Attenuation of RA 27/3 Rubella Virus in WI-38 Human Diploid Cells

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THE PROVENANCE of the RA 27/3 attenuated rubella strain has already been described in print.[1,2] Detailed information was given at the London Conference just three months ago.[3] Therefore we shall only outline the history of this strain, before proceeding to examine its in vitro characteristics and its behavior when inoculated in man.

In order to avoid the problem of passenger viruses, the RA 27/3 strain was isolated directly from naturally infected material in WI-38 human diploid fibroblast~.~

Explant cultures were made of the dissected organs of a particular fetus aborted because of rubella, the 27th in our series of fetuses aborted during the 1964 epidemic. The third explant, which happened to be from kidney, was selected arbitrarily for further study. Fibroblast cells that grew out from this explant could be subcultivated after several weeks. The presence of rubella virus in the supernatant fluids was confirmed. After four subcultivations of the infected kidney fibroblasts, the supernatant fluid was inoculated directly into a WI-38 culture. Once transferred to WI-38, the RA 27/3 rubella strain was passaged further in the same cell strain.

The RA 27/3 strain was tested again after four and eight passages in WI-38 incubated at 35 C. Subcutaneous inoculation of virus provoked much virus excretion, rash, and spread to contacts.

At this point, two sublines were developed, as illustrated in Table 1, the first by passage in WI-38 cells incubated at 35 C, and the second in the same cultures incubated at 33 C. After reaching the 13th WI-38 passage, the second subline was passaged in cultures incubated at 30 C.

Virus pools at four medium-passage levels were tested in man: the 11th and 14th passage levels of the first subline, and the 15th and 17th passage levels of the second. Although only two subjects were tested for each pool, the results were nevertheless striking (Table 2). The passages of the 35 C subline produced more virus excretion and more clinical reaction than the passages of the 30 C subline. In view of these results, the 35 C subline was

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Table 1.—Passage History of RA 27/3

passage No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16									
Dilution passed (C)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35									
passage No.	...	...	...	...	...	...	...	...	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Dilution passed (C)	...	...	...	...	...	...	...	...	33	33	33	33	33	30	30	30	30	35	30	35	30	30	30	30	35

dropped, and further work was done on the low-temperature subline.

Tests in man were conducted with 25th passage virus, which had been cloned five times by terminal dilutions, and revealed desirable properties of a vaccine strain.

Accordingly, we decided to use the 25th passage-level RA 27/3 virus as seed material for later pools and to expand the trials with virus at about this level. The results will be described below.

Characterization of RA 27/3 Virus

In vitro characteristics of the RA 27/3 virus have been investigated, and several markers have been noted.

Because the vaccine strain is adapted to replication at 30 C, we compared different passage levels with respect to growth at 37 C and 30 C. As illustrated in Table 3, RA 27/3 virus adapted to growth in RK_{13} cells replicated better at 37 C than at 30 C, which was also true for RA 27/3 through the 14th passage in diploid cells. Later passages in WI-38 cells of RA 27/3 vaccine virus showed definitely better replication at 30 C than at 37 C.

Plaque formation in RK_{13} cells [5,6] under various overlayers has revealed certain attributes of RA 27/3, as shown in Table 4. Here the RA 27/3 strain is compared to HPV-77 and a low-passage fresh isolate. RA 27/3 produced relatively small turbid plaques, similar in appearance to the low-passage strain. However, in the case of the low-passage strains plaqued under agar overlayers, good numbers of plaques could be produced only with the addition of diethylaminoethyl (DEAE)-dextran to the overlayer, while RA 27/3 produced plaques with or without the presence of DEAE-dextran in the agar.

A system for plaque formation was developed using BHK21 cells which were infected with RA 27/3 and then suspended in agarose.[7] Whereas plaques were produced by the RA 27/3 virus growth in BHK21 or in WI-38, no plaques were produced by other strains, as shown in Table 5. Neither Cendehill nor HPV-77 attenuated strains, nor five unattenuated strains produced plaques. Plaque formation did not occur with the original RA 27/3 virus or with the same strain after eight passages in WI-38 cell cultures. However, by the 15th passage the RA 27/3 virus had acquired the ability to produce plaques in this system. Isolates from the throat of subjects vaccinated with RA 27/3 also formed plaques in BHK21, whereas, in contrast, isolates from Cendehill vaccinees did not.

Clinical Results

Initially, the RA 27/3 was inoculated subcutaneously. Trials in institutionalized children and in normal families yielded no consistent clinical reaction except for an increase in palpable cervical lymph nodes. As shown in Table 6, which illustrates family studies, almost all vaccinees developed antibodies in the range of 1/80 to 1/60, but no spread occurred to seronegative contacts.

Late in 1967 we tried administration of RA 27/3 by a different route—the intranasal one.[8] Attenuated viruses administered parenterally multiply in the nasopharynx. However, HPV-77 [9] and Cendehill [10] attenuated viruses have not regularly induced antibodies when given intranasally. It was therefore interesting