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Facts and Theories on Viruses Causing Cancer and Leukemia

(vertical transmission/oncogenic viruses/oncogene theory/protovirus hypothesis)

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ABSTRACT **It is possible to explain the familial incidence of cancer developing in several members of the same family tree, within the same as well as in successive generations, by an assumption that tumors and leukemia are caused by oncogenic viruses transmitted in a latent form from one generation to another in many animal species, presumably also in man. The term "vertical transmission" was coined to describe this form of transmission of pathogenic agents. In most instances the oncogenic viruses are invisible and harmless to their carrier hosts. Occasionally, however, under the influence of endogenous or exogenous inducing factors, these viruses become activated and cause cancer or leukemia. According to this concept, the law of obligate communicability established for all common infectious agents applies also to oncogenic viruses: each tumor or leukemia can be traced to another similar tumor which developed in one of the preceding generations as a result of "vertical" passage of the same oncogenic virus. This concept postulates that at one time, perhaps centuries ago, these viruses entered from outside the animal hosts and that, since then, they have been transmitted from one generation to another.**

The theory of vertical transmission of latent oncogenic viruses is consistent with experimental and clinical observations made during the preceding two decades. We consider this concept to be a logical and most promising approach to the problem of viral etiology of neoplastic diseases.

During the first half of this century, the viral theory of cancer and leukemia was almost in disrepute; it was difficult to obtain funds or laboratory facilities for research projects dealing with studies on the hypothetical virus origin of cancer. In fact, very few investigators were interested at that time in pursuing this approach to the problem of tumors and leukemia. During those early years, only a few tumors, such as chicken leukemia and chicken sarcoma, mouse mammary carcinoma, frog kidney carcinoma, and some of the warts and papillomas in rabbits, dogs, cattle, and humans had been found to be caused by transmissible, filterable viruses (1).

Isolation of the mouse leukemia virus. Transmission of the virus through embryos from one generation to another

In 1951 we demonstrated that mouse leukemia is caused by a transmissible virus (2). Filtrates prepared from leukemic mouse tissues induced leukemia or lymphosarcomas after inoculation into newborn mice of a nonleukemic inbred strain. The puzzling phenomenon of the development of "spontaneous" leukemia in successive generations of mice of certain inbred lines, such as Ak or C58, previously explained by complicated genetic theories, found its simple and logical explanation as soon as it was demonstrated in our laboratory that the mouse leukemia virus is transmitted in a latent form from one generation to another directly through the embryos (3), presumably through the germinal cells (4).

It became apparent, therefore, that a mouse born to Ak or C58 parents already carries, at birth, the seeds of the latent disease. Later in the life of the carrier host, the virus, triggered by some obscure factors, becomes activated, causing the development of leukemia and killing its host; however, the passage of the virus to another host has already been assured, since transmission of the virus from the carrier host to its offspring usually occurs prior to the activation of the virus. From the host's offspring the virus is transmitted to the offspring's progeny, thence in turn to the next generation, and so forth. Frequently, activation of the virus may not occur during the lifespan of the carrier host, and the host may remain in good health, even though it carries the virus and transmits it to its progeny. In some instances, activation of the virus may occur only occasionally, perhaps every few generations. Thus, the virus may pass through several successive generations, without causing symptoms of disease. For that reason, the task of tracing the host-to-host transmission of the latent virus may be extremely difficult, if not impossible, unless adequate records of many successive generations are available.

VERTICAL TRANSMISSION OF ONCOGENIC VIRUSES

A working hypothesis

Since other forms of cancer have also been observed to develop naturally in successive generations of mice and also in other animal species, including humans, it was conceivable to assume, as a working hypothesis at least, that not only leukemia and lymphomas, but other tumors also, such as sarcomas and carcinomas, are caused by latent oncogenic viruses transmitted from one generation to another. These viruses would remain latent in most instances, but could be triggered into action by a variety of metabolic, hormonal, or chemical factors, or by ionizing radiation. According to this concept, latent oncogenic viruses would be widely disseminated in many animal species and presumably also in humans. Submerged and invisible in their latent form, they would be frugal and moderate in their requirements, causing no harm to their carrier-hosts. This host-virus relationship could be changed,

*New members of the Academy are invited to contribute a review summarizing the research for which they have been honored and its relation to the broader field of science on which it has had influence. Dr. Ludwik Gross was elected to membership in April, 1973.

nevertheless, at any time; for unexplained reasons, the hitherto latent viruses could become activated and acquire a pathogenic potential, causing the development of tumors or leukemia, and killing their hosts.

In order to describe graphically the transmission of oncogenic viruses from one generation to another, we suggested (5) that this form of transmission be designated "vertical transmission," as opposed to "horizontal transmission" of contagious pathogenic agents, such as those causing chickenpox, typhoid fever, or measles, which spread rapidly from one host to another within the same generation.

Confirmed by the studies on mouse leukemia, the concept of vertical transmission of latent oncogenic viruses was subsequently expanded and reviewed in more detail in 1954 with projected theoretical ramifications (6). On the basis of certain preliminary observations, we also postulated at that time that in some instances a latent oncogenic virus may be harmless for its own carrier, but may cause malignant tumors or lymphomas when transmitted to other animal species.

It should be added, however, that transmission of oncogenic viruses does not necessarily have to be limited exclusively to the vertical pattern, but may occasionally, in the case of certain tumors, occur to some extent also horizontally such as the horizontal transmission, among newborn chicks, of the herpes virus causing neurolymphomatosis, i.e., Marek's disease (7).

A very old, usually latent infection

It is apparent that oncogenic viruses may represent a very old, usually asymptomatic, latent infection, very common in many animal species, presumably also including humans, and that they have been transmitted from generation to generation for centuries, only exceptionally causing symptoms of disease. It is quite probable that at one time, possibly many centuries ago, some of the oncogenic viruses entered the cells of many animal species from outside and have been since that time propagated from one generation to another, remaining in most instances submerged, invisible, and unrecognized, except for an occasional tumor or leukemia developing here and there in one of the carrier hosts. However, as in the case of many other diseases caused by transmissible submicroscopic agents, the development of disease would represent only an accident in the relation of the virus to its carrier host. The development of a tumor or leukemia in an occasional host, possibly separated by one, two, or several generations, would represent only a few scattered but revealing links in the chain of a continued host-to-host transmission of the causative agents. As long as these viruses exist in a latent form, they may remain invisible; they are probably incorporated in the host's cell genetic material or may be carried in another submerged form, remaining unrecognized by our current methods and laboratory tools. However, oncogenic viruses would be essentially similar to other infectious agents; each oncogenic virus could be traced to another oncogenic virus in a continuous generation-to-generation chain of transmission, similar to the host-to-host transmission of other pathogenic viruses causing common communicable diseases.

Should this concept prove to be true, it would then follow that the number of individuals suffering from leukemia or tumors would represent only a small fraction of those actually carrying the seeds of the disease.

The turn of the tide

The introduction in 1951 of the newborn mouse as a basic experimental tool for the detection, by bioassay, of the oncogenic potential of oncogenic viruses (2) served as an initial guide, followed shortly by the introduction of newborn animals of other species, such as rats, hamsters, and cats, for similar bioassay studies. Within only a short span of 20 years, a variety of tumors and leukemias in several animal species were found to be caused by filterable viruses, transmissible by inoculation to newborn hosts. The mouse leukemia virus induced leukemia not only in mice, but also in rats, and could be serially transmitted from rat-to-rat by filtrates (8). Cat leukemia was found to be caused by a virus transmissible by filtrates not only to cats (9) but also to dogs (10). Another malignant cat tumor, a fibrosarcoma (11), was found to be transmissible by filtrates to cats, monkeys (12), and dogs (13). Latent oncogenic viruses present in normal mice could be activated by total body x-ray irradiation; the causative virus was recovered from radiation-induced leukemia (14) and passed serially by filtrates in newborn mice, inducing leukemia in the inoculated animals (15). The SV40 (simian virus), latent and harmless for its rhesus monkey carrier host, induced malignant tumors following inoculation into newborn hamsters (16, 17). Certain types of adenoviruses, causing only transient inflammatory disease in their human carriers, also caused malignant sarcomas when inoculated into newborn hamsters or rats (18). A strain of a herpes virus latent for its natural carrier, the squirrel monkey, induced malignant lymphomas when inoculated into the owl or marmoset monkeys (19). The remarkable familial incidence of lymphosarcomas in certain families of cattle found its logical explanation when virus particles were found in bovine lymphosarcoma cells (20).

Gradually, the tide has turned. The concept of viral etiology of cancer and leukemia became at first respectable, then promising, and gradually gained sufficient impetus to represent at the present time one of the principal approaches in the research effort directed toward the conquest of neoplastic diseases.

Some tumors do not seem to contain virus particles

Some questions remain unanswered. It is difficult to understand why certain tumors and leukemias, such as mammary tumors in mice, or leukemia in mice, chickens, and cats, contain virus particles and can be readily transmitted by filtered extracts to other hosts, whereas similar tumors and lymphomas developing naturally in certain other species, such as rats or dogs, and particularly in humans, do not seem to contain virus particles (1). It is possible that lack of proper experimental methods is responsible for this difficulty; as an example, no virus particles were found in bovine lymphosarcoma until the leukemic cells were placed in short-term tissue culture (20). The fact that a tumor does not contain virus particles when examined in the electron microscope does not necessarily imply that such a tumor was not induced by an oncogenic virus. Sarcomas induced in hamsters or rats with the polyoma virus†, the SV40 (simian virus), or with

† The polyoma virus was initially discovered in our laboratory; some of the newborn mice inoculated with filtered extracts prepared from leukemic mouse organs, developed parotid gland carcinomas, instead of leukemia. We promptly demonstrated

certain types of adenoviruses, usually do not reveal the presence of virus particles on electron microscopic examination even though these tumors were induced by inoculation of tissue culture fluids containing innumerable virus particles (1).

Induction of tumors in normal, healthy mice and rats with carcinogenic chemicals

One of the important reasons why many skeptical investigators have hesitated to accept the concept of viral etiology of cancer is the common observation that a variety of malignant tumors or leukemia could be induced with carcinogenic chemicals or ionizing radiation in mice, rats, and certain other, but not all, animal species (1). These observations could be logically explained, however, by an assumption that those animals in which tumors or leukemia were induced were carriers of latent oncogenic viruses, even though such latent viruses could not be detected by our inadequate laboratory methods.

Normal, healthy mice, rats, and other animal species may carry many latent oncogenic viruses which are not detectable by our current means and methods and which become activated under the influence of a variety of inducing factors. Mice, rats, and many other animal species are literally infested with innumerable parasites and pathogenic agents. Would it be surprising to assume that they also carry latent oncogenic viruses? Certain other animal species, however, such as monkeys, are not readily susceptible to the induction of tumors or leukemia with carcinogenic chemicals or irradiation (1); it is quite possible that such animals may not always carry latent oncogenic viruses or that the potentially oncogenic viruses carried by such animals may be less prone to become pathogenic for their own carrier hosts.

The "oncogene" theory

In order to reconcile some of the difficulties in the interpretation of experimental observations on cancer and leukemia and in an apparent attempt to elucidate the role of viruses in the etiology of cancer, a theory was recently proposed by Huebner and Todaro (21, 22) suggesting that most or all cells of the vertebrates carry, as an essential part of their natural evolutionary inheritance, "oncogenic information" (the oncogene), and that cancer results from the destruction of the normal "repressor system" that keeps both the oncogenic and virogenic information in check in the normal adult cell. They propose that "endogenous virogenes" (the genes for the production of type-C viruses) and the "oncogenes" (that portion of the virogene responsible for transforming a normal cell into a tumor cell) are maintained in an unexpressed form by "repressors" in normal cells. Various agents, including radiation, chemical carcinogens, or the normal process of aging, activate the genes and may transform cells by "switching on" the "endogenous oncogenic information." The "oncogene" theory assumes that the virogene and the oncogene information are intrinsic parts of the natural genetic fabric of all vertebrate cells, that the endogenous virogenes and oncogenes are present with varying degrees of expression in all mice, and that "there is a complete copy of the type-C virus information in the genetic material of all somatic cells" (22).

The implications of the "oncogene" theory

What are the implications of a concept which assumes that an "oncogene" or "information for cancer" may develop from a normal cell? The proponents of the oncogene theory suggest that this potential is a part of a "natural genetic make-up" of all cells of vertebrates. Such an interpretation is only one step removed from an assumption that a normal genetic make-up in a normal cell can be manipulated experimentally or altered by certain inducing factors, such as mutation, carcinogenic chemicals, or hormones, so that under such circumstances tumor-inducing "oncogenes" may develop *de novo*. If so, would oncogenic viruses arise in normal cells *de novo*? Are we back to "spontaneous generation" almost a century after Pasteur? Would it not be more logical to assume that the presumably "normal" cells carry latent oncogenic viruses which may become activated by certain inducing factors?

If the "oncogene" theory assumes that "normal" cells carry latent oncogenic viruses which become activated by certain inducing factors, then such a theory would be very similar to, if not identical with, the original concept of vertical transmission of oncogenic viruses, with the implied assumption that latent oncogenic viruses are ubiquitous and are present in practically all cells of vertebrates, except that the term "oncogenic virus" employed in the vertical virus transmission theory is substituted by the term "viral oncogene" and the term "activated virus" is substituted by the term "switched-on virus." This would be only a matter of semantics, however, without too much significance; we still prefer our original terminology. In this era of molecular biology it may seem more fashionable to call a tumor-inducing virus "genetic information for cancer"; we still prefer, and consider it more accurate, to call a virus, a virus.

On the other hand, if the "oncogene" theory assumes that all "normal" cells carry the necessary components for a *de novo* development of an oncogenic virus, then such a theory would be untenable, unless we would accept the concept of spontaneous generation of oncogenic viruses‡. It is not that long ago that tuberculosis, diphtheria, and typhoid fever were also believed to originate *de novo* (1).

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that the injected extracts contained, in addition to the mouse leukemia virus, another smaller, relatively heat and ether resistant virus, which we designated "the parotid tumor virus," since it consistently induced tumors of the salivary glands, although it was also capable of inducing a variety of other tumors, following inoculation into newborn mice [Gross, L. (1953) *Proc. Soc. Exp. Biol. Med.* **83**, 414–421]. Several years later, the same virus, propagated in tissue culture, was renamed by Eddy and Stewart "the polyoma virus," a term now generally used [Eddy, B. E., Stewart, S. E. & Berkeley, W. (1958) *Proc. Soc. Exp. Biol. Med.* **98**, 848–851].

‡ The protovirus hypothesis of the origin of cancer advanced recently by Temin belongs to the same category. Here again, the "information for cancer," leading to the development of a tumor, may arise *de novo* from normal cell components. Thus, according to Temin, "this protovirus hypothesis proposes apparent vertical transmission of the information for cancer, even though the germ line does not contain this information on its chromosomes (proviruses or oncogenes) or off its chromosomes (virions). The germ line is postulated to contain in its chromosomes the potential for genetic evolution by the somatic cells that may lead to the *de novo* formation of the information for cancer." [Temin, H. M. (1971) *J. Nat. Cancer Inst.* **46**, III–VII.]

Oncogenic viruses arising *de novo*, an old problem in cancer research

The concept of oncogenic viruses arising *de novo* from presumably normal cells has plagued the cancer problem ever since the early studies of this disease. Such a concept is actually similar to the theory of cancer developing as a result of "somatic mutation" which was popular some years ago (1).

Bittner, who first reported that mouse mammary carcinoma is caused by an agent transmitted through the milk of nursing females (23), later identified as a filterable virus, also postulated in his initial studies that this tumor-inducing agent may develop *de novo* in susceptible mice (24). In his early studies, Bittner called the mouse mammary carcinoma virus the "milk influence," since the virus was transmitted through the milk and since at that time the virus theory was still in disrepute. Only several years later did the "milk influence" change its name to "mouse mammary carcinoma virus" (1).

Obligate communicability of all pathogenic viruses

The assumption that "all cells of vertebrates carry the oncogenic information" and that, given a proper stimulus, oncogenic viruses can develop in any normal cell, is tantamount to the concept of spontaneous generation and is simply not consistent with the generally accepted principle of obligate communicability of all infectious diseases caused by microbial or viral agents. It is immaterial whether transmission of the virus occurs horizontally or vertically, or whether the virus is latent and occasionally fails to cause disease in an afflicted host. A child develops measles after having been exposed to another child with measles, the latter having contracted that agent from another similar case, and so on. The same is true for smallpox, influenza, and all other communicable diseases. It is only logical to assume that oncogenic viruses are not different, except that they are transmitted "vertically," from one generation to another. Accordingly, each virus, oncogenic or not, can be traced to another similar virus, like all other infectious agents in animals and in man. In mouse leukemia, for example, one case can be traced to another, in a continuous chain of host-to-host transmission of the leukemia-inducing virus. This virus has a distinct morphology of a spherical particle, which can be studied in the electron microscope; it can be grown in tissue culture or in a susceptible host. It can be passed indefinitely by serial mouse-to-mouse passages, inducing leukemia in the inoculated hosts. It has antigenic potency. It can be inactivated by heating. The same is true for other oncogenic viruses, such as those causing leukemia or sarcomas in cats or chickens, or mammary carcinomas in mice, or a variety of sarcomas or carcinomas in rats, hamsters, and other species (1). These oncogenic viruses are not different in their biological properties from other viruses causing a variety of common communicable diseases in animals and in man.

The concept of vertical transmission of oncogenic viruses postulates that at one time, perhaps centuries ago, these viruses entered from outside the animal hosts and that they have been propagated since that time, and passed from one generation to another, like spotted fever rickettsiae transmitted through the eggs in the tick (25), or the mosaic disease virus transmitted from one generation to another in tomatoes or in certain other plants, through the seeds (26).

CONCLUSIONS

The viral theory of the origin of cancer, held almost in disrepute throughout most of the first half of this century, has subsequently gained gradual recognition and is now generally considered to be one of the most promising approaches leading to the understanding and control of neoplastic diseases.

It is possible to explain the familial incidence of cancer or leukemia developing in several members of the same family tree, in successive generations, by an assumption that tumors and leukemia are caused by oncogenic viruses transmitted from one generation to another in many animal species, presumably also in man. The term "vertical transmission" was coined to describe graphically this form of transmission of oncogenic and other pathogenic agents (2). In most instances, oncogenic viruses are invisible and harmless for their carrier hosts. Occasionally, however, under the influence of endogenous or exogenous inducing factors, the latent viruses become activated and cause cancer or leukemia. Individuals developing tumors or leukemia represent probably only a small fraction of those actually carrying the seeds of the disease. It is assumed that all malignant tumors and leukemias are caused by oncogenic viruses. The law of obligate communicability established for all common infectious agents applies also to oncogenic viruses. Each tumor or leukemia caused by an oncogenic virus could be traced to another similar tumor which developed in one of the preceding generations. This concept assumes that at one time, perhaps centuries ago, these viruses entered from outside the animal hosts and that they have been propagated since that time and passed from one generation to another. Oncogenic viruses apparently represent a very old, latent infection, which has been carried in many animals, including humans, for many years.

Latent oncogenic viruses appear to be ubiquitous, particularly in certain animal species. They very seldom become pathogenic under natural life conditions, but many of them can be activated by obscure endogenous factors, or by carcinogenic chemicals, hormones, or ionizing radiation. Some of these latent viruses maintain consistently a harmonious relationship with their hosts and will not cause cancer in their own carriers, but may cause malignant tumors when transmitted to other animal species.

The recently introduced "oncogene" theory (21, 22) may at first sight appear to be similar to the original, previously proposed concept of vertical transmission of oncogenic viruses (6). However, there is a fundamental difference between these two theories. The original concept of vertical transmission of oncogenic viruses assumes that oncogenic viruses at one time entered from outside the animal hosts and have been since that time passed from one generation to another as a latent infection. On the other hand, the "oncogene" theory postulates that the genome responsible for producing the infectious virus is part of the inherited genetic material of all cells of normal vertebrates. Does such a concept imply that all cells of normal vertebrates are actually infected and carry latent oncogenic viruses? Or would the oncogene theory assume that oncogenic viruses develop *de novo* from normal, noninfected somatic cells of all vertebrates? In the first case, the oncogene theory would be very similar to the original concept of vertical transmission of latent oncogenic viruses with only some insignificant changes in terminology and with the assumption that latent oncogenic viruses are present in all or almost all somatic cells of vertebrates. On the other

hand, if the oncogene theory assumes that infectious viruses can be produced from normal endogenous components of healthy, normal, not infected cells, then such a concept would be untenable unless we go back to the old concept of spontaneous generation of infections agents. Temin's protovirus hypothesis belongs essentially to the same category, since it postulates that oncogenic viruses, such as the Rous chicken sarcoma (1), may develop *de novo* from normal cell components‡.

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