REVIEW

Immunodiagnosis of Cancer

By V.V. Deshmukh*

THE concept that the neoplastic cells are immunologically differnent from normal, and are thus subject to host resistance, dates back to the nineteenth century. This century has seen the remarkable evolution of this concept mainly from the transplantion experiments and animal tumours. The role of immunity in human cancer is exemplified by roughly six-fold evidence.

Clinically, it has long been known that there are rare but dramatic cases of spontaneous regression of an established malignant neoplasm. Carcinoma of the breast, malignant melanoma, Kaposi's sarcoma, extra-abdominal neuroblastoma are some of the examples. Some of the recent cases of spotaneous regression have been studied in detail and with immunological responses — both of cellular as well as humoral type have been demonstrated. However, it cannot yet be said that the mechanism of spontaneous regression has been understood to any great extent.

A second large group of indications have come from the autopsy room. Here the pathologists found an overwhelmingly large number of malignancies of prostate and thyroid and neuroblastomas as incidental findings i.e these patients had no clinical evidence of cancer during life and had died of unrelated causes, but neverheless harboured unmistakably malignant tumours. The incidence of such occul cancer in autopsy series was found to be 40 times the incidence of clinically overt cancer. This further strengthened the belief that majority of cancers arising in the body possibly remain clinically dormant as a result of host resistance.

With the recognition of congenital immunodeficiency syndromes, further evidence has come forth. The incidence of malignant tumours in these children is about10,000 times that in normal controls.

The advent of transplanation has provided the opportunity to study iatrogenic immunodeficiency. Even here, we come across a much higher incidence of cancer than the normal population.

The higher incidence of cancer in auto-immune diseases is possibly a reflection of deranged immunological system in these patients.

Lastly, the experimental pathologists have conclusively demonstrated the susceptibility of neonatally thymectomized mice to viral and chemical carcinogens and also to transplanted tumours.

Where there is smoke there is fire and where there is immune response there must be an alien antigen involved. Thus the immune response against cancer led to the intensive search for tumour antigens.

The various antigens, known as neoantigens or tumour — specific tissue antigens (TSTA), and the immune response to these have been widely studied. This has not only added to our understanding of carcinogenesis and nature of neoplastic process but has already made significant contributions to diagnosis and therapy of human cancer. In this paper, we shall concentrate only on the diagnostic aspects of this subject.

If, by immunodiagnosis we mean the ultimate 'cancer test' which will detect any malignancy at the earliest stage of its inception, we are far from our goal. No such test exist at present. The scope of immunodiagnosis as it is today is strictly limited and can be reviewed as follows :

1. Earlier detection of recurrences and metastases than is possible by traditional methods of tumour detection.
2. To aid in the assessment of rate of progression of established metastases.

* Department of Pathology
Salmaniya Medical Centre

3. To evaluate the response to treatment.
4. To help in prognosis.
5. Such a diagnostic test will be of further value if it is quicker and technologically simpler.

The first step in arriving at such a test or a group of tests is to identify and characterize suitable tumour-specific antigens. There are three criteria that the antigen must satisfy before it can be of any diagnostic value . (1) It should be common to several histological types of tumours. (2) It should not be found in normal adult tissues. (3) It's concentration or the immune response of the host should have quantitative relationship with the activity and/or extent of the malignancy.

Once such an antigen is identified, it will be possible to use it either in the form of live tumour cells or extracts thereof to develop antibodies or specificially sensitised immunocytes in suitable hosts. These will form the reagents for the test. It will then be possible to test patient's serum, exudates or tissue fluids for the presence of :- (1) the antigen using the specific antibodies or immunocytes, or (2) the antibody or immunocompetent cells using antigenic extracts of tumour cells.

In this presentation, no attempt will be made to be comprehensive but it is proposed to concern ourselves mainly with the commoner neoantigens or established value and some others which look promising. The techniques for detection of these antigens will be considered only in brief with more emphasis on clinicopathological correction.

VIRUS INDUCED ANTIGENS

Among experimented animals, it is well known that virus-induced tumours carry antigens that are specific to the virus, irrespective of the tissue of origin. However, these antigens are quite distinct from the virus particle (or virion). In contrast, we find that the tumours induced by chemical carcinogens develop neoantigens that bear no relationship to the inducing chemical. Thus tumours caused by the same chemical in two different tissues, will develop two separate neoantigens.

This observation has a bearing on human tumours. Some of the tumours consistently carry a common neo-antigen characteristic of the tumour. These tumours are probably caused by a virus. In fact, in some of these, independent evidence of viral etiology has already accumulated. Examples of the virus-induced neo-antigen in human tumours are Burkitt's lymphoma, post-naso-pharyngeal carcinoma, carcinoma of urinary bladder, osteogenic sarcoma and possibly some other sarcomas and neuroblastomas (1).

Humoral as well as cellular responses to these antigens in human subjects have been demonstrated in vivo as well as in vitro tests. However, these experiments have not so far developed into a diagnostic test due to the lack of clinico-immunological correlation. For example, Fass and co-workers in 1970 (2), developed a skin test with antigenic preparation from human tumours. In case of Burkitt's lymphoma, a positive test was obtained in the early stages of the disease or during remissions. With the advancement or reactivation of disease the test become negative.

Humoral tests of Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) reveal three types of antigens which are associated with the Epstein-Barr virus, alleged to be aetiologically responsible for these cancers as well as infectious monoucleosis (IM) (3, 4).

1. *Early Antigen (EA).* Antibodies to this antigen are present in high titres in most patients of BL. Persistence of this antibody is associated with poor prognosis, and a rising titre may mean imminent relapse. This antibody is also present transiently in cases of infectious mononucleosis (IM).

2. *Virus Capsid Antigen (VCA).* Antibodies to this antigen are present in quite high titres in cases of BL, NPC as well as IM. Thus the test is not tumour-specific. Furthermore, the high levels persist for long periods after regression of tumours or recovery from glandular fever. Thus this antibody is not of much diagnostic value for BL or NPC.

3. *Membrane Antigen (MA).* Anti-MA is present in high titres in almost all cases of NPC & BL but not in normal controls. Rising titre, in contrast to anti-EA, signifies regression of tumour, the higher titres being maintained during remission. Any fall in the titre of anti-MA thereafter usually heralds recurrence of the tumour.

COMMON CELL CODED ANTIGENS

The most commonly encountered antigens in association with human neoplasia belongs to this type. These are also present in embryonic tissues and are therefore known as *Oncofoetal Antigens.* These antigens normally disappear early in the extra-uterine life. The neoplastic process in some way, reactivates the genetic cell code for the synthesis of these antigens. As these antigens are recognised as self by the immune system of the body, they do not give rise to any auto-antibodies with the exception of gamma-

fetoprotein. For this reason these are detected by means of hetero-specific antisera usually raised in rabbits.

1. *Alpha Foetoprotein (α-FP).* This is one of the major serum proteins during foetal life found in most mammalian species described first in mice (5) and later in patients with hepatocellular carcinoma (6). Human Alpha—FP has a molecular weight of about 70,000. Purified preparation have been found to contain 4% sugar i.e. this is a glycoprotein (7). This is raised in amniotic fluid in association of fetuses having neural defects. Also it is known to occur in pregnancy in plasma specially in association with Rh-immunization and diabetes. Over a decade ago, this protein was reported to be present in a large proportion of cases of liver cell carcinomas. The reported percentages vary from 30% to 100%. There seems to be some racial variation in the occurance of this protein in cases of hepatocellular carcinoma, there being higher incidences among Africans race (50% — 80%) and lower in European races (20 — 40%).

Its levels in cases of hepatitis are not related to tumour size and also it may occur, in a small proportion of other neoplastic and non-neoplastic conditions viz. carcinomas of biliary tract, pancreas, stomach, colorectum, lung etc. as well as in some inflammatory conditions viz. viral hepatitis, cirrhosis, ulcerative colitis and Crohn's disease (8). The diagnostic significance of this test should be evaluated with these constraints in mind.

Another area where the α-FP estimation is useful is differential diagnosis of gonadal neoplasm. High levels of α-FP are found in most cases of malignant testicular teratomas whereas seminomas do not produce this protein. Therapeutic remissions of teratomas are associated with depressed levels and recurrence rising titres.

Occasionally high α-FP levels are also found in other cases of malignancy but usually hepatic metastases are present. However, some FP producing g-i neoplasm can be monitored by serial α-FP estimation.

2. *Garcinoembryonic Antigen (CEA).* Carcinoembryonic antigen (CEA) was demonstrated by Gold & Freedman in 1964 (9), as a specific antigen found in the carcinoma of human colon. This is also a glycoprotein with a molecular weight of 200,000. It is found in alimentary tract, liver and pancreas of fetuses between second and sixth months of pregnancy. Raised CEA levels have been reported in a wide variety of histogenetically different tumours (10). These include cancers of colon and rectum (73%), pancreas (92%), liver (67%), bronchus (72%), breast (52%), Uterus (53%) and ovary (37%).Actual levels of CEA will depend upon the extent and site of tumour spread and upon tumour size. However, its occurence in non-neoplastic conditions viz. ulcerative colitis, Crohn's disease (25%) cirrhosis (42%), chronic respiratory disease (25%) and fibroadenosis of breast (7%) limits its diagnostic value (11%). Sequential quantitative analysis may overcome this handicap as neoplastic conditions are associated with rising levels of CEA while non-neoplastic conditions are associated with declining or steady levels.

Of special significance are the rising levels of CEA following surgical, chemotherapeutic or radiotherapeutic extirpation of CEA-positive cancers. These herald a recurrence and may guide therapy, particularly in cases of colorectal cancer. Even preoperative levels of CEA have been found to be of prognostic significance in some recent studies, high levels signifying poor prognosis.

The common technique for estimation is radio-immunoassay.

The field of oncofoetal antigens and their applications is fascinating and ever expanding. Of the more promising oncofoetal antigens a few may be mentioned here.

3. Gamma-foetoprotein found in 75% of practically all types of malignancies.

4. Foetal sulfoglycoprotein associated with gastric carcinoma and secretions.

5. Alpha-2-H-Foetoprotein related to several childhood neoplasms.

6. Beta-S- Foetoprotein formed by hepatomas.

7. Leukemia-associated which is also associated with lymphomas.

The present state of knowledge regarding the above and other oncofoetal antigens is summarized in Table 1.

TSTA'S OF UNDETERMINED ORIGIN

These are the antigens which are fomed by isolated tumours and are naturally less useful. They have mainly been studied in research institutions and have not found much clinical application except in evaluation of specific immune response to be presently discussed. These TSTA's are found in association with melanomas, certain tumours of brain, urinary tract, breast, leukemias, lymphomas,

TABLE I

Clinical Significance of Oncofoetal Antigen

Antigen	physiological occurence	Associated Benign conditions	Associated malignant tumours
Carcino-embryonic antigen	Foetal alimentary system. Foetal plasma	Girrhosis, ulcerative colitis, Crohn's disease, fibroadenoma of breast.	Pancreas, colon, rectum bronchus, liver, breast, neuroblastoma, genito-urinary tract, Leukemias & Lymphomas
Alpha-foeto-protein	Foetal liver & plasma Plasma of pregnant women.	Hepatitis, cirrhosis, ulcerative colitis, Crohn's disease.	Hepatoma, Teratocarcinoma, Choriocarcinoma.
Gamma-foeto-protein	Foetal gut, thymus, spleen, placenta.	—	75% of all malignancies
Alpha-2-H Foetoprotein	Foetal liver & plasma. Adult plasma	—	Childhood neoplasms Some adult neoplasms.
Beta-S-foeto-protein	Foetal liver (not serum)	Cirrhosis (plasma)	Hepatoma ? Other tumours
Leukemia-associated antigen (LAA)	Foetal tissues & Plasma.	—	Miscellaneous Leukemias, Hodgkins disease.
Foetal Sulpho-glycoprotein.	Foetal alimentary tract.	Peptic ulcer mucosa and secretions	Gastric carcinoma & Secretions (Not in plasma)
Placental alkaline (Regan) phosphatese	Placenta, Pregnancy Plasma	—	10 - 20% of tumours of breast, bronchus and alimentary tracts (Plasma)

(Modified from Kay 1975)

sarcomas etc. The only common factor among them is their undetermined origin.

EVALUATION OF THE IMMUNE RESPONSE

After reviewing the neoantigens and their role in diagnosis, let us now turn to the other aspect of immunodiagnosis namely the evaluation of the immune response of the patient. At the beginning of this presentation, a reference has been made to the role of immune surveillance to carcinogenesis. Further immunological studies have not only confirmed this but have found that in many neoplastic diseases the progression of disease is at least partly the function of the immune response of the patient to tumour.

The classical concepts of tumour immunity and host defence against cancer involve host control via thymus dependent T-lymphocytes with direct killing of target cells. Humoral antibodies were generally thought to 'block' the lymphocyte attack except in leukemias where cytoxic effects of humoral antibodies have been well known for some time. However, recent years have seen the end of this simplistic framework. A variety of immune mechanisms are known to be concerned in tumour suppression in addition to T cells and antibodies viz. activated macrophages, cell bound antibodies, the role of helper and suppressor cells etc. Also the blocking effect of soluble tumour antigens has became apparent. The various factors that have thus come to light have been subjects of study and a number of laboratory procedures have been evolved to study these. Only a few of these have well established or plausible clinical significance. Of these also, all are not technically perfected to be able to

cross the research threshold and be available in a clinical laboratory (12). only the barest mention of the more promising techniques is all that is attempted here.

These tests could be classified into those for (i) evaluating general immuno-competence of the patient and (ii) those for evaluatig the tumour-specific immune response.

Table 2 shows the various tests for non-specific immuno-competence separated into three groups testing the T-cell, B-cell and mixed immune responses. (The tests marked with asterick are available in our laboratory. The triangle-marked tests can be performed only in a special laboratory. The remaining could be performed if necessary and if the reagents are made available in our laboratory).

The T-cell dependent tests viz. primary and recall types of delayed hypersensitivity, allograft rejection, blastogenic response to phytohemagglutinin are all impaired in *Hodgkin's Disease* (13). Also the immunologic deficiency as assessed by these tests has been used for staging of the disease and correlated well with poor prognosis before it is markedly improved as a result of chemotherapeutic and radiotherapeutic advances.

As opposed to the above, in *Chronic Lymphocytic Leukemia* we observe almost exclusive deficiency of B-cell dependent immunity i.e. impairment of antibody production. The primary delayed hypersensitivity and other parameters of T-cell dependent immunity remain unaffected (14).

In the same manner, in *Multiple Myeloma* the B-lymphocytes seem to be primarly affected though, as the disease progresses, the T-cells may get secondarily involved.

In *Acute Leukemias* however, the B-lymphocyte function appears to remain intact till quite advanced stages of the disease. Not only is T-cell impairment obvious in early stages but also correlates well with poor prognosis (15). Particularly significant are sudden changes in cell-mediated immune functions. A sudden decline heralding relapse and a sudden improvement accompanying a recurrence. It should be noted however, that with cytotoxic chemotherapy the whole immune complex comes to be deficient in the later course of disease.

Mention may here be made of the immunologic classification of leukemias-especially the lymphatic leukemias. Though still in developing stages, the characterization of leukemias on the basis of the immunologic activity of the leukemic cell is increasingly becoming accepted as a diagnostic and prognostic aid (16). Thus

TABLE 2

Tests for Evaluation of non-specific Immunocompetence

System	Antigens/Reagents used
1. T-cell Test dependent system	
Primary delayed hypersensitivity	BCG, KLH, DNCB
Established delayed hypersensitivity	Dermatophytin, Candida
Skin allograft reaction	Allogenic skin
Blood T-lymphocyte level△	Sheep red cells
II. B-cell dependent system	
Serum immunoglobulin levels*	Immunoplates
Primary & secondary antibody response△	KLH, Flagellin, Diptheria and Tetanus toxoids*
Blood B lymphocyte level△	Fluorescein anti-Ig-G
III. Non-specific	
Lymphocyte blastogenesis	Allogenic lymphocyte; PHA
Blood lymphocyte level*	Routine
RES particle clearance△	Labelled IgG aggregates
Local inflammatory response△	Glass cover slip

* Available in Bahrain

△ Mostly confined to research laboratories

majority of leukemias and adult lymphomas seem to be of B-cell type; the T-cell leukemias appear to have poor prognosis and high incidence of mediastinal masses.

Unfortunately the status of these immunity tests in *Solid Tumors* is far from clearly established. Not all patients with solid tumours are immuno deficient. When present, immunodeficiency is more likely to involve the cellular component rather than the humoral one (12). Further, the immunodeficiency is likely to be less severe in certain histological types (viz. sarcomas, melanomas, adenocarcinomas of breast, colon and GI tract and genitourinary tumours) than in others viz. bronchogenic carcinoma or carcinomas of head and neck. It is of interest to note in the later group specially in bronchogenic carcinoma, even cured patients or patients under remission fail to regain immunocompetence, i.e. the immunodeficiency is irreversible. In such cases, there is usually a history of chronic exposure to carcinogens (viz. cigarettes, alcohol) which may have a direct influence on immunodeficiency. No further attempt will be made here to discuss the nature and causation of immunodeficiency in relation to solid tumours.

Table 3 shows the list of tests available for evaluation of tumour specific immunity. In these tests, the patient's own tumour provides the antigens viz. frozen sections, cell cultures, cell extracts etc. These are used to test the patients sera and lymphocytes to study the humoral or cellular immunity or the whole immunity complex.

1. *Test for humoral response* are probably the least complicated and include such tests as immunofluorescence, complement fixation, complement dependent cytotoxicity and immune adherence (10). These techniques have been applied recently to human melanomas and sarcomas. High antibody titres are correlated with good remission. However, false positives are a problem and one has to remember that all that fluoresces is not antigen !

2. *Tests for cellular response* include an intradermal hypersensitivity test using autologous tumour antigen, a positive test indicating good immunological response and hopefully, better prognosis. This test (also known as Makarie-Tee test) ha been studied in Leukemias, lymphomas, sarcomas and melanomas (17).

The technique of skin window coverslips bearing tumour extracts are repeatedly applied to microabrasions on patient's skin. The cell response thus elicited is harvested on the coverslips which are stained and examined. Presence of basophil-associated monocyte (BAM) aggregates signifies good response (18).

3. *Tissue culture techniques* have also been widely applied to study either the T-cell, B-cell or mixed immune response. In these tests the tumour cells or extracts and lymphocytes and / or sera of patients are allowed to interact in tissue cultures in different sets of conditions. Thus inhibition of tumour cell proliferation by patients lymphocytes or serum can be studied by colony-inhibition test (19). Similarly cytotoxic effect of patients serum, T-lymphocytes or B-lymphocytes can be studied by cytolysis, chromium release or dye-exclusion tests (20). The immunocompetence of the lymphocytes can be evaluated by their exposure in tissue culture to tumour antigens to observe blastogenesis.

Most of the above techniques are still under development in the research laboratories. Only a few of them have been standardized, but still have not become generally

TABLE 3

Evaluation of Tumour Specific Immunity

Response tested	Type of immunity	Techniques
Lymphocyte blastogenesis	T & B cell-mediated	Tissue culture
Toxicity to tumour cells	T or B cell mediated	Colony inhibition, Proliferation inhibition Cr-release, dye-exclusion
Anti-tumour antibody	B-cell mediated	Complement-fixation Immunofluorescens Cytolysis Immune adherence

available. The relative merits of each test are yet to be established. Hopefully in future an optimal battery of tests for evaluation of immuno-competence will emerge and provide an effective and reliable tool for immunological monitoring of tumour therapy.

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