The International Handbook of Medical Science

SECOND EDITION

A daily reference book for medical practice

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Editorial

The International Handbook of Medical Science is designed to provide a practical and intelligent source of daily reference for medical practice. The first edition proved particularly successful and we were most appreciative of the many letters and comments we received.

The second edition is a development of the first. The two major sections on drugs in current use and on the therapy of common diseases have been up-dated and much new information has been included. A discussion on the use of drugs in pregnancy is also included. In addition, however, there are a considerable number of completely new features. The reference section in particular has been considerably expanded and now covers such important subjects as drug addiction, alcoholism, modern contraceptive practice and electrocardiography.

It has been decided to reduce the number of articles on recent advances in medical science but instead to choose for coverage a limited number of subject areas with particular current interest to a wide readership.

We hope that this second edition, with its many innovations, proves to be a worthwhile development of the first. The aim of the volume remains the same: to provide in an intelligent and constructive way much of the information that is needed in medical practice but which it is often difficult and time-consuming to obtain.

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March 1972

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Section A

Recent Advances in Medical Science

Sir Macfarlane Burnet presents a detailed account of current developments and contemporary research into immunological response to cancer. Possible applications at the clinical level are described.

Recent Advances in the Study of Cancer Immunity

by

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At the present time there is a high level of interest both by immunologists and by surgeons in the topic of cancer immunity. This is based on the findings from a wide range of malignant disease, both clinical and experimental, that immune responses can occur against the patient's or animal's own primary (autochthonous) tumour. Responses are not always demonstrable and even when they are there is often little evidence that their presence can inhibit tumour growth. Nevertheless there are strong reasons to think that the process which I called immunological surveillance some years ago is constantly in action to inhibit at least a proportion of initiated neoplasms and that in the absence of an immune response there would be strikingly larger numbers of tumours and a shift of their incidence to lower ages. In principle at least there are potentialities for the application of immunological methods in the therapy of some forms of cancer.

The basic experimental findings

In 1953 Foley [1] observed that when fibrosarcomas were induced in mice by injection intramuscularly of methyl-cholanthrene (MCA) the tumours could be transferred to other mice of the same pure line (syngeneic) strain and that if a tumour was excised after a period of growth the animal was significantly resistant to inoculation with cells of the same tumour. It showed, however, no resistance to the cells of a similar tumour induced by the same dose of MCA in another mouse of the same strain. This type of experiment has been widely used in mice, rats and guinea pigs and the broad pattern of the results can be succinctly stated as follows.

Within a pure line strain tumours induced either as fibrosarcomas after intramuscular inoculation or as hepatomas after feeding or intraperitoneal injection of a carcinogen show varying degrees of immunogenicity, and of malignancy as judged by the latent period before a

standard sized tumour develops. Some of the tumours produced are not demonstrably immunogenic, but when an immune response occurs it is specific to the strain of tumour. It seems likely that there is some limit to the number of new antigenic specificities which can be manifested by such tumours, but there is as yet no record of two identically patterned tumours being produced in different animals. The immunity is of the cell-mediated type involving cells of the T-system and is analogous to homograft immunity. With guinea pig tumours it has been shown directly to be associated with typical delayed hypersensitivity. In some instances no immunity can be shown while a tumour is actually growing or for a few days after its removal. After a week or two immunity becomes evident by the failure of a standardized inoculum of tumour cells to give rise to a visible tumour.

A wholly different result is obtained when tumours are produced in suitable laboratory rodents by the standard oncogenic viruses, polyoma and SV40. If polyoma virus is inoculated into new-born mice or hamsters, multiple tumours in various organs are produced, predominantly in the salivary glands of mice and in the kidneys of hamsters. Inoculated into adult mice the virus produces no tumours. When, however, a typical tumour has been produced in a mouse inoculated neonatally it can be transplanted serially in mice of syngeneic strain. In the process of passage the virus disappears, i.e. its presence as virus – technically as virions – is not necessary to the continued malignant character of the cells.

By quite straightforward experiments it can be shown that the virus-free tumour has developed two or more new antigens, the most important of which from our point of view is the TSTA (tumour-specific transplantation antigen). These antigens are common to all polyoma-induced tumours and are assumed to result from the incorporation of part of the virus DNA in the chromosomes of the malignant cell. The simplest way to demonstrate TSTA is to transplant polyoma tumour cells, induced and transferred in strain A, into a genetically different B strain of mice. After an initial 'take' the tumour is destroyed by a typical homograft reaction since it carries the foreign histocompatibility antigen A. In the process, however, host B develops immunity against TSTA and when subsequently tested with cells of polyoma raised in B no tumour results. It is also found that when an adult mouse of strain A is inoculated with cell-free polyoma virus of any source it becomes resistant to later inoculation with polyoma cells from an A mouse.

This is important because it indicates that in the adult, initially non-immune mouse the virus 'transforms' cells so that they develop the characteristic TSTA and produce small foci of potentially malignant cells. A rapid immune response against TSTA, however, ensures that the foci are destroyed before they become evident. In the neonatal mouse the capacity to respond immunologically is very poorly developed and the tumours enlarge. By the time immune competence should be attained sufficient TSTA is being produced by the tumour to paralyse the immune response. The animal is now tolerant to all the new antigens of the tumour and it grows unhindered.

Similar phenomena are shown with SV40 virus and tumours, but the new antigens induced are quite distinct from those of polyoma. Very recently it is being reported that more detailed experiments indicate that some of these virus-induced tumours may show other new antigens in addition to the major ones for which the virus is responsible.

Clinical evidence for the importance of cancer immunity

No direct experimental study remotely resembling what can be done in pure line mice is possible with human cancer and evidence for the existence and significance of immunity against malignant cells must be obtained by rather indirect and statistical methods. In general it is limited to observations on the frequency of spontaneous malignant disease in defined populations and serological or other *in vitro* evidence of immune response in an individual to his own tumour. It is, however, highly significant that a tiny proportion of established histologically-diagnosed cancers retrogress spontaneously, and on a larger scale there seem to be more recoveries after standard treatment than would be expected if every surviving cancer cell could re-initiate a malignant process. There are several features about these findings which suggest that immune responses are concerned.

If the process of immunological surveillance is of any importance we should expect to find an excess of tumours in persons whose immunological system was grossly deficient or paralysed by immuno-suppressive drugs. This is in fact the case. All the major immuno-deficiency syndromes which with adequate treatment can allow the affected infant to survive for more than a few years show a disconcertingly high incidence of malignant disease with lympho-reticular neoplasms conspicuously frequent. Even more striking has been the experience of patients who have received a transplanted kidney and

survived over a year. The most recent figures I have found (Schenck and Penn [2]) record 52 primary malignant tumours in about 5,000 renal transplants, and suggest that their own experience in Denver covering eight years, with 11 tumours in 184 cases, indicates that the real incidence must be considerably higher than the 1 per cent indicated by the global figures. Of the 52 tumours there were 18 superficial carcinomas of skin, lip or cervix and 10 epithelial tumours of viscera. There were 22 lymphomatous tumours, including reticulum cell sarcoma, of which 11 involved the brain. All these figures are very much higher than the expected incidence in the relatively young subjects concerned. The average age of mesenchymal tumour patients was 30 and of those with epithelial tumours 37 years. The incidence of miscellaneous epithelial tumours is also well above the expected level, but experienced investigators, e.g. Richard Doll, feel that there may be reasons for not taking some of these figures at their face value. No one, however, has the slightest doubt that both in genetically immunodeficient children and in patients maintained for a year or more on immuno-suppressive drugs lymphomas and related tumours are far more frequent than in normal people. The simplest interpretation is that immunological surveillance is of special importance in relation to incipient malignancies of lymphoid cells.

Spontaneous cure in relation to cancer immunity

It is convenient to combine the discussion of spontaneous cure of cancer with evidence for immune responses in man. The main evidence that spontaneous cure is an immunological process depends on the fact that it is just those cancers most frequently showing retrogression which show the best evidence for an immune response by conventional techniques. Everson and Cole [3] found acceptable evidence of spontaneous regression of cancer in 176 individuals. Four tumour types were responsible for 98 of those cases – hypernephroma 31, neuroblastoma 29, malignant melanoma 19, chorioncarcinoma 19. In 1966 Burkitt's lymphoma had only recently been described and it is not mentioned in Everson and Cole's study. This is the well known tumour widely prevalent in the malarious areas of tropical Africa and now known to occur in other parts of the world. Since then every aspect of Burkitt's lymphoma has been intensively studied and it is almost certain that if early diagnosis and follow-up studies were adequate a considerable proportion of spontaneous cures would be observed. David and Burkitt [4], for instance, reported long-term remission

and probable cure of four patients treated with hexamine in 1961. There is also the well known ease with which a substantial proportion can be cured with cytotoxic drugs such as cyclophosphamide. Burkitt lymphoma will therefore be added to the other four types of tumour for further discussion.

For some reason little immunological work has been done with hypernephromas, but there is an extensive literature about each of the others. Malignant melanoma has probably been the most widely studied. Antibody in patients' serum has been demonstrated by fluorescent methods and by cytotoxicity for autologous tumour cells (Lewis *et al.*, [5]). Antigens extracted from the autologous tumour gave reactions of cutaneous hypersensitivity in patients with localised disease but not in those where the tumour had generalised (Fass *et al.*, [6]). Cell-mediated immunity was shown by Hellström *et al.* [7], by using patients' lymphocytes against autologous tumour cells and assessing colony inhibition.

The bad reputation of malignant melanoma is clear enough evidence that these various immune responses are rarely effective in eliminating the tumour. On general grounds one would expect that cell-mediated (T) immunity would be the important controlling factor and the cutaneous hypersensitivity tests of Fass *et al.* suggested that as long as this was demonstrable the tumour remained localised. The circulating antibody studied by Lewis *et al.* behaved in much the same fashion and in some cases antibody present when first tested disappeared as the disease progressed. They distinguished two types of antigen by the type of fluorescence shown on target tumour cells. One was highly specific, being shown only with autologous tumour and serum; the antigen was restricted to the cell surface. The second antigen-antibody reaction showed almost complete cross reaction between different patients and the antigen was present in the cytoplasm. Cross reaction was also seen in the Hellströms' experiments with a colony-inhibition technique, but there is a suggestion in some of their protocols that autologous cells were somewhat more susceptible than allogeneic ones.

Burkitt lymphoma results are difficult to interpret owing to the frequent association of the herpes-like EB virus with tumour cells. If we forget about this and consider the tumour simply as a clone of malignant cells with a new antigen, the results described by Fass *et al.* [8] are of special interest. Thirteen patients with Burkitt lymphoma were skin-tested with autologous cell extract before and after treatment with cyclophosphamide. The results fell into three groups. One case

only was positive before treatment and remained so after effective treatment. Seven cases negative before became positive after treatment; all responded satisfactorily to the drug. Finally, five cases were negative both before and after cyclophosphamide; four of these relapsed. The suggestion is strong that an effective T-type response is necessary to 'finish the job' after treatment with a cytotoxic drug.

Chorioncarcinoma is unique amongst malignant tumours in being composed of cells derived from the foetus and therefore genetically distinct from the patient. On general grounds, therefore, one would expect that the presence of alien (paternal) antigens on the tumour cells would result in their rapid immunologically-based rejection. It is evident, however, that a chorioncarcinoma has a similar lack of antigenicity to that shown by trophoblast cells from which it is derived. This has been ascribed to the presence of a sialic-acid compound, presumably a glyco-protein, on the cell surface. The resistance to immune response is, however, not absolute and again the frequent satisfactory outcome with cytotoxic drugs makes it likely that immune processes play a part once most of the malignant cells have been destroyed.

Finally, a few words may be said about neuroblastoma, a common tumour of infants and children, well known to be particularly amenable to surgical treatment. If histological study of the adrenal is made as a routine in autopsies of children under 3 months of age, many more neuroblastoma foci are found than would be the case if all developed into clinically evident tumours. Beckwith and Perrin [9] found the incidence was approximately 10,000 times that of the clinically evident tumour. Although it has been suggested that the failure of such foci to develop results from the maturation of the neuroblastoma to a benign ganglioma, it seems more likely from Hellström's work on cell-mediated immunity in patients with such tumours that immunological surveillance is responsible for their disappearance.

The nature of tumour-specific antigens

There is no doubt that a tumour may carry antigens not present in the normal tissues of the individual in which it has arisen. The origin of such antigens is, however, controversial and there are several possibilities which for the most part are not mutually exclusive. There are three groups of tumour in which the situation seems to be reasonably clear.

(1) Most carcinomas of the human colon produce a tumour-specific antigen which appears to be identical with a foetal protein normally present in various parts of the gastrointestinal tract.

The other two are the experimentally-induced tumours already mentioned in the introductory section.

(2) Tumours produced by chemical carcinogens which show a wide range of individual antigens and a spectrum of immunogenicity ranging from high to nil.

(3) The virus-specific tumour antigens induced by some of the oncogenic viruses.

Before considering human tumours from this point of view it is worth discussing some of the theoretical possibilities as to how these antigens arise. If such discussion is not to be interminably qualified, certain conditions must be accepted for the time being as if they were axiomatic.

(1) A tumour is a clonal proliferation initiated from a single cell which has undergone an inheritable change expressible as a somatic mutation and not interfering with the viability of the cell line.

(2) Somatic mutation in the broad sense becomes clinically demonstrable only when it results in a proliferative advantage to the altered cell and its descendants.

(3) Every somatic cell carries the genetic potentialities of the whole organism to which it belongs, including potentialities normally expressible only at particular stages of embryogenesis and development.

With this background something may first be said about the possible origin of somatic mutations. I should like to introduce a suggestion here, initiated so far as I am concerned by Professor Richard Doll, in discussing informally the curious regularity of the age-specific incidence of cancer by which (with some 'fiddling' in a few cases) it takes the form of a straight line when plotted logarithmically on both axes. Almost the only necessary conditions for such behaviour are that rare and random circumstances should act with equal likelihood *over the relevant period* to produce some continuing and cumulative effect. Doll showed that most of the apparent exceptions could be brought into line by making some reasonable assumptions about the period over which the mutagenic [carcinogenic] agent was operative.

When the rule holds effectively over the whole of life one must assume that the mutagenic influence is always acting and we can postulate thermal agitation or background ionising radiation from cosmic rays or terrestrial radioactivity. The deviations of lung cancer

age-incidence can be rationalised by considering the experience of cohorts of contemporaries in relation to the changing history of cigarette smoking. The remarkably steep straight line for prostatic cancer can be brought down to the standard slope of 4–5 by assuming that the mutagen concerned is active only from the age of 35 onward.

This approach fits neatly with some suggestions on the nature of carcinogenesis that I have recently elaborated (Burnet, [10, 11]). They are based on the very generalised hypothesis that a wide variety of 'non-biological' molecules, i.e. substance for which there is no evolved mechanism capable of handling them effectively in the body, may find opportunity to enter the genetic compartment of the cell and damage information-carrying DNA while still leaving the cell viable. Such damage must necessarily be minimal if the mutated cell is to survive and have descendants. It will be based on *chemically* definable changes but informationally the changes are likely to be as random as those of any spontaneous mutation. Where experimental oncogenic viruses are concerned, the intrusion into the nuclear mechanism will in general be at random but complicated by the possibility of viral nucleic acid being fused into the cell genome.

The origin of new antigens which can provide a basis for immunological surveillance or perhaps future immunotherapy of cancer can now be formulated:

(1) By definition any informationally random episode, if it is to induce a cell to initiate a malignant clone, must so modify the cell that it and its descendants have an inheritable proliferative advantage over its unmutated congeners. Usually this will be associated with a cell surface change – loss of contact inhibition for example – presumably involving both the function and detailed structure of one or more genetically coded receptors in the cell membrane. Similar random changes may also involve other cell membrane proteins not directly concerned with malignant character.

If the damage is to structural genes, change expressed in the cell membrane proteins will be in amino-acid sequence. Depending on circumstances, these may or may not modify function or confer autoimmunogenicity on the protein. Either may happen without the other.

(2) Foreign intrusion into the genome is at least equally likely to cause inheritable disturbance in non-structural operational aspects of the genetic system of the cell. In the book already referred to I have discussed at length the variety of 'inappropriate' components that can be produced by cancer cells (Burnet, [10]). It appears that once a malig-

nant clone is initiated there is either some secondary loss of integration of nuclear function or an increased vulnerability to casual mutagens. Every somatic cell exposed to an appropriate degree of random damage will be capable in principle of sometimes (even if the likelihood is almost infinitely rare) producing any protein that any cell – embryonic, foetal or adult – of the organism can make. If the protein in question is a highly active pituitary hormone it will have an immediately evident clinical effect if the clone producing it expands to more than minimal size. If it is a tissue-specific 'inaccessible' antigen to which normal immune tolerance is not provoked, severe autoimmune disease may be initiated. There are steadily increasing numbers of clinical reports of conditions that can probably be ascribed to such processes.

(3) Finally we come to a matter of great current significance: the appearance with some regularity in tumours of colon and rectum of a foetal antigen normally produced by cells ancestral to the intestinal epithelium. It would be by no means unlikely that most active cancers should produce proteins characteristic of foetal cells of the particular system concerned. The special interest of cancers of the lower bowel may be due merely to the fact that the antigen CEA (carcino-embryonic antigen) can readily be detected in the circulating blood. If the embryonic antigen had disappeared well before birth, the body is unlikely to have any intrinsic tolerance and there will be possibilities that antibodies may be produced against it or clones of T-immunocytes of appropriate specificity develop.

The manifestations (clinical and laboratory) of immune response in malignant disease

It is only too clear that any immune response to clinical malignant disease is usually ineffective and there is plainly scepticism in many quarters as to whether immunological surveillance is 'really' of any human significance. Nevertheless I can also sense that with the established facts of specific tumour antigens and of effective immune responses in a number of experimental situations there is an even wider optimism that some form of immunotherapy for at least a proportion of cancers will be developed. If this is to happen it must be by an understanding and exploitation of the types of immune responses to malignant cells which can be studied in the laboratory.

It is probably desirable first to recapitulate for readers not familiar with recent ideas in immunology the modern division of immune responses into two systems. The T-system is so called because the cells