Formal Discussion: Biologic Aspects

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I should like to consider briefly another type of obstacle that might influence leukemia control. I refer to the experimental obstacles in evaluating the possible role of viruses in human leukemia.

The possibility of viral etiology of the human disease seems reasonable in view of the conclusive evidence for viral leukemogenesis in a number of lower animal species. Whether leukemogenesis in these animals always requires the participation of a virus is unsettled, but this is not germane to this discussion. As Dr. Kaplan pointed out, no virus has yet been linked to human leukemia. However, the past failure to demonstrate such a virus may be due more to the formidable experimental obstacles than to a defect in the hypothesis itself.

Studies of the leukemia viruses in experimental animals have not only provided a hypothesis for etiology of the human disease; they have also defined the methodology for demonstrating leukemia viruses in these animals. The successful approaches have been quite straightforward. The material containing a suspected leukemic virus was inoculated into members of the species of origin. Initial isolation usually required the use of very young or newborn animals; the latent periods often approximated $\frac{1}{3}$ to $\frac{1}{2}$ of the animal's life-span. Subsequent passages permitted crossing of species barriers in some instances. Virulence of the virus as determined by shorter latent periods and susceptibility of older animals also increased with passage. The value of cell culture studies, electron microscope investigations, and so on has depended in large part on the ability to correlate these parameters with leukemogenesis in the animal. For example, propagation of a leukemia virus in cell culture was confirmed by production of the disease in a susceptible host (7). Particles of a certain morphology were identified as the responsible viruses by their ability to produce leukemia in the animal (3). In other words, the reference parameter for all such studies has been the production of leukemia in the host of origin. This has created serious problems with the human studies, since it is not possible to utilize an analogous *in vivo* assay system. As a result, the most important aspect of the established animal methodology has been compromised by the necessity to use laboratory animals for leukemogenic assay of the human material.

Since the mouse has been such a valuable tool for the study of experimental leukemias, it has been the most widely used animal in the human studies. Like any other experimental animal, it has a number of theoretical and real disadvantages in studies of this type. The most apparent disadvantage is that the mouse simply may not be susceptible to the hypothetical human leukemia virus. Therefore, if virus detection depends on leukemia induction in the mouse, it will be missed. Even more disconcerting is the realization that even if leukemia (or some other disease) were produced in the mouse by a human virus it would not necessarily follow that the same virus would produce the same disease in the human. It is well established that different hosts may respond differently to the same agent. An analogous situation presently exists with human Adenoviruses 12 and 18. Here we have human viruses that produce malignant tumors in hamsters and mice, but there is presently no evidence that these same viruses are carcinogenic for the human.

Another very real problem with the mouse is that it frequently harbors latent murine leukemia viruses. Therefore even if a leukemia virus is isolated in the mouse, it may be very difficult to ascertain its origin. The fact that there are no wholly satisfactory and convenient serologic methods for identification of the murine leukemia viruses compounds this problem. In a recent study in our laboratory we isolated a leukemia virus from Ha/ICR mice during the course of blind passages of organs from animals that had been originally inoculated with human leukemic bone marrow (1). Parallel passages of normal organs from normal mice of the same strain and age were negative. Although the evidence still isn't absolutely conclusive, it now seems likely that this virus is of murine origin. The blind passages apparently somehow activated or enhanced the potency of a latent leukemia virus in our test animals.

We are presently evaluating the germ-free mouse as a test system (6). However, since vertical transmission is known to occur with some murine leukemia viruses, it is questionable whether these animals will circumvent the problem of indigenous leukemia viruses. Newborn subhuman primates are now being used more widely in human studies. Hopefully, some of these may provide more suitable test systems, but these possess the same theoretical disadvantages as the mouse and other lower animals.

The fact remains that it has not been possible to investigate directly the virus etiology of human leukemia by methods analogous to those used in lower animals. If the same limitations had governed investigations in the chicken and mouse, it is doubtful that a leukemia virus would have yet been isolated. It seems appropriate, therefore, to view the negative human studies in this perspective.

By extrapolation of knowledge from the murine leukemia viruses, one might predict that attempts to link a virus to human leukemia by conventional indirect virologic methods will encounter substantial obstacles. The feeble antigenicity of these viruses in their natural hosts could

seriously interfere with serologic linkage by the usual technic of seeking an anti-viral antibody in the patient's serum. Low level virus replication in susceptible cell cultures without production of cell damage makes detection difficult in the absence of an *in vivo* leukemogenic assay system. Immunofluorescence, electron microscopy, and other methods may prove very helpful, but the value of these technics is again hampered by the lack of correlation with *in vivo* disease production.

Electron microscopy has provided some very intriguing and provocative observations about the human disease. Investigators from several laboratories have observed particles in human leukemic plasma that are morphologically similar to the murine leukemia viruses (2–5). The use of concentration and purification procedures, such as density gradient centrifugation, has markedly increased the number of "positives" (2). These particles are occasionally seen in control plasmas but usually in smaller numbers. The interpretation of these observations must await correlation with other parameters. For example, it is not known whether these particles represent viruses and, if they are viruses, whether they have any significance in the disease. However, the consistency with which they are found and the striking similarity to the murine leukemia viruses provide a substantial impetus for further investigation.

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