Continuing Medical Education Series No. 6.
Ed. K. Pavithran, p. 34-39, 1988.

Immunology of cancer

D. M. Vasudevan

Immunology as a research speciality, particularly in the area of cancer study has become one of the very versatile branches of science. Ehrlich in 1909 (1) and Bashford in 1910 (2) were the first investigators to study the phenomenon of rejection of transplanted tumours, who thus laid the foundations for the immunology of cancer. A new era in this field was ushered by the demonstration of antigens by Foley in 1953 (3) and Prehn in 1957 (4). Concommittant with the malignant transformation, it is assumed that certain new antigens also appear, which are called the tumour associated antigens, and if the presence of these antigens are demonstrated by transplantation techniques, they are often called as tumour specific transplantation antigens. In most models studied, there appear only weak tumour associated antigens. The tumour antigens to which a host can most effectively respond, and hence reject, are those located on the cell membrane rather than in the interior of the cell. Klein, 1966)5

These antigens may represent either products of altered genes, or the expression of de-repressed genes that are dormant in normal cells. Neoplasms evoked by chemicals usually do not cross react at all even in tumours provoked by the same chemical at different sites in the same individual.(6) Generally speaking, antigens of chemically induced tumours are individually specific. On the contrary, the tumours induced by a viral agent usually possess cross antigenicity with tumours produced by the same type of virus elsewhere.(7) The viruses interact with nucleic acids of the host in a specific manner, resulting in the cross reactivity of antigens in viral-induced tumours. Continued presence of virus-derived genetic information was seen in the transformed neoplastic cells.(8) A third type of tumour associated antigen is the appearance of embryonal antigen on malignant cell. This is probably due to the de-repression of genes concomn.ittant with the de-differentiation process during the malignant transformation. The best examples of such embryonal antigens are the carcino-Embryonic Antigen which appears in circulation of patients with carcinoma of colon (Gold and Freedman, 965) (9) and the Alpha Feto-Protein detected in circulation of hepatoma patients(Abelev, 1968) (10) Many such embryonal tumour associated antigens are described recently such as Carinofetal ferritin, Beta chain of Human Chorionic Gonadotropin, Lactoferritin and Beta Onco Fetal Antigen. Since they are embryonic, such tumour-associated products are not antigenic in the tumour bearieg host, and hence are not rejection inducing. However such embryonal antigens are very useful for follow-up purposes.

Evidence for existence of tumour associated antigens in human tumour cells came from in vitro experiments showing that patients with tumour had circulating antibodies and mononuclear cells which were capable of killing their own tumour cells cultured in vitro.(11) They show a cross reactivity of a type which has not so far been encountered in animal tumours. Thus, tumour specific antigens of all meianomas appear to cross react but are distinct from antigens of other carcinomas. Carcinomas of the bladder do have a tumour-antigen; but it is different from antigens expressed on renal carcinoma cells.(11) Thus in general, human cancers of different histogenic origins have different tumour associated antigens, but those of the same histogenic origin show the same tumour specific antigens (13) It seems that these human tumour antigens detected by in vitro tests may be the expression of organ-specific embryonal antigens.

The concept of cell-mediated immunity being a fundamental defence mechanism against neoplasia, is put forward by Burnet(14) in his theory of the 'immune surveillance'. Malignant cells continually arise within the host from spontaneously occurring genetic mutations or from the action of chemicals, hormones, physical irritants or viral factors. These malignant cells will have altered cell surface antigens and so are recognised by the immune system and are rapidly annihilated. In accordance with this theory, it has been demonstrated that there is an increased incidence of viral and chemically induced tumours in neonatally thymectomised animals (15) or in persons receiving immuno-suppressive drugs (16).

The immunological effector mechanism operating against the tumour cells could be any one or in combination of the following different systems: 1. sensitised and cytotoxic T cells; (2) complement dependent antibody mediated system; (3) antibody dependent cell mediated cytotoxicity (ADCC); (4) normal killer cells (NK); (5) macrophages.

The target cancer cells could be directly lysed by the sensitised cytotoxic T cells. (17) This mechanism is independent of the presence of antibodies or complement. This cell mediated immune response is considered to be the most important mechanism for tumour allograft rejection. (18) An intimate contact between lymphoid cells and target cells is very essential before target cell destruction. Presumably the contact is achieved by specific receptors on the immune lymphoid cells. Cytotoxic effect of sensitised lymphocytes requires Ca++and Mg++, and is dependent on consumption of energy. T cell mediated cytolysis is exquisitely specific; non-antigen bearing bystander cells are not at all lysed (19) Simultaneous with the activation of T cells by the antigens, a group of soluble protein substances are released into the surroundings by the T cells. These are collectively known as lymphokines, which include Macrophage Migration Inhibition Factor; Macrophages are assuming increasing importance as an important factor in the tumour cell destruction. (20) Macrophages can kill the tumour cells either after activation by soluble factors released by sensitised T cells or after opsonisation with the help of antibodies. (21) The antibodies will cause target cell destruction by the following mechanisms (a) complement fixation (b) attracting the ADCC killer cells and (c) by opsonisation of the target cells and thereby making them more susceptible to macrophage activity. It is shown that leukemias are usually very sensitive to the cytolytic action of humoral iso-antibodies, while sarcomas are resistant. Thus cells having a higher concentration of surface antigens are more sensitive to cytotoxic antibodies. Similarly an increase in the ratio of antibody to tumour cells will also increase the cytotoxic effect of antibodies (22). These antibodies are very useful in preventing the blood-borne metastasis of cancer. The antibodies and complement components are freely available in blood, and so the metastatic malignant cells could be rapidly lysed intravascularly. However the antibodies may not reach in extravascular spaces in adequate quantities, and so their cytolytic potential will be restricted in the case of solid tumours.

The very existence of antigenic tumours implies that neoplastic cells have escaped the immunological surveillance mechanisms. Even though the body is mounting an immune resistance against cancer cells, often it seems inadequate, thus leading to growth of the tumour. There are many theories put forward to explain the possible routes of escape of tumour cells from the immunological restraint.

A general depression of cell-mediated immunity is noticed in malignancies. (23) Whether this depression is the cause or the effect of carcinogenesis is not very clear. (24) Specific tolerance to tumour antigens may be another reason for the non-rejection of the tumour. This aspect has ceen extensively studied in virally induced tumours. Inadeqate local response due to abnormal localisation of immunocompetent cells may be another reason for his depression of immunity in cancer patients. (25) Cytotoxic lymphocytes directed specifically against the tumour cells, when injected, did not reach into the tumour, but homed mostly to the gut and lymphoid organs. Such a behaviour gives a severe anatomical limitation

to immune response. If sufficient number of active cytotoxic lymphocytes are available at the tumour site, the tumour may regress. The beneficial effect of local painting of B. C. G. on the tumour tissue may be due to the recalling of the lymphocyte populations to the required sites. Moreover evidences suggesting the abnormal activity of suppressor T cells are also accumulating in the literature. (26) Another well-substantiated hypothesis is that the tumour specific antigens are masked by siala-glycoproteins, so that the immune lymphocytes could not recognise the malignant cells. (27) Removal of sialic acid from the tumour cell surface by enzymes leads to increased antigenicity of tumour cells in a wide variety of tumours. (28) The present theories regarding the escape mechanisms are mainly centred around the finding that soluble antigens released by the cytotoxic lymphocytes, thereby allowing the tumour mass to grow. (29) This inhibition of cytotoxicity of lymphocytes by the serum factor is shown to be reduced immediately after the removal of the tumour mass. (30) Further the inhibitory serum factor has shown to combine with cytotoxic lymphocyte but not the tumour cells. Thus the antigens released from the tumour cells can effectively combine with the receptors on the effector lymphocytes. The inhibitory factor may be the antigen-antibody complexes (31).

A balance of so many factors in the immunological effector mechanism will determine crucial question of whether a tumour is rejected or not. The immune system may act as a schizophrenic personality. Whereas the immune "Dr. Jekyll" protects the host from cancer cells, the perverse "Mr. Hyde" apparently protects the cancer cells from hosts attack. This dichotomy in immune activity reflects its ability to form cytotoxic cells and antibodies on the one hand, and enhancing the inhibitory factors on the other.

The standard forms of treatment such as radiotherapy, chemotherapy and surgery leave some cancer cells in the body. These residual cancer cells will then start to multiply leading to recurrence and culminating in fatal outcome. It is here, the immunotherapy promises a bright future. When the tumour load is reduced by the conventional treatment procedures the remaining cells could be destroyed by an appropriately activated immune response of the body through a well-controlled immunotherapeutic schedule. Immunotherapy can be an effective tool to tackle with the problem of residual cancer cells provided technically safe and operationally effective, therapeutic schedule is being formulated. A somewhat sceptical examination of the achievement of immunotherapy todate reveals a startling lack of success. However, it is such a potent idea, such an exciting concept, that it cannot be simply set aside. A lot of work has been done on nonspecific immunotherapy using B.C.G., levamisole, and such other immunostimulants, without much encouraging results. Active specific immunotherapy by using purified tumour associated cell surface antigens still remains a long term ambition. A very promising approach to the problem is immuno targetting.(32) We are working on these lines. Our aim is to isolate specific antigen from tumour cells(33) produce specific antibody, tag the antibody with anticancer drugs, then to develop the antibody-drug complex with liposomes. These liposome encapsulated drug is being injected to tumour bearing mice. In these case, the antibody will direct the anticancer drug specifically towards the cancer cell, so that even small doses of the drug may be curative, with minimal undesirable side effects. The initial results on this immunotargetting approach are very encouraging.(34)

It will take a long time to get a clear answer on viruses with human cancers. Many pioneers like Gross are of opinion that all human cancers are of viral origin; equally great authorities on the subject are convinced that no viruses are related with human cancers. Perhaps the truth may be in the middle of these two extremes; some human neoplasms may be associated with viruses. In research into human diseases, epidemiology often has to replace direct experimentation. The cell membrane of fresh and cultured Burkitt's tumour cells have been shown to possess new antigens not pressent on the surface of bone marrow cells from patients. All patients with Burkitt's lymphoma have Epstein—Barr viral antibodies

in very high titres, whereas in normal children of the same age and tribe, antibodies are usually absent or low in titre. It is interesting to note that the E. B. virus is not demonstrated in Burkitts lymphoma cells until they have been grown in culture for some time. This shows that the infective viral particles are produced only when the effect of the immunological mechanisms are removed. Similarly very high titres of anti-EBV antibodies are shown to be present in circulation of nasopharyngeal carcinoma patients, especially of Chinese origin. During the last 10 years evidences are accumulating on the asscciation of herpes simplex virus as well as human papilloma virus with the genesis of human uterine cervical carcinomas. Probably the latency of the virus and the malignant potential are very much linked. It is known that herpes virus can remain inside the host cells for many years. The biological process of transformation into malignancy is also known to take many years. Perhaps the latent virus remaining so long time, side by side with the host DNA, has got an increased chance to be integrated with the cellular DNA, thereby progressing into malignant transformation. The idea is attractive enough as a hypothesis. But the co-factors, promotors, and initiators of the transformation processes are yet to be identified.

Oral cancer constitutes about 30% of all human cancers seen in our region. (35) Out of 1000 oral cancer patients, 60% showed the presence of HSV_1 antibodies, as against 39% in 300 healthy control subjects. (36) It may be argued that any virus could non-specifically invade the cancer cells leading to these results. To rule out this possibility, as a negative control, antibodies against adenoviruses in 200 oral cancer cases and 151 controls were tested. Both HSV_1 and adenovirus infections are common in this region, as shown by the control values. But only HSV_1 antibodies, but not the adenovirus antibodies did show any increase in oral cancer group. When HSV_1 antibody titres in cancer groups compared with normals, the percen age positivity at each titre was more in oral cancer patients, as compared to normal controls. Thus the anti-HSV antibodies are not only more prevalent in cancer group, but also that the patients had increased titre values.(37) The HSV_1 antibodies in patients who came for follow up at regular intervals were assessed. Among the 20 patients thus studied, 60% had decreasing titres during the follow up period. This shows that the stimulation to produce antiviral antibody was lost when the tumour was clinically removed. In certain patients, the antibody titre, was seen to be increased, may be as the forerunner of the recurrence of the disease. Fluorescence studies were also conducted using anti-HSV_1 antibodies.(38) HSV_1 infected vero cells served as positive controls and non-infected vero cells as negative controls. Among the 20 normal control specimens 77% showed brilliant fluorescence. The specificity of the reaction was confirmed by the loss of fluorescence when the antiserum was treated with HSV_1 infected vero cells. Anti-HSV_2 serum showed positive fluorescence in 30% specimens, which could be explained by the similarity between the two viral strains.(39) Since the HSV_1 antibodies are more in oral cancer patients, and the HSV_1 antigens are seen on the oral cancer cell surface, it is worthwhile to test whether the HSV genome is present in oral cancer cells. For this, the DNA from cancer specimens were extracted, denatured, precipitated on nitrocellulose membrane and hybridised with radioactive nicktranslated cloned HSV probes under stringent conditions. It is seen that HSV probe was hybridised with 56% of 100 oral cancer cases studied and 38% of 50 cervical cancer cases (40) Further human papilloma virus (HPV_{16}) was seen to be hybridised with 10% oral cancer and 37% of cervical cancer specimens. The normal cells did not hybridise with viral probes. The HSV and HPV genes are generally mutually exclusive, as very few samples contain both viral genes concurrently. The above experiments strongly shows the relationship of HSV with oral cancer. The HSV antibodies are consistently higher in oral cancer patients, the HSV related antigens are shown to be on the oral cancer cells and the HSV probe could be hybridised with the DNA from oral cancer, specimens In view of the present study, it can be assumed that the HSV may play a role as promoter or co-factor in the etiology of

oral squamous cell cancers. Further work is in progress to assess whether the HSV genome is integrated into the host DNA.

At first scientists showed that certain DNA sequences of animal oncogenic viruses are required for transformation ability, and these are denoted as oncogenes. Hower later on it has been shown that similar DNA segments are available even in normal cells. Oncogenes are now shown to be having certain essential functions in the cell cycle of normal cells. It is hypothesised that viruses are introducing alternate promotor sequences to the already present oncogenes in the cells, thereby giving endless duplicating ability to the transformed cell.

After all viruses are jumping genes. Virus is the only efficient mechanism for horizontal transmission of genes. And evolutionary process is quicker, and perhaps possible only by rapid horizontal transfer of genes. Paleogeologists are of opinion that the organisms with exoskeleton has appeared throughout the world almost simultaneously. For the production of exyskeleton, alkaline phosphatase enzyme is necessary. It is assumed that the gene for alkaline phosphatase is evolved in a few organism, which has been very rapidly transferred horizontally within a very narrow geological time interval. There are also many other arguments to show viruses are very essential for evolution. In that case, cancer is the deferred payment by the individual members of the society for the benefit accrued by the Society as a whole.

Our immediate task is to show conclusively whether herpes viruses are oncogenic or not. In the case of EBV and Marek virus this has been more or less done, though co-factors are yet to be identified. Other members of the herpes group are to be analysed systematically, and their co-factors are to be evaluated. If virus has been shown to be a causative agent to cancer, prevention may be possible by immunisation. Immunological intervention has already been successful in Marek's disease. Scientists are actively thinking on a similar embarkation on EBV related diseases. When the cause is known prevention can be tried. Knowledge is power and our immediate task is to acquire fresh knowledge on suspected oncogenic viruses

It is correctly said that human destiny is in cross roads. Our generation has acquired the capacity to superkill our own species, by thermonuclear reactions, which can also be utilised to make an utopia on earth. In the macrocosmic level, our generation has witnessed the first human landing in moon; followed by unmanned landings in other nearer planets and the voyager journeying outside the solar system. In the microcosmic level, discovery of so many subatomic particles together with the quantam philosophy have erased the boundary between physics and metaphysics. In the realm of biology, ours is the first generation which had acquired the knowledge and power to carry out genetic engineering. Already we have instructed *E-coli* to produce human insulin We are doubly fortunate to be alive at a time when we could understand the ways of nature in a better manner, and we could influence our surroundings logically.

References

1 Ehrlich P: The collected papers of Paul Ehrlich, Vol.2, Immunology and Cancer Research, P. 550, Pergamon Press, London.

2 Bashford E J: Proc Roy Soc London, Series B, 1910; 82:298.

3 Foley E J: Cancer Res, 1953;13:835.

4 Prehn R T: J Nat Cancer Inst, 1957; 18:769.

5 Klein G: Ann Rev Microbiol, 1966; 20:223.

6 Globerson A and Feldman M J:J Nat Cancer Inst 1964; 32:1229.

7 Klein E and Klein G:J Nat Cancer Inst, 1964;32:547.

8 Klein G, Clifford P and Klein E: J Nat Cancer Inst, 1967;39:1027.

9 Gold P and Freedman S O: J Exptl Med, 1965;121:439

10 Abelev G I: Cancer Res, 1968;28: 1344

11 Hellstrom I, Hellstrom K E, Sjogren E O et al: Int J Cancer, 1971; 7:1.

12 Bubenik J, Perlmann P, Helmstein K. Int J Cancer, 1970; 5:39.

13 Bubenik J and Perlmann P: Int J, Cancer, 1970;5:310.

14 Burnet M F: Brit Med J, 1965;1:338

15 Law L W,: Cancer Res, 1966;26:551,

16 Zukoski C F, Killen D A and Ginn E.: Transplantation, 1970;2:71.

17 Vasudevan D M, Brunner K T, Cerottini J C.: Int J Cancer, 1974;14: 301.

18 Brunner K T, Plata F, and Vasudevan D M.: in Host Defence Against Cancer and its Potentiation, Ed, D. Mizuno et al, Univ of Tokyo Press, P.43,1975

19 Vasudevan D M, Brunner K T. and Cerottini J C,: British J Cancer, 1973; 28: (suppl) 35.

20 Prabha B Kumari T K, and Vasudevan D M.: Europ J, Cancer, 1984;20:891

21 Vijayakumar T, Ankatail R, Remani P and Vasudevan D M: Cancer Immunol Immunotherapy, 1986;22:76.

22 Ravindran A, Remani P, Vasudevan D M, and Vijayakumar T: Cancer Journal, 1986; :135.

23 Vijayakumar T and Vasudevan D M,: Cancer letters 1985;27:339.

24 Vijayakumar T, Sasidharan V K and Vasudevan D M: Ind J Cancer, 1984;21:7.

25 Vasudevan D M and Vijayakumar T: Ind J Cancer, 1977;14:345.

26 Prabha B and Vasudevan D M: Cancer, 1983;52:1837.

27 Pillai S, Sasidharan V K and Vasudevan D M.: Indian J. Cancer, 1981 18, 258,

28 Vasudevan D M, Balakrishnan K and Talwar, G.P.: Int. J. Cancer, 1970, 6,506.

29 Vijayakumar T, Remani P and Vasudevan D. M.: J. Exp. Clin Cancer Res., 1986, 5, 257.

30 Raghunath P. N, Joseph C. D. and Vasudevan D. M.: Ind. J. Clin. Biochem 1987, 2, 85.

31 Raghunath P. N., Joseph C. D. and Vasudevan D. M.: J. Exptl. Clin. Cancer Res., 1987, 6, 173.

32 Vijayan K. K., Remani P. Vasudevan D. M.: J. Exptl. Pathol., 1987, 3, 295.

33 Abdulkader M., Ravindran A. and Vasudevan D. M.: Ind. J. Med. Res. 1981, 74, 428.

34 Nirmala K. and Vasudevan D. M.: Ind. J. Cancer, March 1988.

35 Padmanabhan T. K. and Vasudevan D. M.: Ind. J. Cancer, 1982, 19, 189

36 Kumari T. V., Shanmugam J. and Vasudevan D. M.: Ind. J. Med. Res. 1982, 75, 590.

37 Kumari T. V., Tankamani H. and Vasudevan D. M.: Ind. J. Cancer, 1984, 21, 137.

38 Kumari T. V., Vasudeuan D. M. and Ankathil R.: J. Exptl. Pathol., 1987. 3, 75,

39 Thankamani V., Kumari, T. V. and Vasudevan D.M.: J. Exptl, 1985, 2,123

40 Vasudeuan D. M., Kumari T. V. and Kuruvilla K.: Proceedings of Indo-US Workshop on Viruses and Human Cancer, p.37,1986.