Theoretical and Literary Evidence for the Existence of the Passive Antitumor Defence System

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It is well documented that despite global abnormalities of the immune system in AIDS and other immune deficiency diseases or in immunosuppressed patients the incidence of only a few kinds of tumour increases and even in the development of tumours in question the degree of immunosuppression seems not to be a critical factor. It results from this that the known immune system has no significant role in the mechanism that prevents the development of tumours. Consequently, the fact that tumours do not develop in the majority of the population during their lifetime, indicates the existence of other defence systems. We assumed that the defence is made by small substances of the circulatory system. Substantial evidence exists that the uptake of the majority of these substances is increased and unregulated by tumour cells and proportional to their availability. This feature of tumour cells may be fatal when the number of cells is still low and there are abundant substances in their environment because some substances may be toxic if their concentrations can reach high level in the cells. Thus, the arising cancer cells die in the majority of the population during their lifetime if the number of cells arisen is not too high, or the concentrations of the required substances are not too low. To our hypothesis, the above effects of the physiological mixture of the given molecules in the blood form the Passive Antitumor Defence System (PADS). This hypothesis is confirmed by our experiments and supported by epidemiological, clinical observations and other literary data.

Key Words: Immune response in AIDS; immune system; small substances of the circulatory system; synergistic tumour cell killing effect; passive antitumor defence system; PADS.

INTRODUCTION

It is well known that full-blown AIDS is associated with substantial loss of virtually all cellular and humoral immune responses. The degree of that is well demonstrated by rejection-free renal graft survival in a patient with AIDS despite the significant and prolonged withdrawal of the usual immunosuppressive agents. Relying upon these findings, the incidence of all or almost all kinds of tumours should increase in AIDS populations if the known immune system were the only mechanism to prevent the development of tumours. However, the incidence of only some kinds of tumours, mainly Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL), has significantly increased. On the basis of time-related changes in the percentage of AIDS-related KS and the observed large differences in incidence between homosexual and heterosexual,
haemophilic, and injection drug user AIDS patients and that an increase in the incidence of KS and NHL has not yet been described in children with AIDS," it can be stated that even in the case of the above-mentioned tumours the reason for the high incidence is not the defects of the immune mechanism but other agents." These findings strongly support that the known immune system has no significant role in the mechanism preventing tumour development. This statement is further supported by the following observations: The majority of clinically relevant tumours are not or are only weakly immunogenic. There is no increase in common tumours (carcinomas of the breast, colon, lung, prostate, etc.) in primarily immunodeficient children or in immunosuppressed adults (usually transplant recipients). Also in immunosuppressed organ allograft recipients only some kinds of malignancies, primarily KS, have a higher incidence than in normal populations. Patients with selective immune deficiency disease (e.g., lepromatous leprosy) show no evidence of increase in tumours. The explanation that natural killer cells and macrophages have a tumour preventive role in the above cases is refuted by many publications. The diminished cytotoxic capability and pool sizes of natural killer and lymphokine-activated killer (LAK) cells, abnormal function of monocytes and macrophages, etc., and the change of tumour incidence independently from these in AIDS supplies additional evidence for this refutation.

Were death of "nonimmunogenic" tumour cells an absolutely accidental event, the simultaneous development of a number of primary tumours in organs should be a relatively frequent occurrence. However, the development of even double synchronous primary tumours is rather rare. Thus, the fact that tumours do not develop in the majority of the population during their lifetime can only be explained by the existence of other defence systems.

THE PASSIVE ANTITUMOR DEFENCE SYSTEM

It is obvious that the components of a general defence mechanism (a "surveillance") must be in the circulatory system. It is well known, that different small substances (amino acids, monosaccharides, nucleobases, vitamins, membrane permeable intermediates of the cell metabolism, etc.) of the circulatory system can reach and enter both normal and tumour cells because their presence is fundamental for cell functions. Their uptake by normal cells is regulated, but there is abundant evidence that the uptake of the majority of these substances by tumour cells is increased, unregulated and proportional to their availability. A net flux of them occurs towards the tumour cells and they may attain high levels relative to the levels in surrounding normal tissues. Thus, the cancer cells have a selective reproductive advantage over their normal neighbours in the competition for these substrates. According to our speculation, this feature of tumour cells provides them an advantage over normal cells only when there are many tumour cells, and they have to compete with normal cells for these substances. The same feature, i.e., the increased, unregulated uptake of some of these substances may be fatal for arising tumour cells, when the number of cancer cells is still low and the amount of the given substances in the environment of cells is abundant. Under these conditions the tumour cells accumulate the above substances and it can be assumed that some of these substances may be toxic for cancer cells and can kill them if their concentrations can reach high level in the cells. To our hypothesis that happens with arising tumour cells in the majority of the population during their lifetime if the number of cells arisen is not too high (absence of strong carcinogenic effects) or the concentrations of the required substances are not too low (healthy subjects, balanced food intake). Otherwise, the number of tumour cells arising can exceed a critical value (critical cell number) at which the divisions of the cells overcompensate the killing of cells by the mentioned substances, and it is most likely that a tumour develops.

It is common knowledge that tumour cells differ largely from normal cells. However they differ only in degree, not in kind. To the best of our knowledge, there is no absolute, qualitative difference between normal cells and tumour cells at all. This precludes the possibility that a single substance can kill the tumour cells selectively, only more than one co-operating molecules can be effective. Obviously, the various kinds of tumour cells differ from normal cells in numerous ways, and therefore the type and amount of the substances effective against them also differ to a certain extent. However, the physiological mixture of the substances in the blood is probably generally effective against all kinds of tumour cells.
These substances are able to perform a continuous and a relatively constant level of "surveillance" because in normal conditions their concentration in the serum is retained within a narrow range by physiologically regulated anabolism, catabolism, reabsorption and excretion. Their actions in the given situations form the Passive Antitumor Defence System (PADS).

The function of these "killer" molecules is generally not the protection of the organism. They play such a protective role only when tumour cells arise and exist. This dual role is very similar to the protective role of substances (i.e., fatty acids, porphy- rings, lactic acid, etc.) which are bactericidal to certain pathogenic micro-organisms. Only the killing of tumour cells needs the collective, simultaneous, synergistic effect of more than one substance, because the altered-self cells do not differ to such an extent from the non-altered cells, like the nonself pathogenic micro-organisms.

**DISCUSSION**

We supported this hypothesis by being able to select experimentally thirteen substances of the circulatory system from 69 molecules examined, using the synergistic tumour cell-killing effect as criteria. The mixture of them had significant toxic effect on various tumour cell lines in vitro and on leukaemia and solid tumours in vivo, but it had no toxic effect against a normal cell line in vitro and against the animals in vivo. Testing other eighteen compounds of the circulatory system we could select three additional substances. The mixture of the sixteen substances could destroy a certain number of cancer cells in vitro when the components were used in the same concentration as they occur in the blood. On the basis of the above results, it can be stated that these substances existing together in the circulatory system can really kill the arising tumour cells in the body if the number of the cells is not too high (critical cell number) or the concentration of the given substances are not too low. The mixture of the substances caused apoptosis of different tumour cell lines but the same substances when we used them singly had not effect on tumour cells. In turn, the above mixture did not induce apoptosis of normal cells. The same amounts of other physiologically and chemically similar substances of circulatory system did not cause apoptosis of tumour cells either alone or together.

The existence of PADS is also supported by many epidemiological and clinical observations, as well as other literary data. On the one hand, epidemiological studies accumulated evidence that consumption of different vegetables and fruits is associated with a decreased risk of cancer, but it was also observed that the intake of total carotenoids, retinol and total vitamin A was weakly and inconsistently related to risk and the protective effect of vegetables and fruits is made not only by ascorbic acid or carotenoids but other food constituents also play a role. Taking into account that the substances are found in vegetables and fruits, and that the capacity of intestinal transport of all the amino acids, carbohydrates and most water-soluble vitamins increases when there is a large food intake, it can be assumed that the substances of PADS may be among the above-mentioned "other food constituents." Thus, appropriate nutrition can positively affect the amount of these substances and the operation of PADS. On the other hand, malnutrition can affect negatively the amount of substances and the operation of PADS. However, the majority of the substances of PADS selected by us have an endogenous source. Thus malnutrition does not cause a substantial loss, but only a decrease in the effect of PADS. This means that PADS always operates, but at a lower level in cases of malnutrition than in normal conditions and the operation of it always depends on the quality of the nutrition. From the above the probability can be seen that the positive effect of proper nutrition is larger than the negative effect of malnutrition. Thus, the significant effect of malnutrition on the tumour incidence can probably be observed if prolonged malnutrition occurs in a large population. This speculation fits in well with the observation that the risk of cancer related to poor nutrition in the poorly fed Moslem populations of Central Asia may be considerable, even without other detrimental effects.

Of course, not only nutrition and malnutrition can influence the amounts of PADS substances and effect of PADS. For example, it has been observed by epidemiologists that alcohol increases the risk of cancer, although the mechanisms by which alcohol induces cancer in humans are not clear. At the same time, heavy alcohol abuse is associated with an inadequate intake of proteins and vitamins, and impairs absorption, utilization, storage and excretion of nutrients, and enhances pyridoxine degradation.
presence of a growing tumour because the tumour cells are always subjected to some effect of the "defence" molecules. This is supported by the observations that the cell death rate is still high within non-necrotic tumour tissue and that 70% to 90% of newly-produced tumour cells in humans die spontaneously by a mechanism that is yet poorly understood and that in some cases spontaneous regression of tumours occurred even in AIDS patients and is also supported by our recent experiments (Gy. Kulcsár). Furthermore, in maximally immuno-depressed recipients almost 80% of the papillomas regressed, although cellular or humoral immune capacity in the animals could not be detected by conventional means.

It is also probable that PADS has a role in the defence against metastases. This is supported by the observations that the development of metastases is an inefficient process and a very small percentage (<0.01%) of circulating tumour cells initiate metastatic colonies. It was observed that metastases do not necessarily develop even when large numbers of viable tumour cells regularly enter the blood in patients with peritoneovenous shunts. These observations show that host factors other than surveillance by the immune system were responsible for local encouragement or suppression of metastatic growth because no cellular immune response was detected in response to micro metastases or isolated tumour cells. Mechanical factors such as blood turbulence or deformation of the cells in the microvasculature can also participate in the destruction of a part of released cancer cells, but, considering that more than 104 allogeneic, non-immunogenenic MCa1V tumour cells are required to transplant the tumour, (when there are no considerable mechanical factors) into 50% of 6 Gy whole-body irradiated athymic NCr/Sed nude mice, it can be stated that the remaining cells are being killed due to the PADS. Large tumours can probably cause the decline of PADS, thus facilitating the formation of metastases. However, this influence of the tumour ceases after removal of the tumour mass. This is supported by observations that the removal of the tumour restores to normal the abnormal profiles of plasma amino acids found during the tumour-bearing state and micro metastases in breast cancer patients became undetectable for a while after surgery, both in treated and untreated patients.

It is well known that tumour cells released into the bloodstream are rapidly distributed to many organs, but they only grow to form metastases in certain sites because the microenvironental conditions of the various organs (e.g., the concentrations of nutrients, orgaderived inhibitory factors) influence the survival of the tumour cells. To our speculation, certain PADS substances also have a role in this process. The amount and kind of PADS substances in the various organ environments probably differ, depending on the function and metabolism of the given organ. Cells released from various kinds of tumours may be sensitive to these substances in different ways. This assumption is supported by the findings that some organs rapidly and effectively diminish the number of live tumour cells. These in vitro experiments clearly demonstrated that the inhibitory effects are dose-dependent, and due to the presence of soluble, dialyzable, non-immunologic agents of small molecular weight diffusing out of organs. The effects of the given organs on the given tumour cells in vitro were in clear agreement with the in vivo observations. It can be found in other reports as well that diffusible inhibitors of tumour growth may be released by certain tissues.

The immune function declines with advancing age. Looking at the incidence of cancer in AIDS and in other immune-deficient patients, it might be expected that only some kinds of tumour increase in incidence with advancing age. Contrary to expectation, the incidence of most types of cancer increase. This contradiction shows that a different and more general defence mechanism than the declined known immune system protects the living system against tumour development and that this defence mechanism also declines with advancing age. All of these are in consonance with the observations that the molecules of PADS also decline with advancing age and this decline is prolonged. Namely, the prevalence of digestive disorders is increased in the elderly. Intestinal absorption of nutrients, including amino acids shows a decrease with aging. Ingestion of an amino acid load from protein hydrolysate resulted in serum amino acid concentration a lower rise in older human subjects than in young adults. Even the fasting serum level of all amino acids occurring in PADS was significantly lower in elderly people as compared to young subjects. Malnutrition is relatively frequent among elderly ambulatory patients and the elderly have more likely than young
adults low or deficient levels of ascorbic acid,\(^{58,59}\) riboflavin and pyridoxine.\(^{58}\)

The investigations of the mechanisms of apoptosis inducing effect in tumour cells of the substances of PADS are currently underway in our laboratory.

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