months period of observation has never been higher in the control population than 5%. At times of such minor prevalence of influenza, a dilution of the latter by the inclusion of other diagnostically confusing illness seems inevitable. So that, on the whole, it has been surprising that a reduction of illness has been demonstrated at all (Table II). Yet

TABLE II.—*British Influenza Vaccine Trials*

Epidemic	Population	No.	Vaccine	% Attack Rate	Protection Ratio	Diagnosis
(1) A (1953)	Industry, Services nurses, students	6,370 6,340	B A	4.9 3.0	1.63	Clinical
(2) A (1956)	Industry	2,499 2,487 2,509	B A (monovalent) A (polyvalent)	5.0 3.5 3.3	1.4 1.5	"
(3) A (1956)	School	344 100 100	Unvaccinated A (1955) A (1954+1955)	20.0 8.0 2.0	2.5 10.0	Clinical and laboratory

(1) Report of M.R.C. Committee on Clinical Trials of Influenza Vaccine (1953).

(2) Report of M.R.C. Committee on Clinical Trials of Influenza Vaccine (1957).

(3) Hawkins, Hatch, and McDonald (1956).

though vaccination in 1953 and in 1956 lowered the attack rate only by 30 to 40%, the effect was statistically significant. In contrast, a single trial in 1956 at a boys' school, where the attack rate of influenza A in the uninoculated boys was 20%, showed a ratio of protection closely similar to that found by the U.S. Commission (Hawkins, Hatch, and McDonald, 1956). Perhaps part of the disappointment afforded by the M.R.C. field trials may thus be blamed on nature, but some at least is undoubtedly due to our poor methods of measurement of protection.

The Future Prospect

In view of what has thus far been accomplished, it is obviously unrealistic to visualize the control of influenza with immunizing agents such as those at present available. "Control" is a word which implies ability to curtail spread as well as to limit manifestations of the infectious process. The present era may indeed be the first phase in our gropings towards control of influenza, and so we must continue to experiment with different sorts of vaccines and also with field trials.

So far as the present threat of Asian influenza is concerned, it must be obvious that in the short time available we can do little but prepare for as good a field trial of protection by formalized egg vaccine as can be devised. Such a trial must be preceded by tests of the antibody-forming power of an inactivated vaccine made from the Asian virus. As the latter has certain unusual properties, it is clear that we cannot be sure that the facts gathered about vaccine made from older viruses will necessarily hold good for the future.

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THE NATURE OF CANCER ANAEMIA, AND ITS BEARING ON THE IMMUNOLOGICAL THEORY OF CANCER

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A preliminary account of this work has already been reported (Green, 1957b) in a general survey of the immunological theory of cancer (Green, 1954). This ascribes the neoplastic change to loss of tissue-specific antigen(s). An important part of these investigations was to study the nature of tumour haemolysins and haemagglutinins and determine the response of many kinds of experimental tumours to the parenteral or intra-tumoral injection of foreign and autologous red cells. Some of these results have been reported (Green, 1957b), and a more detailed account will appear in a separate publication. Here we present the results of tests on the blood of cancer patients and discuss them in the light that these and other findings throw on the immunological theory in general.

This theory implied that an immune reaction of the tumour to certain cells of the host was a possibility. If so, the anaemia of cancer, often so prominent a feature at some stage of human and experimental cancer, might be due to an "auto-antibody" produced by the tumour mass. Such an antibody, if coating the red cell, might be detected by the use of specific antiglobulin sera. Accordingly direct Coombs tests were made on the blood of cancer patients, but only very occasionally was a positive result obtained at a titre positive with sensitized Rh+ cells. However, on using much lower dilutions of antisera it was found in many cases that the speed of agglutination was much greater than that of the red cells of normal subjects. While this work was in progress it was becoming evident (see Discussion) that the anaemia of human cancer was not primarily a by-product of haemorrhage, infection, malnutrition, etc., but a true haemolytic anaemia induced by an agent of unknown origin. In terms of the immunological theory this suggested that the tumour itself could on occasion react against cells of the host possessing an antigen which the tumour cells lacked. Such a possibility made the occurrence of what seemed a relatively feeble antibody coating the red cell a matter of great practical and theoretical interest.

Methods

Direct Coombs Test.—This is performed in the standard way (Dunsford and Bowley, 1955), mixing 1 drop of 50% packed cells and 1 drop of diluted antiglobulin serum on an

opal tile. Much lower dilutions are used than are needed to elicit a full positive test with sensitized Rh+ cells. A serum with an Rh titre of 1:80 was tested at 1:10, 1:20, 1:40, and of 1:200 at 1:20, 1:40, 1:80. Higher dilutions were made if needed. Two sources of antiserum (Burroughs Wellcome, and a goat anti-human-globulin serum supplied by Mr. I. Dunsford) were available. Three controls, test cells and saline, Rh+-sensitized cells (O) and non-sensitized normal cells (from laboratory staff) are set up. After standing one minute at room temperature the tile is gently rocked and readings are made over a period of five minutes. Scoring of agglutination is made roughly as follows: ++, immediate; +, complete within two minutes; ±, agglutination occurring definitely at an earlier period than the control non-sensitized cells. It should be noted that normal control cells often agglutinated in five minutes or more, probably due to an anti-species agglutinin effective at low serum dilutions. If the test was positive (++ or +) it was repeated using serum dilutions up to the maximum giving a ++ reaction with sensitized cells.

Indirect Coombs Test.—Two tubes containing 6 drops of test serum mixed with 2 drops of a 50% suspension of once-washed normal O Rh+ cells are incubated, one at 37° C. and one at 4° C., for 1½ hours. The cells are tested for sensitization as above.

Elution of "Antibody."—Heavy suspensions of cells are washed (×3) in ice-cooled saline and placed in a Kahn tube to about a third of its depth. A similar quantity of saline is added and the mixture agitated in a 56° C. water-bath for seven minutes. The cells are sedimented rapidly in a centrifuge containing water at 56° C. in the buckets. The eluate is removed and immediately tested with once-washed normal Rh+ O cells. Half the eluate plus 2 drops of 50% suspension of the cells is incubated at 37° C. and half at 4° C. for 1½ hours. They are then tested for sensitization directly.

TABLE I.—Direct Anti-Human-Globulin Tests on Red Cells of Cancer and Non-cancer Patients

Group	Total No.	No. Positive Tests		% Positive ++ to +	Significance of Difference P
		++	+		
Leeds					
Cancer ..	294	49	91	48	} <0.001
Non-cancer	188	3	26	15	
Normal ..	66	0	2	3	
Sheffield					
Cancer ..	157	13	42	35	} <0.01
Non-cancer	26	0	3	12	
Leeds and Sheffield Combined					
Cancer ..	451	62	133	43	} <0.001
Non-cancer	214	3	29	15	

Results

Direct Tests

There is statistically a highly significant difference between the percentage of positive tests (++ or +) in cancer and non-cancer patients (Table I). Nor can this difference altogether be ascribed to a large preponderance of groups with a high incidence of positive tests. If we omit two such groups (Table II), advanced cancer and tumours of the

TABLE II.—Incidence of Positive Antiglobulin tests in Various Groups of Patients

Group	No.	No. Positive (++ to +) Tests	% Positive	Significance of Difference P
(1) Cancer excluding (2)	343	133	39	<0.001
(2) Advanced cancer	108	62	57	
(3) Cancer of R.E. system, Leeds	58	41	71	
(4) Cancer of R.E. system, Sheffield	43	12	28	0.01
(5) Combined (3) and (4)	101	53	52	
(6) Cancer, excluding (2) and (5)	242	80	33	
(7) Non-cancer patients	214	32	15	0.001

reticulo-endothelial (R.E.) system, a significant difference between cancer and non-cancer cases still remains. It is clear, however, that the greater the cancer mass the more likely is the test to be positive. The advanced cases included all those in which there was a known metastatic focus, local or remote. Obviously the overlap is considerable, for, on this basis, some cases classified as "not advanced" would in fact be so. All but one of the eight Leeds cases labelled carcinomatosis gave a positive test (usually ++). When, in Sheffield, a surgeon was asked to select some widespread cancer cases the incidence of strongly positive tests rose sharply. In this connexion the lower incidence of positives in the Sheffield as compared with the Leeds results may be noted. This is probably associated with the difference in the source of the cases. In Sheffield the majority were ambulant cases from the Radiotherapy Centre, and contained a number, probably considerable, in which the cancer mass, owing to treatment, would be small or non-existent. In Leeds the blood samples were taken in general hospital wards shortly before or after treatment had begun. In the earlier stages of the investigation the Leeds and Sheffield results were much wider apart than they were later (Table I), and at this time the difference in the source of material was more accentuated.

Tumours of the Reticulo-Endothelial System

It will be noted (Table II) that not only advanced cases, but at Leeds the group comprising tumours of the R.E. system, showed a much higher incidence of positives than the cancer group as a whole. This is emphasized if comparison is made with this latter group, from which the advanced cases are removed. Again there is a discrepancy between the Leeds and Sheffield figures, the R.E. tumour group showing no increased incidence in Sheffield, possibly again because there was a preponderance of ambulant cases under treatment in Sheffield. It should be particularly noted, however, that 5 hospitalized cases of multiple myeloma (3, Sheffield; 2, Leeds) all gave positive direct and indirect tests—in 4 so strong as to be reported a frank positive Coombs test.

The high incidence in the R.E. tumours is mainly attributable to the lymphoid tumours, chronic lymphatic leukaemia, and lymphosarcoma. Of 20 such cases in Leeds 16 were positive and a few* gave a frank positive Coombs test—that is, positive at the optimal serum dilution for Rh+ sensitized cells. When these cases are excluded the number of cases in individual tumour groups is too small for exact analysis. The incidence of positives in the 34 Leeds cases of myeloid leukaemia, Hodgkin's, and other reticulososes was 50%, and the figures suggest that chronic myeloid leukaemia may ultimately reveal a somewhat higher, and Hodgkin's disease a distinctly higher, figure than cancer in general—particularly if the advanced cancer cases are omitted.

Other Cancer Groups

The cancer cases in general comprised almost every type of cancer, but the groups were usually too small for discrete analysis. The preponderating group was breast cancer, and of a grand total of 84, 36 (41%) were positive, a proportion very close to the combined total figure (see Table I) for cancer as a whole. Of 34 cases of lung cancer 47% were positive, though the Leeds figure (69%) alone, together with several very strongly positive reactions, suggested that the incidence, possibly because of inadequate treatment, may be higher than average. Otherwise, except for two small groups, skin and testes, which showed a not statistically significant high percentage of positives, the incidence was rather strikingly similar to the common average in all types of cancer. Excluding lymphosarcoma, there were far too few cases of sarcoma to make a comparison with carcinomata possible.

*Any unprecise statement such as this is due to the fact that a record book containing the larger part of the Leeds findings was removed from the laboratory by an unauthorized person and never returned. Fortunately, the results had already been summarized, but not in every detail.

The incidence of positive tests in non-cancer is significantly lower than in cancer patients. This group consisted mainly of surgical operations (hernia, etc.) and surgical cases of infection (appendicitis, etc.). The latter provided the bulk of the positive tests. Of the 29 positives in the Leeds non-cancer group 20 were cases of infection, many suppurative in type.

It is obvious (Table I) that normal subjects rarely give a positive test. These samples were mainly derived from groups of laboratory technicians in the earlier part of the investigation to obtain a rota of normal controls. In later tests only one such blood sample was used as a negative control. It was noted that subjects giving a weak positive ($\pm$) test had usually a mild type of infection (for example, acute sinusitis).

TABLE III.—Comparison of Auto- and Iso-antibody Titres in Sera of Cancer and Non-cancer Patients

Case Type	Auto-antibody Titre Higher	No Difference, or Iso-antibody Titre Higher
Reticulo-endothelial tumours ..	12	6
Advanced cancer ..	9	4
Other ..	9	16
Non-cancer ..	1	21
	30	26

Indirect Tests

Of 74 cancer patients giving a positive direct test the indirect test was positive in 56, thus showing the presence of circulating antibody in the majority. It is a "cold antibody," for, though sensitization was attempted at 37° C. and 4° C., only at 4° C. was it achieved. Comparison of the titres at which positive cancer and non-cancer sera were active (iso-antibody) with the titres of the direct test (auto-antibody) showed the preponderance of stronger auto-antibodies in the cancer group. This was particularly evident in those cancer groups with the highest incidence of positive tests (see Table III).

Elution of Antibody

In blood samples giving a strong (++) reaction elution of the antibody was successfully achieved in 8 of 15 instances. Here again sensitization was more effective at 4° C., though it could be obtained at room temperature but not at 37° C.

Effects of Transfusion and Irradiation

In only two cases, both giving a strong positive test, was there a distinct possibility of a recent blood transfusion(s) being a factor, but in one of these, in which repeat tests were possible, the reaction had not weakened several weeks after the last transfusion. In five other transfused cases the test was not positive (++ or +). Although this point was overlooked in the early stages there is now ample evidence that positive tests are not usually due to mildly incompatible transfusions.

As to radiotherapy, of the 89 Leeds patients receiving this treatment at the time of blood-sampling the incidence of positives was 51%, a figure differing little from the Leeds average for all cancer patients. The reason for this particular inquiry was that Rosenthal *et al.* (1955) found that in lymphoid tumours x-ray therapy seemed to precipitate the onset of abnormal haemolysis.

Nature of the Antibody

From the above data it seems that we are dealing with a cold incomplete "antibody." Absorption with an equal part of 1:1,000 pure serum gamma-globulin,* which completely removed activity on Rh+ sensitized cells from the antiglobulin serum, left the activity on the many cancer or non-cancer sensitized cells tested intact. Absorption with 1:1,000 human serum or with a mixture of alpha- and beta-serum globulins removed all activity. If the alpha- or beta- (or both) globulin adsorbed on the red cell is an antibody it is related to the type found in auto-immune acquired

haemolytic anaemia rather than to the type found in the anaemia of the newborn.

The possibility existed that the cold antibody was anti-H (Dacie, 1955), which is adsorbed from the serum by cooled group O cells, but routine ABO blood grouping showed that the group incidence in positive cases was close to that of the general population. Neither was the cancer-induced antibody adsorbed by the saliva of H-secretors.

Discussion

It is not proposed to cover here all the relevant literature which suggests the existence of haemolytic anaemia in experimental and human cancer (Green, 1957a, 1957b, for further references). There are a few salient points in the development of our approach to the subject that should, however, be emphasized. In the almost inevitable anaemia associated with rapidly growing transplanted tumours, evidence has accumulated that the primary cause is not infection, haemorrhage, or nutritional competition, but a process whose activity bears a linear relationship to the tumour mass. We have shown that the anaemia induced by chemical carcinogenesis is greatly accentuated on the appearance of the tumour and increases progressively until death.

Mrs. Savigear and ourselves have also shown independently that the red cells from 50% of rats bearing either transplanted or chemically induced tumours agglutinate with rabbit anti-rat-globulin serum. No correlation was found between the degree of anaemia and the presence of such a positive test. Nor, it may be noted, was there any direct correlation in the human tests described here. This may possibly be due to varying levels of blood regeneration, for in lymphoid neoplasia Ross *et al.* (1951) and Rosenthal *et al.* (1955) state that evidence of accelerated erythropoiesis may be found. Moreover, the rate of regeneration may very well be modified by such variable factors as an infected tumour, etc.—in other words by factors usually thought to be the direct cause of the anaemia.

The degree of anaemia in man associated with any particular tumour type appears to be related to the size of the tumour mass, but even where the tumour invades the bone marrow the well-known hypothesis that displacement or destruction of blood-forming tissue is the cause cannot be substantiated (Collins and Rose, 1948; Shen and Homburger, 1951). And yet there is no doubt that a significant degree of anaemia occurs in at least half, and accelerated red-cell destruction in all, or nearly all, advanced cases of cancer. It has been clearly demonstrated by the Ashby technique (Brown *et al.*, 1944; Brown, 1950) and the transfusion of isotope-labelled red cells (Ross *et al.*, 1951) that this destruction is sometimes exponential in type and not just an acceleration of the normal disposal mechanism. Moreover, in one particular tumour group, those of the lymphoid system, the evidence is strong that an auto-immune antibody is present in the blood. Not only is there evidence of very great acceleration of red-cell removal (Brown *et al.*, 1951), but the Coombs test is often positive. Rosenthal *et al.* (1955) found it in all of 20 cases of chronic lymphatic leukaemia and 4 of lymphosarcoma, but we have not found the incidence so high, nor did Ross *et al.* (1951). Rosenthal's group found other evidence of haemolytic anaemia such as reticulocytosis, spherocytosis, bilirubinaemia, and hyperplasia of both erythroid and lymphoid bone marrow. No explanation was found, but a review of the literature suggested that some component of the lymphoid system was the source of the antibody. Others have suggested that in auto-immune anaemia in general there is abnormal protein production which stimulates antibody production and that malignant lymphoid tissue provides one example of this (Dacie, 1955; Rappaport and Johnson, 1955).

When the present investigation was started we saw the problem mainly as one possible approach to testing the validity of the immunological theory of cancer (Green, 1954). The idea was that if malignant cells are so because they lack tissue-specific antigen(s), then those derived from cells normally capable of antibody production might well retain that function. The idea was reinforced by the evidence that

*Kindly supplied by Miss McKay, of the Lister Institute.

neoplastic cells of the R.E. system do retain some functions. Reticulum-cell sarcomas show phagocytosis and reticulin formation (Miller *et al.*, 1952), and multiple myelomata cells produce Bence Jones proteins. Moreover, there is suggestive evidence that malignant lymphoid cells, like the plasma cell, may produce abnormal periodic-Schiff-positive proteins (Rappaport and Johnson, 1955). If antibody is produced by such cells it would be directed against cells containing the antigen they lacked—that is, their normal prototypes and cells developmentally related to them. Tissue identity is almost certain to be defined by a mosaic of antigens, one or more of which will form part of the pattern in other types of cell. When early in the course of the work it was found that red cells from patients with tumours of the R.E. system, particularly lymphoid and reticulum-cell tumours, were giving a high incidence of positive anti-immune globulin tests, it made the idea of an immune response of the tumour to its host not too improbable. When, at a later stage, 5 cases of myelomatosis gave a strong positive reaction (4 Coombs-positive in the accepted sense) the possibility was reinforced, for the plasma cell has now a strong reputation as an antibody producer.

It was, however, now becoming obvious that the red cell from diverse types of cancer, particularly advanced cases, yielded positive results, albeit rarely at so high a serum titre. Ultimately, as we have seen, the difference between the cancer and non-cancer groups became statistically highly significant. It is difficult to believe that two entirely different processes are involved in the anaemia of reticulo-endothelial and other kinds of tumour—particularly since, as already noted, there is ample evidence that haemolytic anaemia of some degree is a common feature of all types of cancer, especially of advanced cases. If the tumour is not the source of the antibody found by us, then this is likely to be the position with lymphoid and plasma-cell tumours also. The usual view that the red-cell antibody may be a response to abnormal proteins formed by R.E. tumours must in that case be extended to cover malignant tumours in general. On the other hand, we have accumulating evidence of antigenic lack in tumour cells (Green, 1957a, 1957b), and there is even some direct evidence of lack in the neoplastic lymphocyte itself (Seligmann *et al.*, 1955). Using the Ouchterlony technique, two antigens were found in the lymphocytes of acute lymphatic leukaemia, compared with three in the normal. Similar evidence of our own for other types of cancer is gradually emerging (see Green, 1957b). Weiler (1956a, 1956b) has demonstrated the absence of organ-specific antigens in experimental liver and kidney tumours. In fact, the evidence, considered as a whole (Green, 1957a, 1957b), strongly favours our view that a malignant cell derived from an antibody-producing cell *would* have no choice by immunological law but to produce antibody against normal cells of its own, and probably related, type—that is, all provided it retained its normal capacity for antibody synthesis. If this does happen, as in theory it must with certain tumours, then the possibility of its happening in all becomes more of a reality. It should be noted that the cold incomplete type of antibody found on the cells and in the serum of some 30–60% of groups of cancer patients is usually a weak one except in certain R.E. tumours and sometimes in advanced cancer. A relatively mild malignant state like chronic lymphatic leukaemia, or a relatively small mass of sarcomatous lymphoid tissue, apparently produces an immune response only equalled by a very large carcinomatous mass. It could be supposed that this is so because lymphoid tissues are either better antibody producers or more closely related antigenically to red cells than other tissues. There is no sound evidence that the normal lymphocyte does produce antibody, but perhaps it is potentially a more efficient producer because it derives from a stem cell capable of differentiating into antibody-forming cells. On the other hand, the plasma cell is most probably an antibody-forming cell and probably retains this property when malignant. The fact that in myeloid and acute leukaemias and Hodgkin's disease there appears to be not much difference in haemolytic antibody formation from cases of advanced cancer in general

suggests that a greater capacity for antibody production, potential or actual, is the possible reason for the greater frequency of positive tests in lymphoid and plasma cell tumours.

The Auto-immune Haemolytic State

If, however, other tissues possess a latent capacity for antibody production when challenged by a tissue containing a specific tissue antigen they do not possess, then malignant cells, if they do lack such antigen(s), might well be the only kind of cell in the body ever to manifest this property. All healthy cells might be neutral to one another as a result of acquired tolerance developed during foetal life. In other words, the essential nature of the malignant state may reveal a property inherent, but never manifest, in normal cells. The mechanism of such an "auto-immune" state may prove to be of quite a different order to the classical reaction to foreign nucleo-proteins (Green, 1957a). If this assumption proves true, a major theoretical difficulty concerning the relation between cancer anaemia and antigen deficiency in the cancer cell would be overcome.

The auto-immune haemolytic state is not yet regarded as a feature of malignancy generally. The prevailing opinion is that only lymphoid tumours, and rarely Hodgkin's disease, can be included amongst the many conditions which may on occasion precipitate it. Such conditions are broadly covered by a few virus diseases like infectious mononucleosis and pneumonia, liver cirrhosis, and "cortisone-responsive" states like lupus erythematosus and rheumatoid arthritis. They are possibly all due to the production of abnormal proteins which function as auto-antigens. An auto-immune state is compatible with other clinical features of many of these diseases. Where a micro-organism is responsible it is likely that some fraction of it unites as a haptene, with tissue protein or polysaccharide, the auto-antigen(s) so produced resembling a red-cell antigen in sufficient degree for the antibody elicited by it to be attracted to this cell. This may not, in minor degree, be an uncommon event, for in our own group of non-cancer cases 15% gave a positive antiglobulin test, though very rarely so strong as in many cancer cases. Such positives were predominantly associated with acute infections. This particular relation may be the result of fortuitous selection of control cases from surgical wards, but it shows that even acute appendicitis fairly frequently produces conditions which temporarily sensitize the red cell to antiglobulin serum.

The autohaemolytic anaemias have been divided into two groups—one with clinical and laboratory evidence of an immune state, and one in which the only evidence of haemolysis is that the survival time of the red cell is reduced. The malignant state, apart from the special tumours mentioned, would, on past evidence, fall into the latter category. It now looks doubtful, however, whether the distinction between, for instance, the anaemia of lymphosarcoma and that of general carcinomatosis is justified. As we have indicated, it may be just a question of the antibody-producing potency of the tissue concerned. Theoretically it would be expected that if malignant growths do produce antibody to host red cells the amount would vary according to its antigenic relationship to the red cell; it might therefore in many cases be nil. Such variations were in fact found in the response to the injections of autologous red cells into a variety of experimental tumours (Green, 1957b). The prototype cell might contain no antigen related to one in the red cell, or, if it did, it might not be lacking in the cancer cell.

This may explain, together with varying capacities for red-cell regeneration, the very different degrees of anaemia in advanced human cancer in which, occasionally, there is no anaemia at all. There was no difference between the average blood (72%) Hb levels of positive and negative cases of the cancer group as a whole; yet there were more cases (21%) of severe anaemia (<50% Hb) in the positive than in the negative group (4%). Tumours of the R.E. system, however, showed a greater average degree (59%) of anaemia in the positive group, and all with severe anaemia gave a positive test. If, then, cases with severe anaemia are excluded,

members of the positive group were on average less anaemic than the negative. One explanation is that up to a critical point the more active the haemolytic process the greater the relative degree of haemopoiesis. In the R.E. group there is also the possibility that blood regeneration is inhibited, which Collins and Rose (1948) have suggested may happen in leukaemia. In terms of the immunological theory, cytolysis of blood-forming cells by some tumours of this group might be expected. In our cases there was no evidence that tumours of different organs, apart from certain R.E. tumours, differed greatly as source of antibody, for the numbers of positive tests were fairly equally distributed. The possibility exists, however, that in some cases with increasing malignancy there is a decreasing functional ability to produce antibody.

The lack of individual correlation between the degree of anaemia and the result of the test could also be due to the fact that the antisera used may not be the most sensitive for our purpose. They differed distinctly in potency in that the percentage of positives varied from batch to batch in apparently comparable groups of cases. In every instance the globulin coating the red cells was of non-gamma-globulin type, whereas the Coombs antiserum is rich in anti-gamma-globulin. Immunization of rabbits with positive cells or their eluates is in progress in the hope that more active antisera will be obtained.

An interesting point was that in more than half the cancer cases, when the comparison was possible, the antibody was more active on autologous than on homologous cells, whereas in non-cancer cases this was rarely so. In tumours of the R.E. system and in advanced cancer this distinction was much more evident. This suggests that the antibody globulin found in cancer serum has a greater affinity for the patient's own red cells, and differs, even if only slightly, from that formed in various other diseases. This would be in line with the hypothesis that in cancer the normal red cell, and in the non-cancer case a modified tissue protein, provides the antigenic stimulus. The antibody in the latter case might well show no autologous specificity.

Structure of the Antibody Globulin

More precise knowledge of the structure of the antibody globulin is being sought by electrophoretic analysis of red-cell eluates, but we already have some indication of its nature. In auto-immune haemolytic anaemia the frequency of false-positive Kahn and Wassermann reactions has often been noted. We found a high incidence of such positive reactions, particularly of the Kahn, during experimental carcinogenesis, and some years ago a similar investigation was started in human cancer. This revealed *inter alia* a similar tendency, reflected in a nearly doubled incidence of positive Berger-Kahn reactions (46% of 206) in cancer as compared with non-cancer patients (26% of 441) (see Green, 1957b). At a later date no correlation with positive antiglobulin tests was found. There were, however, indications of a direct correlation with a positive complement fixation test using human tissue phospholipid as antigen, though such positives (14 of 43 tests) were few in number. There are many indications that there are very frequently, both in the sera of animals with cancer and in cancer patients "antibodies," capable of binding with lipid substances. Evidence has been obtained pointing to lipo-proteins with strongly determinant phospholipid groups as important cellular antigens. Moreover, lipo-proteins and phospholipids derived from malignant tissues affect the red cell in ways very similar to those produced by true antibodies. The evidence for all these statements is discussed in detail by Green (1957a, 1957b), and has led to the suggestion that some type of antilipid "antibody" may appear in cancer serum. The speculation is tempting that the antibody globulin detected in the present work is part of a lipo-globulin antibody whose presence may ultimately be detected by other serological tests in which the lipid, and not the globulin, acts as antigen.

If the hypothesis is correct that the cancer itself is the direct cause of red-cell lysis it would seem unlikely that the

effect would be restricted to this cell. In this connexion the possibility has to be remembered that only cells in intimate contact with cancer tissue may provide an efficient antigenic stimulus. Any type of blood cell, provided it has any antigenic similarity to the normal cell from which the cancer derived, could in theory be involved. It is therefore interesting that Mrs. Savigear, working in our laboratory, found that rat-tumour extracts, in addition to their effect on the red cell, also lysed blood leucocytes and splenic lymphocytes and plasma cells. The effect was not given by spleen or liver extracts from the same animal. It was not seen in tumour extracts made just prior to regression in all other tumours of the group—that is, it was manifest only by fully viable cells. On the plasma cell it was much more evident in the transplanted than in the chemically induced tumour, possibly owing to the fact that plasma-cell infiltration in the homologous transplanted tumour is a common phenomenon. If indeed cancer cells are capable of damaging these and related cells *in vivo* it might explain the decline in immunity in general which has frequently been noted in cancer, particularly perhaps in Hodgkin's disease.

Such possibilities indicate that the problem of cancer anaemia, if it proves to be immunological in nature, will have much wider implications both in theory and in practice, in the cancer problem as a whole. It would raise the question of whether many of both the local and the systemic effects of cancer are the result of immune responses on the part of the tumour. In terms of the immunological theory the cancer cell would then be correctly regarded as an aggressive parasite in the sense that it is capable of invading, and perhaps damaging, host tissue because it is, immunologically speaking, a new race of cells. It may possibly prove that not only the anaemia but the general emaciation of cancer is in part due to this immune aggressive reaction of the tumour to the host.

It has long seemed significant to me (H. N. G.) that tumours of the reticulo-endothelial system are particularly prone to produce systemic effects. Not only, as we have seen, are they potent in producing anaemia, which in some types may be the first severe warning of their presence, but they are particularly associated with febrile disturbances and symptoms suggesting the existence of an inflammatory lesion. Fever is observed perhaps more in lymphadenoma (Pel-Ebstein pyrexia), but is not uncommon in any of them. In fact, this disease, like the leukaemias, was long regarded as an inflammatory state both on clinical and on pathological grounds. The pronounced local inflammatory-like changes in Hodgkin's disease may be an expression of an immune reaction of the tumour to host tissue promoted by a cell which, by nature of its origin, is potentially a strong antibody producer. If so, we may have to look at many of the lesser degrees of cellular infiltration in and around cancer tissue in quite a different light from that of the mechanical theories long held.

Validity of the Immunological Theory

The main purpose of this work was to test the validity of the immunological theory (Green, 1954) at a practical level. Direct and indirect evidence in its favour has accumulated greatly since it was first put forward, and more precision has been given to the nature of the antigen(s) lacking in the cancer cell. These, it seems likely, are tissue (organ)-specific antigens with very probably a lipoprotein basis (Green, 1957a, 1957b).

If the suggested mechanism of cancer anaemia proves basically true, then the truth of the theory would be apparent. If another explanation proves true, then of course the theory is not disproved. In fact many immunologists might say that its suggested bearing on cancer anaemia is irrelevant and untenable because it rests on the unproved assumption that many cells, apart from one or more found in lymphoid tissue, have a potential ability to produce antibodies. There exists, however, evidence suggesting the possibility of this happening even in normal tissues. In the experiments of Oakley *et al.* (1949, 1951, 1954, and 1955),

who quote the relevant literature, many tissues, such as breast, uterus, appendix, fat, skin, and striated muscle, were shown to produce antitoxin when challenged with soluble toxin. There was no doubt about the response, but the possibility of invading inflammatory cells as the source of the antibody could not be excluded. Similar interpretative difficulties were found in our own work (Green, 1957b) on tumours. A wide range of spontaneous (animal and human), induced, and transplanted tumours were found to contain haemagglutinins and haemolysins, often more active against cells of the host than other cells. Such properties could be augmented, or specific new ones induced, by challenging certain tumours, particularly by intratumoural injection, with autologous or homologous and heterologous red cells. In this case the response to autologous cells suggested that the cancer cell might be itself producing the anti-cell factors.

Whether this proves true or not, the tumour cell may be, immunologically speaking, in quite a different category to normal cells. As we have seen, in theory it is unique in being the only body cell whose antigenic make-up has altered in postnatal life, and thus immunological tolerance has not, in respect to this alteration, been acquired. If the change in the tumour cell was antigenic gain, then of course some host cells would react against the tumour; if antigenic loss, as seems highly probable, then the tumour could react against the host. In other words malignancy may by its very nature either confer on the cell the property of reacting immunologically or provide an opportunity for this latent power to be demonstrated. It would react only to the challenge of the tissue-specific antigens it had lost. It is possible that the property of reacting to foreign nucleoproteins is a specialized property of normal antibody-producing cells. We have obtained some evidence that tumours do not produce significant amounts of antibody to such antigens. In any case, spontaneous as distinct from transplantable tumours are usually not deficient, to any great degree, in iso-antigens (see Green, 1957a). If antibody is produced by them it would be expected to be directed against membranous and cytoplasmic lipo-proteins. Such ideas accord with the finding that the tumour haemolysins and haemagglutinins belong to this class (Green, 1957b) and suggest where we should search for the precise nature of the antigenic deficiency in the cancer cell.

Conclusion

It is stimulating to realize that more knowledge of the immune mechanism concerned in cancer anaemia may provide a definitive clue to the nature of the antigen(s) lacking in the cancer cell and thus to the fundamental nature of cancer; for it should be noted there is now quite strong evidence that antigenic deficiency is a feature of the cancer cell. The facts presented here, though only a small section of the whole, closely correspond to those predicted by the immunological theory. This indicates that specific antigenic deficiency is not just a pointer to immaturity in the cancer cell, but the cause both of this immaturity and all the properties of the malignant cell.

Finally, it should be made clear that some of the rather dogmatic statements made here cannot be appraised without reading the detailed evidence for the immunological theory of cancer, presented by Green (1957a, 1957b).

Summary

The immunological theory of cancer maintains that the malignant cell profoundly differs from the normal in its antigenic make-up owing to lack of a tissue-specific antigen(s). It was therefore postulated that, because of this antigenic difference, malignant cells, if derived from antibody-producing cells, should be able to produce antibody to the corresponding, and certain antigenically related, normal cells.

This idea was supported by the fact, confirmed here, that certain tumours of the lymphoid system produce an

auto-immune haemolytic state as revealed by a positive Coombs test. It was reinforced by obtaining positive tests in cases of multiple myeloma, a tumour derived from the antibody-forming plasma cell.

Using anti-human-globulin sera at lower dilutions than the standard, it was found that positive tests were not restricted to these special types of cancer.

This modified antiglobulin test was positive in very significantly more (43% of 451) cancer patients than in non-cancer patients (15% of 241). Both the number and strength of positive tests was greater the greater the tumour mass.

If the groups (tumours of lymphoid tissue and advanced cancer in general) which gave the highest percentage of positive tests were excluded, the difference between cancer and non-cancer patients was still highly significant.

The antibody coating the red cell was of the cold, incomplete non-gamma-globulin type, and was found in the serum of many patients giving a positive direct test. It thus resembles that found sometimes in auto-immune haemolytic anaemia in non-cancer patients. Reasons are given for the prediction that it may prove to be a lipo-globulin complex.

It is concluded that the anaemia of cancer in general, and not just of certain lymphoid tumours, is primarily of an auto-immune type.

Both fact and theory point to the tumour, and not the host, as the source of the haemolytic antibody.

It is argued that the production of auto-antibodies is a special and peculiar property of the cancer cell, because not only does it lack certain specific tissue antigens, but, in addition, it has no acquired tolerance to the antigenic incompatibility occasioned by this lack.

These findings were predicted from theory, and thus support the validity of it. If it is found, as the evidence strongly suggests, that cancer cells are immunologically aggressive to the host, it would not only explain the present findings but many other local and systemic effects of cancer. It would also show that the immunological theory of the nature of cancer (Green, 1954, 1955, 1956, 1957a, 1957b) is basically correct.

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