[CANCER RESEARCH 33, 1417-1418, June 1973]

Summary of Informal Discussion on General Aspects of Herpesviruses

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Dr. Spiegelman opened the discussion by asking Dr. Roizman two questions: one, what was the actual concentration of the DNA in the reaction mixture; for that is the key to interpreting the kinetic curves, as it is necessary to know the C_0t value that was actually measured. The other question was where are the controls, how many normal DNA's have been examined in the same way, and is there any hybridization with DNA from normal uteri.

Dr. Roizman replied that Dr. Spiegelman's questions fall into 2 classes. One has to do with experimental design and the total maximum amount of DNA in a system. Our reaction mixture contained a maximum concentration of DNA of approximately 5 mg/ml, in a volume of the order of 25 to 50 μl. Second, we had deliberately chosen not to plot the data according to C_0t because we believe our method of data presentation to be far more sensitive.

Dr. Roizman stated that Dr. Spiegelman's 2nd question had to do with the significance of finding herpesvirus-specific DNA. In reply, he stated that 2 controls were used: the cells in which the virus was grown and human liver. The relative unavailability of uteri was the reason for the use of human liver. Furthermore, at the time that the experiments were being performed, it was uncertain whether virus-specific sequences would be found; therefore, no serious attempts were made to obtain normal human uteri. However, Dr. Roizman continued, these attempts are now being made and more tumors will be tested in the future. The complementary sequences are in the uterine tumor cells that were tested, but they are not in human liver or in the cells in which the virus was grown.

Dr. Roizman feels that the HeLa cell story is very interesting. The HeLa cell as it is now in common use bears very little relationship to the original HeLa cell, isolated by Dr. George Gey. The original HeLa cell grew very slowly, in clumps or chunks, and fetal cord serum was used for growth. Dr. Roizman made several attempts to get original HeLa cells: in fact, Margaret Gey promised him some early-passage cells, if available, but he has not yet received them. For obvious reasons, HeLa cells obtained from commercial sources will not be used by him.

Dr. Stern commented that, in a very recent article, a pathologist at Johns Hopkins reported reviewing the HeLa cell and the original tumor from which the HeLa cells were cultured. The pathologist seems to have concluded that this is not a squamous cell carcinoma but rather an adenocarcinoma or a columnar cell carcinoma.

Dr. zur Hausen then stated that 1 of his associates, Dr. Schulte-Holthausen, tested 13 biopsies of cervical cancer by using herpes simplex virus type 2 complementary RNA. The sensitivity of this method was about 1 to 2 genomes per cell, much less sensitive than that of the method that Dr. Roizman reported. By using this assay he was not able to find any specific hybridization within those cervical carcinoma cells. Of course, if these tumors contained fragments of the size that Dr. Roizman reported, he would not have observed them. Dr. zur Hausen said that he believed that the results indicate at least a basic difference in the reaction of herpes simplex virus type 2 with cervical cancer cells, as compared to another herpesvirus, Epstein-Barr virus. In Burkitts lymphomas and nasopharyngeal carcinomas, the tumor cells seem to be loaded with viral genomes, and obviously the complete viral genomes are present in those cells. Thus a basic difference seems to exist between these 2 systems.

Dr. Roizman replied that he has not attempted to use complementary RNA because, unless it is very well characterized to show that it actually is transcribed from the entire DNA molecule, it is really of limited value.

Dr. Subak-Sharpe asked Dr. Roizman whether he might be working with satellite DNA, which has unique patterns of nearest-neighbor frequencies of bases. Dr. Roizman replied that he had extracted the whole-cell DNA from the tumor without fractionating it.

Dr. Benyesh-Melnick described some recent results, obtained in collaboration with Dr. P. A. Schaffer in her laboratory, on mutants of herpes simplex virus type 1. About 60 mutants have been obtained, 22 of which have been placed in 15 complementation groups. By doing 2-point reciprocal crosses with 11 mutants in 7 complementation groups, they have obtained a preliminary genetic map.

Dr. Subak-Sharpe pointed out that he was wary of accepting data on recombination when 2-point crosses are done. In his laboratory, only 3-point crosses are now used. Dual infection, for instance, with each virus at a multiplicity of 5 plaque-forming units/cell, may be quite different with respect to physical particles. Thus it is necessary to have an independent measure of output.

In the experiments that he described in this paper, all the combinations were performed simultaneously. In each, the total multiplicity was 10 plaque-forming units/cell but in the ratios of 3:1, 1:1, and 1:3. Under those conditions, he accepts data in which the output suggests that there had been approximately 1:1 input of effective genomes.

He stated further that his laboratory also has tested mutants, but he could not indicate as yet how many

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complementation groups that they have found. His laboratory uses several different complementation tests to check each mutant. Absence of complementation does not necessarily mean that both input viruses are mutated in the same gene. His mutant B, for instance, does not induce synthesis of very much viral DNA.

Dr. Nahmias asked Dr. Stevens about the possible similarities or differences between latency and potential oncogenicity of herpes simplex virus. He pointed out that Dr. Stevens had suggested that the virus might remain latent in cervical cells; yet all of the animal data suggest that the virus remains latent in the nerve ganglia. Also, Dr. Nahmias raised the question of whether the phenomenon of latency is the same as the phenomenon of transformation.

Dr. Stevens replied that, as indicated by Dr. Roizman's data, having part of the genome in cervical tumor cells might be defined as latency but does not fit the usual meaning. He went on to suggest that perhaps the virus is latent in sensory ganglia and that may be how it is continually reapplied to the cervical area. If the virus does come from the sacrospinal ganglia over a long period of time, it could produce latency or partial "latency" involving only a portion of the viral genome. Some cervical tumors might differ from the 1 reported by Dr. Roizman and contain the entire viral genome.

Dr. Aurelian then reminded the group that her laboratory had found viral antigens in cervical tumor cells and had isolated a virus from 1 tumor. Another cell line had been established from an invasive cancer. This one did not yield whole virus but contained viral antigens. They also found antigens in cervical tumor cells obtained directly from patients; however, these cells did not contain virus particles. Dr. Aurelian suggested that the difference between the 2 cervical tumor cell lines in her laboratory may be due to their origin and the transcriptional program of the persistent viral genome in the 2 lines. She said that, whereas the line that yielded virus was obtained from a carcinoma *in situ*, that which did not yield the virus was obtained from an invasive cancer. She further mentioned that most of the exfoliated tumor cells that were studied in her laboratory and that did not show evidence of virus particles, in spite of the presence of antigens, were also obtained from cases of invasive carcinoma. She suggested that the state and expression of the persistent viral genome may be a function of the stage (preinvasive or invasive) of the cervical neoplasia.

Dr. Roizman agreed with Dr. Nahmias that it is important to determine whether the transcriptional program is the same in a latent infection as in a tumor cell, in terms of viral events. He emphasized that he considered it necessary to accept the idea that the virus is an important agent in the oncogenic process. For instance, the role of the virus in the different stages of cancer development, starting with carcinoma *in situ*, should be considered.

Dr. Naib indicated that he had surveyed old sections of cervical biopsies on patients who had had a genital herpes infection and those who had not. He noted that 45% of patients with history of genital herpes infections had single transformed cells in the endocervix and glands. These cells had a thick, dense cytoplasm and large nuclei not typical for viral changes as we know them.

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Cancer Res 1973;33:1417-1418.

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