The Role of Viruses in Human Leukemia
A Summary

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It is certainly a hard task to summarize discussions dealing with virological aspects of human leukemia. The diverging views, the different approaches render it almost impossible to review comprehensively the data presented. It is unavoidable, in addition, that the biased views of the author are applied to the issues raised at this meeting.

Is there any progress visible as compared to the last meeting 2 years ago at this very place? Progress in the elucidation of the role of viruses in human leukemia? Progress in our understanding of the mechanisms leading to virus-induced leukemia in general?

It appears to be easier to start with the second question first: many elegant studies were reported dealing with virus-induced leukemogenesis in avian as well as in mammalian systems. BALUDA pointed out the importance of the target cell for a specific response in terms of cell transformation and stressed the acquisition of new DNA sequences in transformed cells (1). Nontransformed cells contain DNA which reveals 60 % of homology with avian myeloblastosis virus (AMV) sequences only. His data were somewhat contrasted by GRAF's studies who presented evidence for the cell specificity of various AMV strains, each of them transforming different target cells (2). Based on his experiments GRAF claims that the different types of tumors observed in BALUDA's studies are due to a mixture of different AMV strains present within the original inoculum.

In this respect it was interesting to learn that the helper RAV-virus, present in preparations of defective erythroblastosis virus (2), induces by itself severe anemia in chicken, but no erythroblastosis. This may have some relevance for human leukemias, where (as stressed by MOLONEY) refractory anemia or even pancytopenia frequently is a preceding disorder.

The avian systems was also investigated in DUESBERG's studies in order to identify the localization of transforming sequences within the avian sarcoma virus genome (3). Sarcomavirus-specific sequences were identified in 3 different strains of sarcoma viruses by selecting specific fragments of partially degraded viral genomes and subjecting them to fingerprinting after further partial digestion. These studies come close to experiments reported by BISHOP's group in isolating sarcoma-specific sequences by hybridization techniques (4). I hesitate to agree to call such sequences "oncogene" since transformation of lymphatic cells occurs naturally by leukosis viruses which appear to lack the respective sequence.

BAUER and HOFSCHNEIDER reported the isolation of a new particle from the allantoic fluid of embryonic eggs (5). It seems to differ from known avian leukosis viruses in that it does not share antigens with AMV. It also reveals distinct properties of its RNA-dependent DNA polymerase.

Turning now to the mammalian systems, the situation becomes increasingly complex: many of the newly isolated mammalian oncornaviruses offer the fascinating possibility to study their evolution across the species-barrier. As explained by TODARO, endogenous viruses of baboons are also found in a number of cat species (6) and permit a rough calculation when an infectious process took place from the baboon to the cat or vice versa. This as well as similar systems may provide us with an entirely new approach to study the evolution of certain species. It should not be overlooked, at the same time, that most of these studies are performed with material derived from laboratory animals. It is abvious, therefore, that the possibility of inadvertent contaminants has to be excluded.

JAENISCH presented extremely interesting data on genetic control of oncornavirus information in the mouse system (7). He studied the infection of embryos at the 4–8 cell stage and looked into the presence of virus-specific DNA within the germ line as well as within somatic cells at later stages of development. This seems to offer a new approach in the regulation of virus-specific information in mammals. It was interesting to learn at this occasion that cells at very early stages of embryonic development are non-permissive for those viruses he tested (murine leukemia viruses and SV 40). One wonders whether there exists a specific mechanism which protects such cells and possibly also germ line cells against early genetic damage.

Studies on the role of FRIEND leukemia virus in the differentation of mouse pluripotential stem cells into erythroblasts were reported by OSTERTAG. HARDESTY also alluded to this question (8). The ingenious cell separator used by OSTERTAG, based on laser-beam scanning and computer-directed deflection of drops, appears to represent an elegant and important tool in the elucidation of cell differentation. This was also convincingly demonstrated by GREAVES (9) experiments. OSTERTAG's statements on the possible role of DMSO in the induction of viral and globin messengers RNA-synthesis by affecting repressor binding within the cell may deserve further studies.

Transfection experiments revealing the existence of DNA proviruses were rather briefly discussed at this meeting. BENTVELZEN made the interesting observation that DNA from spleens of Rauscher virus leukemic mice transfects and transforms efficiently when applied under appropriate conditions. In this respect it seems interesting to note that similar studies have not yet been reported with human leukemic cell DNA. One could imagine that similar events may take place in tissue culture or by transfecting cells of primates in vivo with DNA originating from human leukemia cells.

Interesting new aspects were contributed by BURNY in his studies on the viral etiology of bovine lymphosarcoma (11). The epidemiology of this disease resembles the spread of feline leukemias which were, unfortunately, not discussed at this meeting. It is of interest to note that 100 % of animals developing disease revealed antibodies to viral antigens. This in part to such an extent that they can be measured by relatively insensitive immunoprecipitation methods. This appears

to contrast markedly the situation in human leukemia, where the demonstration of even leukemia-specific antigens, as pointed out by GREAVES (9), is presently either impossible or requires difficult manipulations.

The presence of bovine oncornaviruses in commercially available batches of calf serum, as observed by BURNY (11). should be another word of caution in claims of new oncornaviruses from tissue culture cells maintained with such reagents.

Turning now to human leukemia and lymphosarcoma, isolates from human disease naturally require special attention. Two claims of successful oncornavirus isolations were reported at this meeting (12) and others are found in the literature (13, 14, 15). GALLO described extensively the successful isolation of such viruses from a patient with acute myelogenous leukemia (12). According to his studies the agent appears not to be an endogenous virus of man or certain primates. It shares many characteristics with the simian sarcoma virus and it is not yet entirely clear whether it can be differentiated at all from this agent. Although repeatedly isolated from the same patient, there are some disturbing observations which are difficult to reconcile with a role of this virus in human myelogenous leukemia:

(i) recent studies reveal the presence of two different oncornaviruses in these isolates. One of them appears to be idential with simian sarcoma virus, the other shares features with baboon endogenous virus (16).

(ii) no convincing levels of antibodies directed against these isolates can be demonstrated in the patient, nor in other individuals suffering from the same disease, or in healthy control persons (17).

(iii) DNA-sequences related to these agents have not been demonstrated in the DNA derived from spleen cells of the patient from whom the viruses were recovered.

Thus, there remains the possibility, as remote as it may be, of a laboratory contamination. Further studies appear to be essential to clarify the origin of the isolated agents. The second isolate was reported by NOOTER. It has been obtained from a child with lymphosarcoma. This virus has not yet been further characterized. Although the data seem to be intriguing, the use of rat XC-cells for plaquing this agent raises some questions. Endogenous rat oncornaviruses have recently been found in XC-cells.

The third group isolating putative human oncornaviruses was not represented at this meeting. KIRSTEN and PANEM were able to recover a simian sarcoma virus-like agent from human embryonic lung fibroblasts (13).

It is obvious that each of these isolations requires great interest. It appears to be a long way to clarify whether they indeed represent human viruses. If so, it will be an even longer way before they can be implicated in human leukemic disease.

SPIEGELMAN reported the presence of specific DNA sequences, as determined in his endogenous reaction, in almost every kind of human tumors (18). The significance of these findings should be further elucidated, since they are also found in two human malignancies most probably induced by a DNA containing virus (19).

The various isolations of oncornaviruses from primates should support attempts to recover similar agents from human leukemias and lymphomas. It is of particular

interest that oncornaviruses have been isolated from acute myelogenous leukemias in gibbons.

There are, however, certain features of most human leukemias which are presently difficult to reconcile with an oncornavirus-induction. Although anologies to animal leukemias sponsor intensively the current interest in oncornaviruses, it may be worthwhile to consider some of the diverging aspect:

(i) In contrast to most animal oncornavirus-induced tumors it appears to be extremely difficult to demonstrate any kind of oncornavirus-specific molecules in human leukemic cells. This is also shown in GREAVE's study on antigens specific for acute lymphatic leukemia (9).

(ii) Sera derived from leukemic patients appear to lack antibody-activities against known oncornaviruses. This certainly includes the woolly monkey isolate. In regard to all known naturally occurring oncornavirus-induced leukemias and lymphomas it would be exceptional if man would respond without antibody production.

(iii) Human leukemias and lymphomas represent, at least in their vast majority, monoclonal diseases. Thus, the continuous production of transforming particles appears to be somewhat unlikely.

(iv) The failure to demonstrate viral particles in human leukemic cells certainly contrasts the situation in animal systems.

In this respect it was somewhat surprising that the only virus known to be oncogenic in man and consistently associated with specific lymphatic diseases, the Epstein-Barr virus (EBV), played a minor role at this meeting. This DNA-containing herpes group virus was briefly discussed by DIEHL showing that NULL cells apparently lack receptors for EBV-infection (20). It has to be remembered that EBV is found in virtually 100 % of African Burkitt's lymphoma cells, as well as in very few cases of similar histology outside of the African tumor belt; that it infects and transforms specifically B-lymphocytes, but is also found in every epithelial tumor cell of human nasopharyngeal carcinoma (19). This virus induces lymphoproliferative disease in marmosets and transforms and "immortalizes" human lymphocytes efficiently (19).

The most potent and effective leukemogenic agent in primates, herpesvirus saimiri, was also discussed in one presentation only (21). LAUFS reported on prevention of saimiri-induced oncogenesis by prior inoculation of heat- and formaline-inactivated vaccines. It should be noted that herpesvirus saimiri induces lymphomas or acute lymphatic leukemias after short incubation periods in 100 % of inoculated marmosets (22).

Returning to human leukemias, there is presently no good reason to speculate that these diseases are herpesvirus-induced. In such case it would be, most probably, not too difficult to detect virus-specific antigens within the transformed cell or on their surface. The entire lack of these "footprints" in human leukemic cells remains a puzzle in regard to their suspected viral etiology. It could be relevant in this respect that there exists a group of transforming viruses which are most difficult to trace within their transformed host cells, the human papilloma or wart viruses (23). In spite of numerous attempts it has not yet been possible to detect papilloma virus-specific T-or surface antigens within their transformed host cells. Recent results reveal that there exist several types of human papilloma viruses which can

be differentiated by biochemical methods (zur Hausen and Gissmann, unpublished results). There may be other candidate viruses along these lines and it appears to me to be a good bet that at least some forms of human leukemias (if they do have a viral etiology at all) should be due to non-enveloped viruses.

I am stating this because it is my feeling that our intensive search for human analogies to well established laboratory system in animals may misguide us. Most probably it will be worthwhile to persue also different avenues in our search for a viral etiology of human leukemia. If the intensive search for human oncornaviruses fails to provide conclusive evidence we should be prepared to look as well into the role of other agents in the induction of this malignant disease.

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