apply also to the use of antibodies linked with radioisotopes and cytostatic agents. If it were possible to concentrate radioactive isotopes and drugs specifically in tumour cells, their toxic side-effects could be greatly diminished. There are only a few studies showing a preferential localization of antibodies on tumours, but highly specialized techniques have been successfully used in animal experimentation. Assuming that a selective localization of antibodies against tumours could be obtained, conjugates of cytostatic agents with them could potentially be used. However, they present the disadvantage of being recognized by the organism as foreign substances and might therefore be taken up more rapidly by the reticulo-endothelial system. Furthermore, the possibility cannot be excluded that the drug-protein conjugates may be immunogenic and elicit the formation of antibodies against the drug. However, since the antibodies may

react with drug-sensitized cancer cells to cause lysis, this effect could intensify any lethal effect.

Another difficulty is that certain drugs have to be released from a carrier protein before they display antimitotic activity. For this reason bonds must be chosen between drug and antibody that can be split by enzymes present in the tumour. Obviously the success of such a therapeutic approach will depend on the outcome of two opposing factors: the rapidity with which the conjugate is fixed to the antigen and the speed at which intravascular hydrolysis takes place.

Boron compounds attached to antibody globulins have been considered, in view of their ability to emit alpha rays when bombarded with neutrons. So also have antibodies against fibrin labelled with radioactive iodine, as a means of delivering radioactivity to fast-growing transplanted tumours. As yet the results obtained have been insubstantial.

8.3.2 *Passive immunotherapy of human cancer*

The successful use of immune sera or human immunoglobulin in the treatment of infectious disease has stimulated many investigators to try to treat cancer by similar means. Recent investigations in patients with chorionepithelioma have been discussed in section 6. Here other types of tumour are considered.

Heterospecific sera or immunoglobulin. Heterospecific (usually horse) immune serum has been used by various workers. Their work is open to the serious criticism that the observations were not properly controlled and their claims that the patients benefited unconvincing. Moreover, many of the investigators believed that they were using sera specifically active against tumour tissue and inactive against normal tissues, although there is no convincing evidence to support this belief and often quite good evidence to the contrary.

It would be unwise to assume that passive immunization with heterospecific serum will never play any part in the treatment of cancer, but the potential dangers of this type of therapy, including nephritis and serum sickness, and the possibility of the enhancement of tumour growth seem at present disproportionately great in comparison with the meagre possibility of benefit to the patient.

Homologous sera and immunoglobulin. Large quantities of immunoglobulins have been injected into patients suffering from various forms of cancer. In several there were unfortunate side-effects and in none was improvement observed. Only very minimal improvement has been seen in patients with acute leukaemia receiving immunoglobulin therapy.

In one study immunoglobulin was prepared from the serum of a normal subject who had previously received leukaemic blood. The serum

contained antibodies active against the cells from the donor. The immunoglobulin was administered to a patient with chronic myeloid leukaemia without appreciable beneficial effect, except for transitory retardation in the rise of the leukocyte count.

The use of the serum or immunoglobulin of patients in periods of cancer remission has also been suggested. This has been tried in a few patients with Burkitt's tumour or melanoma, but the results do not yet permit an evaluation of the effectiveness of the procedure.

8.4 Cell transfer of immunity

8.4.1 *Cell transfer in animals*

Possible approaches to the use of immunologically reactive cells include the use of: cells from immunized or non-immunized donors; cells from the spleen, lymph nodes, lymphatic duct, peritoneum, or bone marrow; and syngeneic or non-syngeneic donors. The use of non-syngeneic immunocompetent cells introduces the possibility of a continuing reaction of the grafted cells against the recipient (graft-versus-host reaction) if they persist.

Unsensitized lymphocytes might in theory affect tumour cells in two ways. (1) If the tumour-bearing animal is suitably pretreated they can take over its haematopoietic system and effect a general graft-versus-host reaction which will be inimical to the growth of the tumour. (2) If brought into close contact with certain types of tumour cells, they can kill them. The existence of the latter reaction was realized when it was observed that certain tumours grow less well in F₁ hybrids than in the parental strain, and the phenomenon may have similarities to the allergic death described by Gorer. This property of lymphoid cells, the capacity to inhibit allogeneic cells non-specifically, has not so far been exploited for immunotherapy.

The growth of established primary or syngeneic tumours could not be slowed down by the injection of foreign non-immune lymphoid cells under conditions where there was no graft-versus-host reaction. However, when foreign lymphocytes are injected into a host in which a graft-versus-host reaction occurs, the growth of some established syngeneic tumours is significantly retarded. The most striking results have been obtained with murine leukaemias. Allogeneic haematopoietic grafts had an anti-leukaemia effect in mice transplanted with small numbers of leukaemic cells, and some inhibitory effect has been obtained in virus-induced leukaemia. It proved difficult to take advantage of this immunotherapeutic effect because of the graft-versus-host reaction. However, the use of cytotoxic drugs made it possible to limit this reaction, and these

agents can probably have the further effect of being directly anti-leukaemic. Other experiments with allogeneic cells have shown an anti-tumour effect against ascitic neoplasms. These observations formed the basis for a clinical investigation (see section 8.4.2).

The other approach is to use lymphoid cells of syngeneic, allogeneic, and heterogenic origin from donors immunized against the tumour. Lymphocytes derived from donors not syngeneic with the tumour-bearing host have been shown to be active against both the TSTA and the normal transplantation antigens of the host. But it seems likely that the anti-tumour action *in vivo* is mainly due to the anti-TSTA effect. Only in this way can the specificity of the *in vivo* anti-tumour action be explained; the lymphocytes are effective only against the tumour used for immunization. It is surprising that a genetically dissimilar animal (particularly when there is a species difference) detects and reacts significantly against the minor antigenic component specific to the tumour.

The results obtained in different tumour systems suggest that immune lymphocytes can act directly against the tumour cells *in vivo*—this might be called passive cellular immunity since the host does not participate—, as well as by an indirect mechanism. It is possible that different populations of lymphocytes may be involved in the different processes. Three mechanisms may be envisaged by which lymphocytes interfere directly with tumour growth—by cell-with-cell interaction leading to “mutual suicide”, by release or *in situ* formation of cytotoxic antibody, or by a process akin to the delayed hypersensitivity reaction. That immune lymphocytes can interfere with the growth of target cells by cell contact in spite of a major histocompatibility barrier has been convincingly shown in tissue culture, and there are indications that this also occurs when the immune response is directed against minor transplantation antigens. There are no experimental data indicating that the antitumour action of lymphocytes is brought about by circulating antibodies released from the injected cells, this indeed being inconsistent with a great deal of data on the rejection of homografts. Serum from animals immunized with a syngeneic tumour facilitated (i.e., enhanced) the growth of the tumour on transplantation, whereas lymphoid cells from the same animals prevented the establishment of a tumour graft.

The phagocytic action of macrophages on tumour cells has to be considered as an alternative mechanism for the destruction of antigenic malignant cells. This action, which has been demonstrated *in vitro* with macrophages from sensitized animals, is believed to be mediated by antibody acting as an opsonin. This observation, together with the demonstration that some types of antibody are capable of adsorption to macrophages, has shown that opsonizing antibodies may in some cases react with macrophages first and confer on these cells the properties of sensitized cells.

Cell transfer in which the immune lymphocytes colonize the lymphoid organs of the host and produce antibody (free or cell-associated) has been demonstrated for a variety of antigenic stimuli. An essential requirement for prolonged action is that the injected lymphocytes should be genetically compatible with the host or that the host's immune system should have been inactivated in some way. In healthy recipients allogeneic or heterologous lymphocytes either show no immunological activity such as the formation of antibody, or, if they do, cease to do so within a few days after grafting.

Cell transfer in completely syngeneic systems was found in most cases to be ineffective. Only in combination with partial tumour removal by surgery or irradiation did immune cells give evidence of inhibiting tumour growth.

In experiments in rats in which allogeneic and heterogeneic lymphocytes derived either from the thoracic duct or by direct drainage of the efferent lymphatics of a stimulated node from suitably immunized donors have been shown to retard the growth of and, very occasionally, cure chemically-induced primary fibrosarcomas, the action requires the participation of the host but yet does not appear to be due to an actively functioning graft of the foreign lymphoid tissue. The findings are consistent with the view that there is a process by which the injected lymphocytes initiate an immune reaction which is brought to completion by the recipient's own immune system. If an RNA protein complex is necessary to initiate antibody production, there must be some means of conveying such a complex in a protected form from the site of antigen localization to the lymphoid system as a whole. There is obviously considerable scope for examining the specific antitumour effect of cell-free fractions of lymphocytes derived from animals immunized with a portion of the tumour to be treated.

8.4.2 *Cell transfer in humans*

Clinical trials of immunotherapy using the transfer of various types of cells have established the overall safety of these procedures and some beneficial results seem to have been obtained, but no cures have been achieved.

Experimental studies in animals have indicated that cells from immunized donors are more effective in inhibiting tumour growth than those from non-immunized donors. However, this procedure may present special hazards in man since it involves the injection of cancer tissue into other individuals.

Clinical trials of the transfer of immunity by cells have been of six types.

(1) Large numbers of leukocytes from patients with chronic myeloid leukaemia have been transferred to patients with acute leukaemia resistant

to all conventional forms of therapy. This treatment has been followed by complete but short remissions. A marked correlation between the antileukaemic effects and the clinical or histological evidence of graft-versus-host reactions indicates that the effect of the treatment was due to the action of immunologically competent donor cells.

(2) A few clinical trials have been conducted of leukocyte transfusion from cancer patients either cured or in remission to patients with the same type of cancer. The preliminary results were sufficiently encouraging to justify continuing these studies.

(3) A few cancer patients have been treated with allogenic spleen cells injected intravenously or intraperitoneally. In every case favourable changes were noted in the patient's symptoms, the physical signs, or the findings in the final biopsy, but there was no evidence of cure.

(4) Allogenic bone marrow grafts have been attempted after whole-body irradiation in patients with acute lymphoblastic leukaemia—a form of treatment that has been shown to be successful in experimental mouse leukaemias. The cell grafts often grew, but serious problems—particularly a special susceptibility to infection with herpes and varicella—arose as a consequence of the secondary syndrome that occurred in these patients because of the graft-versus-host reaction. Patients who survived the secondary syndrome showed longer remissions than would normally be expected without treatment, thus suggesting an antileukaemic effect from this form of therapy. One patient who died of an intercurrent infection after twenty months showed no histological evidence of leukaemia at autopsy.

(5) Some patients with a carcinomatous pleural effusion received an intrapleural injection of rabbit lymphoid cells from animals previously immunized with the corresponding tumour cells.

(6) A few experiments have been performed utilizing thoracic duct lymphocytes from persons immunized against the cancer tissue of the patient to be treated. In some cases the immunized donors of the lymphocytes were other cancer patients, in others healthy persons. Evaluation of the efficacy or complications of this approach is not yet possible.

These clinical trials can be considered as being of two main classes. One involves injection of cells without any attempt to obtain a persisting graft. Previous treatment of the patient by immunosuppressive measures is not required, but the results are very limited even in leukaemia, the remissions obtained being very short. The other involves attempts to ensure survival of the grafted cells, which appear to be far more effective but have the drawback of being associated with a severe secondary syndrome due to the graft-versus-host reaction.

9. SPONTANEOUS TUMOURS OF ANIMALS

Spontaneous tumours of domesticated animals could provide very useful models for experimental studies of immunotherapy, especially as some forms frequently metastasize and others show regression followed by immunity to re-exposure. Bovine lymphosarcoma, one of these tumours, occurs in several countries in Europe, North America, and elsewhere and is transmitted vertically in infected herds. The venereal tumour of dogs is readily transferable to healthy animals by inoculation of material containing viable tumour cells and it shows spontaneous regression followed by immunity to reinoculation. Metastases are not common, but they can be produced experimentally in the conjunctiva and other visible mucous membranes. The tumour cells of both these neoplasms can also be grown in culture for *in vitro* immunological studies.

Other spontaneous tumours of animals that may prove useful are the mammary tumour and osteogenic sarcoma of the dog, the urinary bladder cancer of cattle, and various animal melanomas. The horn cancer of Indian cattle (restricted to South-East Asia) is a squamous cell cancer that frequently metastasizes.

Dog and cat leukaemias (lymphomas) have been shown to be transmitted by cell-free filtrates, and in cat leukaemia viral particles resembling those of mouse leukaemia have been demonstrated in electron micrographs.

WHO is supporting collaborative studies on the spontaneous neoplasms of domesticated animals and has set up an International Reference Centre for Comparative Oncology (at the Armed Forces Institute of Pathology, Washington, D.C., USA) and three collaborating centres¹. These studies could be enlarged to include immunotherapeutic trials.

10. CONCLUSION

The main objective of cancer research is to achieve better methods of treating and/or preventing cancer in man. The Group believes that immunological methods are likely to contribute significantly to this end. It therefore recommends that experimental and, where appropriate, clinical investigations should be encouraged in the general field of tumour immuno-

¹ The three collaborating centres are : the Department of Pathology, Royal Veterinary College, London, England (tumours of the alimentary tract and animal leukaemias); the Veterinary Pathology Institute of the University of Zurich, Switzerland (tumours of the respiratory tract); and the Pathology Department, Antoni van Leeuwenhoek-huis Vereeniging, Netherlands Cancer Institute, Amsterdam (tumours of the urogenital system and mammary gland).

logy and in particular in the development of immunotherapeutical procedures. Progress in this direction will be greatly facilitated by a better understanding of the basic phenomena of immunology.

The Group recommends international co-operation in clinical trials of immunotherapy for cancer to permit international evaluation of the clinical data, and the provision of background information on animal and clinical experiments for physicians contemplating such trials.

Recommendations relating to particular types of investigations appear in various sections of the report (pages 10, 15, 20, 25).

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