PRODUCTION OF A PRECIPITIN AGAINST RENAL CANCER

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The possibility of provoking antibodies to cancer, apart from its fundamental interest in relation to one type of host resistance, could have therapeutic implications (*Brit. med. J.*, 1959; Goldman, 1961; Southam, 1961; Mellors, 1962; Mihich, 1962). If antibodies to tumours could be obtained they might provide, either alone or after combination with radioactive or cytotoxic chemicals, a new class of specific therapeutic agents. Autoantibodies to tumours, usually in low titre, have been described in several studies, as have apparently successful efforts to raise their titre with vaccines prepared from autologous tumour (Finney *et al.*, 1960). The success of these measures would depend on the presence in tumours of specific antigens absent from normal tissues. Although incontrovertible evidence of such antigens has not yet been obtained, it is not unlikely that tumours with a characteristic morphology may contain specific chemical substances which confer structural individuality. This is not to say that there is necessarily a common specific antigen for all or even several kinds of neoplasm, but merely that histologically or histogenetically related neoplasms may share unique antigenic material.

We thought that human renal cancers with their fairly characteristic histological pictures might on these grounds contain renal-cancer-specific material suitable for immunization. A woman with advanced metastatic renal cancer was injected with an emulsion in Freund's adjuvant of a microsomal fraction of a similar carcinoma from another patient. The procedure had no apparent effect on the clinical course of the disease but resulted in the formation of a weak precipitin against human renal cancer.

Case Record Before Immunization

In July, 1961, the patient, a woman of 47, had her left kidney removed for a carcinoma in the lower pole. The tumour consisted of papillary, tubular, and more solid anaplastic growth with areas of necrosis and only occasional foci with clear cell hypernephroma-like morphology.

In February, 1962, she had commencing intestinal obstruction, and a further laparotomy revealed similar metastatic tumour disseminated in the pelvis and lower abdomen. Her obstructive symptoms recurred in April, but the abdominal neoplasm was found to be too extensive and adherent to the viscera to permit palliation by either surgery or radiotherapy, so it was decided to see if any possible help could be derived from an immunization procedure. A tumour sample was removed at this stage to provide material for subsequent serological tests.

Immunization Procedure

We had a large batch of microsomal material prepared from a morphologically similar renal carcinoma removed at operation from another patient a year earlier. This donor patient, a man of 77 with the same ABO and Rh blood

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groups as the recipient, had had his left kidney and tumour removed in April, 1961. The tumour showed the same papillary histological pattern as the present case but with greater necrosis and slightly more conspicuous hypernephromatous areas. His kidney and tumour had been separated, diced, snap-frozen at -76°C ., and stored at -20°C . until February, 1962, when the microsomal fractions were prepared as in previous studies of tissue specificity (Nairn *et al.*, 1960b, 1962), employing homogenization, ultrasonic disintegration, and differential centrifugation. For immunization the tumour microsomal material (60 mg. protein/ml.) was emulsified with an equal volume of Freund's complete adjuvant. The antigen was sterile on culture, but 50,000 units of sodium penicillin G and 0.05 g. of streptomycin ("crystamycin") were added to each ml. of emulsion to reduce the risk of infection and abscess formation.

Before immunization the patient was tested for sensitivity to tuberculin and to Freund's incomplete and complete adjuvants. The Mantoux test was negative with 1/10,000 and 1/1,000 tuberculin and there was no reaction to the intradermal injection of 0.1 ml. of incomplete adjuvant. A similar injection of the complete adjuvant resulted in local erythema but no general reaction during 24 hours' observation. Thereafter, on April 18, the patient was given a test intramuscular injection of 1.7 ml. of the cancer antigen (100 mg. protein) with complete adjuvant; the total volume of emulsion, including antibiotic, was some 3.5 ml. The ensuing local and general reactions were no greater than might be expected from such a vaccine: there were local swelling and tenderness which subsided over the course of a few days, and a small elevation of temperature which returned to its previous level next day. The first immunizing course was therefore completed by administering a further 6.8 ml. of antigen (400 mg. protein) with adjuvant in two doses at two-day intervals and in several intramuscular sites. After a seven-weeks interval, on June 11, a 0.1-ml. test dose of antigen with adjuvant was given intramuscularly, and as there was no untoward reaction the second immunizing course of 5 ml. of antigen (300 mg. protein) was completed within the next 24 hours.

Clinical Course

At no time was there any clinical evidence of a favourable response to the immunization courses, and continued advance of the abdominal tumour mass could readily be detected by palpation. Methotrexate, 5 mg./day, was given from May 7 for five days in the hope of relieving the progressive intestinal obstruction, but the patient developed toxic signs with severe epistaxis and a rapid fall in the white-cell count. The bleeding was controlled and the white-blood-cell and platelet counts were restored by several packed-cell and concentrated platelet transfusions. There was still no sign of tumour destruction and the patient died on June 25, 1962.

Pathological Findings

Necropsy, six hours after death, confirmed the presence of massive deposits of tumour, weighing more than 1 kg., in the pelvis and lower abdomen; small metastatic tumours were also detected in abdominal lymph nodes, liver, lung, and bone. The other organs were remarkably normal, and death was attributed to inanition and hypotensive renal failure; the terminal blood urea was 120 mg./100 ml.

The tumour showed a similar histological pattern to the earlier biopsies although the papillary structure now predominated more; there was no unusual necrosis nor specially remarkable leucocytic reaction. In the kidney there was mild focal chronic inflammation of the parenchyma but no other significant abnormality; the glomeruli looked normal apart from a tendency to tubularization of Bowman's capsule. Plasma cells were numerous in the spleen and present around the antigen inoculation sites in muscle but were not identified in several sections of tumour or kidney.

Some tissue blocks were snap-frozen at -76°C ., stored at -20°C ., and used for the preparation of cryostat sections which were treated with fluorescent antibody for the detection of tissue-bound globulin. The sections were fixed for 10 minutes in ethanol-ether mixture (equal parts) at room temperature followed by 30 minutes in 95% ethanol at 37°C ., and were stained by goat anti-human-globulin labelled with lissamine rhodamine B (RB 200); appropriate staining controls were included (Nairn, 1962). Accumulation of globulin was detected in plasma cells in the spleen, lymph nodes, marrow, and the muscular injection sites, and extracellularly in the tumour and kidney. In the latter it was mainly found in capillary walls, especially in the glomeruli, but in the tumour it appeared to lie on the surface of the cellular trabeculae (Fig. 1); such deposits were not observed in other tissues.

FIG. 1.—Section of patient's renal cancer treated with RB 200-conjugated goat anti-human-globulin. Human globulin on the surface of the cancer cells is indicated by bright specific fluorescence. ($\times 340$.)

Immunological Studies

Serum samples were examined at weekly intervals from April 10 until the final specimen on June 25, taken one hour after death. The investigations included gel-diffusion on microscope slides (Mansi, 1958), immunoelectrophoresis, complement fixation, and immunofluorescence.

Gel-diffusion Analysis

The sera were tested against microsomal material (25 mg. protein/ml.) and concentrated homogenates of a number of different renal cancers, other cancers, kidneys, and other organs. The various antigen preparations were suspended in phosphate-buffered saline (0.01 M phosphate, pH 7.1) and contained no antibiotic or adjuvant. There was no sign of precipitating activity against any antigen until the fourth week after the immunizing course began. The serum, taken on May 15, showed a faint diffuse precipitin line after 48 hours against the tumour microsomes used for injection, the patient's own tumour microsomes, and microsomes from several other human renal cancers, but not against other tissues, including the kidney microsomes from the donor case. The findings with the next three serum samples, taken on May 21, 28, and June 2, were the same except that the precipitin line was now stronger and appeared within 24 hours. Subsequent sera on June 11, 18, and 25 showed a weak line against normal kidney microsomes, including

those from the donor and recipient cases. This line was very faint and appeared to give the reaction of partial identity with the line against the cancers. No precipitin line was obtained with a microsomal fraction from a necrotic area of another human-kidney cancer, though the corresponding fraction from the non-necrotic part of this tumour gave a line identical with that against the other human-kidney cancers tested.

Serum absorption with A and B red cells had no effect on the precipitins, but absorption with kidney microsomes from either the donor case or the patient prevented the development of the anti-kidney line without materially affecting the anti-cancer precipitin; this could be removed by absorption with renal cancer microsomes from either case.

When the sera were tested against the different kidney cancers and the corresponding normal kidney fractions, obvious precipitin lines showing the reaction of identity were produced within 72 hours with all the cancers; and inconstant faint, possibly cross-reacting, lines occurred with normal kidney (Fig. 2). Crude homogenates were slower to produce precipitin lines than microsomal material. Various preparations of other human organs or cancers showed no sign of reaction with any of the sera: the other materials tested included microsomal fractions of the patient's own liver obtained at necropsy, human colons, colon cancers, skin, thyroid, and hamster kidney and kidney cancer; homogenates of human liver, lung, heart, marrow, spleen, skin, thyroid, and breast and stomach cancers, including some necrotic tissues, were also tested.

FIG. 2.—Diagram of gel-diffusion reactions between the patient's serum (S) and microsomal fractions of kidney cancer (C), kidney (K), and other tissues (O). 1=Patient; 2=Donor; 3=Other patients.

Other Serological Tests

Although the gel-diffusion analysis left no doubt about the specific precipitin in the patient's later serum samples, we were unable to obtain confirmatory evidence of the presence of antibody by complement fixation or immunofluorescence, and attempts to identify the precipitin by immunoelectrophoresis or chromatography resulted in loss of precipitating activity. This failure may have been attributable to low titre and poor avidity of the antibody, though the immunofluorescence tests were largely vitiated by unusually strong non-specific staining reactions exhibited by sections of renal cancer—a phenomenon already discussed elsewhere (Nairn *et al.*, 1960a).

Discussion

The anti-renal-cancer precipitin produced by this patient appears to have developed as a direct result of the immunization course with homologous tumour. Its serological activity against the patient's own tumour could be attributable to autoimmunization promoted by the adjuvant in the vaccine, but seems more likely to be due to a common specific antigen in renal cancers, since identity of precipitin lines was demonstrated with the tumours from the donor and the five other cases. Three of the latter showed a predominant classical clear-cell hypernephromatous picture, but the patterns of the immunizing and the patient's cancers were more heterogeneous, being largely papillary though with some areas of clear-cell type. While all tumours showed various degrees of necrosis, necrotic tissue did not reveal antigenic activity with the present sera.

The later development in the patient's serum of possible weak activity against normal kidney from herself, the antigen donor, and the other individuals suggests either chemical similarity between the kidney and cancer microsomal antigens or persistence in the cancer of kidney-specific material in reduced amount; complete identity between the kidney and cancer microsomal antigens is excluded by the failure of fusion of the gel lines and the results of differential absorptions. The absence of activity against other human tissues or tumours, necrotic or otherwise, indicates that the precipitin to the renal cancer is tissue-specific and not a general isoantibody.

Despite the lack of evidence of a cytotoxic effect on the tumour by the antibody, the fact that globulin, possibly antibody, had localized *in vivo* on the surface of viable tumour cells is an encouraging observation. It suggests that such antibody could conceivably be used as a specific localizing carrier for radiotherapeutic or chemotherapeutic agents. However, the possible cross-reactivity with normal kidney, in which globulin localization was also demonstrated in the post-mortem histological preparations, is a warning that damage to normal organs might result from attempts at active cancer immunization unless complete cancer specificity of the antigen could be assured. On the other hand, we have shown that the anti-kidney activity of the anti-cancer serum was removable by kidney absorptions, which left the anti-cancer activity largely unaffected. Such an absorbed serum should be non-toxic to normal organs and the possibility of its *in vivo* localization in the renal cancer of a passively immunized patient is an obvious subject for future study.

Summary

A patient with advanced renal cancer was immunized with a microsomal fraction in Freund's adjuvant of a homologous tumour with similar morphology.

A precipitin against human renal cancer was first demonstrated by gel-diffusion studies of the patient's serum four weeks after immunization. Immunohistological examination of the tumour *post mortem* showed localization of globulin on the surface of the cancer cells. There was no indication that the immunization procedure had any effect on the tumour growth or the clinical course.

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THE CURE OF HODGKIN'S DISEASE

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The title of this paper has been deliberately phrased because we feel it is time that the pessimism of the past was challenged. This challenge has already been made, for example, by Peters and Middlemiss (1958), but a new generation of medical men is still being taught that this disease is "invariably fatal" (Davidson, 1960). We present here an analysis of data collected at the Christie Hospital, Manchester, which further confirms the conclusion that Hodgkin's is indeed a curable disease. Whatever else it may mean, the word "cure" inspires a sense of comfort and reassurance in the lay mind. In medical writing it is a word not commonly employed, and attempts at definition are always open to criticism. A realistic meaning, however, can be given to the word if cure of a disease is taken to connote that in time—probably a decade or two after treatment—there remains a group of disease-free survivors whose annual death rate from all causes is similar to that of a normal population group of the same sex and age distribution.

It is not easy, in the light of present-day knowledge, to understand how Hodgkin's disease acquired its sinister reputation. It is true, as we shall show, that once the disease is generalized the prognosis is poor. But many patients present themselves at hospital with a lymphadenopathy which is still localized, and it is at

this stage that the opportunity for cure should not be lost.

Clinical Material and Mode of Management

During the years 1934–56 1,189 patients diagnosed as having Hodgkin's disease were examined for the first time in the clinics of the Christie Hospital, Manchester. Of these, 85% received treatment. The remaining 15% consisted of patients for whom radiotherapy was withheld because of advanced disease, and who were referred to their local hospitals for terminal care.

Of 926 treated cases, 822 had the clinical diagnosis confirmed by unequivocal histological reports of Hodgkin's disease. This clearly defined group of cases has been used to determine the prognosis. (A large group of cases with equivocal reports will be discussed later.) The whole of the reported series has a minimal after-treatment period of five years; 446 cases have matured to 10 years, and 191 are capable of yielding 15-year results.

The term "localized" implies that the lymphadenopathy was at the time of treatment clinically confined to one lymph-node region and was amenable to radical irradiation in one undivided volume of tissue. Thus enlarged lymph nodes were confined to one or both