In Vitro Approach to a Correlation of Cell Susceptibility to Viral Infection with HL-A Genotypes and Other Biological Markers[1] (36671)

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It is generally agreed that cells obtained from human fetal tissues may vary in their susceptibility to viral infection in tissue culture (1–5). Differences in the susceptibility of cells obtained from different donors to infection by poliomyelitis virus were initially described by Hayflick and Moorhead (1). Brown and Tyrell (2) and Embil and Faulkner (3) have reported similar observations for rhinoviruses, enteroviruses, adenoviviruses, mumps, measles and Herpes hominis viruses. More recently, Potter, Potter and Oxford (4) and Aaronson (5) prepared fibroblast cultures from explants of skin fragments obtained from newborn children and adults including some subjects with genetic diseases, and assessed the susceptibility of such preparations to infection by viral agents. Variations in the susceptibility of cells obtained from individual donors to infection by any one given virus were documented, although the etiology of such variations was not clear.

Recent observations indicate that a number of genetic factors, and particularly the genetically determined systems of histocompatibility, such as the murine H-2 system, for example, may play an important role in conditioning host resistance or susceptibility to virus-induced neoplastic diseases (6, 7). This consideration raised the possibility that the tissue culture technique, utilizing primary fibroblast lines obtained from the skin of related and unrelated donors genotyped for 17 different genetic marker systems, including HL-A, might provide a useful approach to further studies of this question in man.

Materials and Methods. Normal primary fibroblast lines were prepared from skin explants isolated from 30 Caucasian donors in the course of studies of the responses of HL-A genotyped subjects to skin allografts (8). The fibroblasts were cultured by the plasma clot technique; they were grown in McCoy's 5a modified medium (Grand Island Laboratory) containing 15% fetal calf serum and 100 IU of penicillin, 50 μg of streptomycin and 25 μg of Fungizone/ml of medium. The same medium, containing 5% fetal calf serum, was used for maintenance of the cultures when the cells had formed a confluent monolayer. For passage, the cells were detached from the culture dish by treatment with 0.25% trypsin solution. For preservation of the cell lines, aliquots of each fibroblast sample were frozen by graded cooling in McCoy's medium, with 30% calf serum and 10% dimethylsulfoxide; the cells were then stored in liquid nitrogen. All studies were done with cell lines which had been passaged 10 to 30 times. In addition to these fibroblasts, WI-38 cells were obtained from Flow Laboratories after 21 passages, and were handled in the same manner.

The genetic markers known for each of the fibroblast donors included HL-A, erythrocyte groups ABO, rhesus, MNSs, Duffy, Kell, Lu, Lewis, P, and Secretor; platelet groups Ko^a, Ko^b, Zw^b; serum groups Gm,

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Inv, Gc, Tf; erythrocyte enzyme groups PAc (acid phosphatase), PGM-1 (phosphoglucomutase-1) and AK (adenylate kinase). Fourteen of the donors were members of three families (Vin, Pet and Piq). In the Vin family, fibroblasts were available from the father (No. 36), the mother (No. 309), three genotypically HL-A identical siblings (Nos. 38, 39, 40) and two HL-A different siblings (Nos. 37, 308). Fibroblasts from the mother (Nos. 271) and two HL-A different children (Nos. 269, 270) were available from the Pet family. In the Piq family, fibroblasts from the father (No. 279), the mother (No. 282) and two HL-A different offspring (Nos. 280, 281) were studied. The remaining 16 fibroblast lines were obtained from unrelated subjects of known HL-A genotypes.

Four different strains of viruses known to produce cytopathogenic effects (CPE) in cultures of WI-38 cells were selected for study. Two strains, herpes hominis and adenovirus, are DNA viruses, and the other two, echovirus and measles virus, are RNA viruses. Two of the viruses, herpes hominis and the measles viruses are considered to be enveloped viruses and the other two are considered to be naked (9).[2] All viruses were passed at least once in WI-38 cells in this laboratory before use in the susceptibility tests. The seed virus for all tests consisted of measured aliquots of a pool of infected nutrient medium, which was frozen and stored at −70° until used. The susceptibility of fibroblast lines obtained from individual donors to infection by a given virus was evaluated on the basis of the production of a cytopathogenic effect (CPE). In a typical screening test, 10 fibroblast lines and WI-38 cells, which served as controls, were tested with two or more viruses in the same experiment.

Each cell line was grown in 35 × 10 mm plastic petri dishes (Falcon) until a confluent monolayer was formed. The growth medium was then replaced by 2 ml of maintenance medium, and 0.1 ml of four serial $\log_{10}$ dilutions of the virus under study was added to petri dishes containing fibroblasts from the same donor. Each test was performed in duplicate or triplicate, and was accompanied by one or two preparations of each cell line to which no virus was added (uninfected controls).

The cultures were examined daily for the presence of a CPE. The latter was graded from zero if no CPE was observed to 4+ if the entire monolayer was involved. The occurrence of spotty areas throughout the monolayer was graded as ±; a rating of 1+ referred to involvement of 25% of the monolayer: of 2+ to 50% and of 3+ to 75% destruction of the entire monolayer. The susceptibility of each fibroblast line to a given virus was gauged by a comparison of its CPE at the highest dilution of that virus giving a 1+ or greater CPE in WI-38 control cells at a specific time after infection. This time, which was determined after preliminary tests of each virus with WI-38 cells, was 2 days for the echovirus, 3 days for the herpes virus, and 7 days for adenovirus and the measles virus. Fibroblasts which showed less reaction than the WI-38 cells were considered relatively resistant under these experimental conditions; cells showing a 1+ or 2+ CPE were termed susceptible, and those with a 3+ or 4+ CPE were considered very susceptible.

Serum samples from each fibroblast donor were tested for antibody titers against the four viruses under study. Standard hemagglutination–inhibition tests were used for determination of measles and adenovirus-7 antibody titers; the standard complement fixation test was employed for determination of herpes simplex antibody, and the neutralization test for determination of echovirus-11 antibodies.[3]

Results. The results of a representative screening test are illustrated in Table I. In this particular experiment, the highest dilution of herpes virus giving a 1+ CPE in WI-38 cells after 3 days was 10^{-6}. At this

[2] The herpes hominis Type 1 virus was obtained from Drs. P. Brunell and A. Gershen at New York University; the adenovirus Type 7 was obtained from Dr. Stephen Millian, New York City Department of Health Laboratories; the measles virus was provided by Dr. John Enders, Harvard Medical School.

[3] Lennette, E. H., and Schmidt, N. J., eds., "Diagnostic Procedures for Viral and Rickettsial Infections." Amer. Pub. Health Ass., New York (1969).

TABLE I. Comparison of the CPE Caused by the Same Inoculum of Herpes Hominis Virus in Susceptible WI-38 Fibroblasts and in Skin Fibroblasts Obtained from a Genotyped Panel of Normal Human Subjects.

		Degree of CPE observed at virus conc of:				
Cell line	After inoculation (day)	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Uninfected control cells
WI-38	1	±	0	0	0	0
	2	2+	1+	0	0	0
	3	2+	2+	1+	±	0
279	1	±	0	0	0	0
	2	1+	±	0	0	0
	3	2+	1+	1+	±	0
280	1	0	0	0	0	0
	2	±	0	0	0	0
	3	1+	±	0	0	0
281	1	±	0	0	0	0
	2	2+	1+	±	0	0
	3	3+	2+	1+	1+	0
282	1	1+	±	0	0	0
	2	3+	2+	1+	±	0
	3	4+	3+	2+	1+	0

concentration of the virus, fibroblast culture Nos. 279, 281 and 282 were susceptible, and culture No. 280 was relatively resistant to herpes virus. A similar experiment performed under the same conditions 3 months later gave the same results.

In repeated experiments, the optimal dilution of each virus for comparing susceptibility to infection was found to be 10^{-6} for herpes hominis, 10^{-4} for adenovirus, and 10^{-6} or 10^{-7} for echovirus. In one experiment with measles virus, it was 10^{-2}; in another test, it was 10^{-4}.

The results of screening fibroblast lines from 30 normal human subjects are summarized in Table II. Twenty-seven of the fibroblast lines were susceptible to infection by the herpes virus; 4 of them were very susceptible. Three other cell lines were classified as resistant. Only 2 of 26 cell lines were resistant to adenovirus. These two cell lines were also resistant to the measles virus. All of the 18 cell lines tested were susceptible to echovirus. The resistance of individual cell lines was usually virus-specific; and resistance to one given virus was not necessarily associated with resistance to others.

Cell lines obtained from the parents (Nos. 36 and 309) and three HL-A genotypically identical siblings (Nos. 38, 39, 40) in family Vin (Table II) were equally susceptible to infection by the viruses used in this study. In contrast, fibroblasts obtained from an HL-A different sibling in this family (No. 308) were resistant to the herpes, adeno- and measles viruses. In the Piq family, cells from the parents and one sibling (Nos. 279, 282 and 281, respectively) were susceptible to the herpes, adeno- and echoviruses, while cells prepared from an HL-A different sibling (No. 280) were resistant to the herpes virus.

Interpretation of the CPE as evidence of viral infection of the affected cells was supported by additional studies designed to assess the ability of the cells to support viral growth, as reflected by viral replication and the production of viral hemagglutinins. Figure 1 illustrates a representative experiment. Petri dish cultures of a resistant cell line (No. 280) and a susceptible line (No. 38) were inoculated with 0.1 ml of a 10^{-5} dilution of herpes virus. The preparations were examined at 24 hr intervals, the CPE was recorded, and 2 samples of each cell line were

TABLE II. Summarized Results of Testing the Susceptibility of 30 Different Human Fibroblast Lines to Viral Infection.[a]

Cell strains tested	Virus utilized			
	Herpes hominis	Adenovirus-7	Echovirus-11	Measles
Control cells WI-38	S	S	S	S
Families studied (No.)				
Vin, F 36	S	S	S	S
M 309	S	S	S	S
Sib 37	S	S	S	S
Sib 38+	S	S	S	nd
Sib 39+	S	S	S	S
Sib 40+	S	S	S	S
Sib 308	R	R	S	R
Pet, M 271	S	S	S	nd
Sib 269	S	S	S	nd
Sib 270	S	S	S	nd
Piq, F 279	S	S	S	nd
M 282	S	S	S	nd
Sib 280	R	S	S	nd
Sib 281	S	S	S	nd
Unrelated individuals (No.)				
111	S	S	nd	S
106	VS	VS	nd	S
124	VS	S	nd	S
141	VS	S	nd	S
126	S	S	nd	S
109	S	S	nd	S
6	S	VS	nd	S
123	S	VS	nd	S
100	S	R	nd	R
117	VS	VS	nd	S
153	S	nd	S	nd
252	S	nd	S	nd
154	R	nd	S	nd
265	S	nd	S	nd
118	S	VS	nd	S
121	S	VS	nd	S

[a] F = father; M = mother; Sib = sibling; S = susceptible; VS = very susceptible; R = resistant; + = HL-A identical.

frozen daily at −70°. On day 4, the dishes were thawed, and the supernatant fluid from each set of 2 dishes was removed, serially diluted, and titrated for its viral concentration by inoculating 0.1 ml of serial dilutions of the sample into each of 3 culture tubes containing WI-38 cells. The end point was the highest dilution of each sample giving a CPE at 48 hr after inoculation. As noted in Fig. 1, a 1+ CPE was observed in the susceptible cells (No. 38) after 24 hr; it increased to 2+ at 48 hr and 3+ at 96 hr. The viral content of the frozen and thawed supernatant also increased progressively; its viral titer was 10^{-4} on day 1, 10^{-8} on day 2 and 10^{-9} on day 3. In contrast, no CPE was noted in the resistant cells (No. 280) until day 4, and no virus was detected in the supernatant until day 2, when the viral titer of the supernatant was only 10^{-2}. This result suggests that

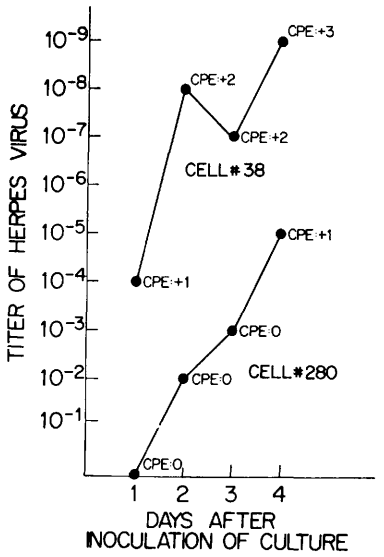

FIG. 1. Comparison of the CPE and rate of replication of herpes virus in a susceptible (No. 38) and a resistant (No. 280) cell strain. Titration of herpes virus in the supernatants obtained daily from cell strains Nos. 38 and 280, performed in tubes of WI-38. The end point was determined as the highest dilution producing a cytopathogenic effect at 48 hr after inoculation.

although resistance to viral infection is relative in nature, there is a direct relationship between the degree of resistance or susceptibility to viral infection, as evidenced by the CPE, and the ability of a virus to replicate in the susceptible cells. The possibility that the resistance of some of the cell strains tested might have been a consequence of interference by saprophytic organisms, such as Mycoplasma, for example, was tested by omitting the thallium acetate from the Hayflick culture media in a number of experiments, thereby removing its inhibitory effect on the growth of nonhuman strains of myoplasma. Both liquid and agar media were used, and no evidence for the presence of PPLO organisms was found in three resistant cells strains (Nos. 100, 154, 280). More rigorous tests will be required, however, to conclusively eliminate this possibility.

Evidence of a correlation between the CPE and the production of viral hemagglutinins, as an expression of the rate of intracellular replication of the virus in susceptible cells, was sought in another experiment. For this purpose, petri dishes containing monolayer cultures of cell line No. 308 which had been found susceptible to echovirus were inoculated with serial dilutions of this virus. Pairs of dishes were frozen at —70° at 24 hr intervals after inoculation. These were then thawed and centrifuged, and the supernatant, which contained intracellular and extracellular components, was tested for the presence of viral hemagglutinins at 4° with human blood group O erythrocytes. The supernatant obtained at 24 hr after inoculation, when no CPE had been observed, contained no detectable viral hemagglutinins. In contrast, supernatants obtained on days 2 and 3, when the CPE was 2+ and 3+, respectively, contained hemagglutinin titers of 1:32 and 1:64, respectively.

There was no apparent correlation between the presence of humoral antibodies against measles, adenovirus, and echovirus 11 and the *in vitro* susceptibility of fibroblasts obtained from these donors to infection by these viruses. On the other hand, three individuals (Nos. 124, 141, 117) who were very susceptible to infection by the herpes simplex virus had antibody titers exceeding 1:8 against this virus.

Discussion. Recent studies of the susceptibility of mice to oncogenic viruses have provided convincing evidence that the host genome, and particularly genes closely linked to or identical with the H2 locus play an important role in conditioning host resistance to viral infection (6, 7). Previous reports suggest that human fetal and adult fibroblasts may differ in their *in vitro* response to viral infection (1–5). These observations have raised the possibility that a systematic investigation of the HL-A genotypes and other biological markers present in the individual cell donors may provide some information regarding hitherto unrecognized host variables capable of conditioning human susceptibility to viral infection. Variations in the susceptibility of cells obtained from different normal individuals to infection by one or more viruses have been observed during this study. The number of subjects studied thus far is evidently insufficient to warrant any

conclusions regarding possible relationships between individual host cell resistance to viral infection and the biological markers of that subject. It is of interest, however, that the resistance of an individual cell line to a given virus appeared to be specific for that virus, and did not imply resistance of the same cells to other viruses. This observation supports the contention that the variations in cell susceptibility to viral infection described in the present study were not a consequence of nonspecific mechanisms of host resistance to viral infection. Rather, such variations may have been related to genetic factors conditioning the host cell membrane properties.

It is of interest that some correlation has been noted between fibroblast susceptibility to infection by herpes virus and the titer of humoral antibodies against this virus in the cell donor. This preliminary observation raises the intriguing possibility that there may be a relationship between levels of circulating antibodies against a given virus and its ability to replicate in the host cells. Extensive further studies will be required, however, to confirm this possibility.

The further screening of cell lines isolated from a large number of families genotyped for 17 different genetic marker systems (8) may be of value in delineating possible patterns of inheritance of resistance to viral infection. The possibility that the host's HL-A genotype or genes closely linked to the HL-A chromosomal region may play a role in conditioning resistance to viral infection may be particularly deserving of further study. No pattern of dominant inheritance has evolved as yet from the limited studies performed here. It is of interest, however, that cells obtained from donor No. 280 (family Piq) were resistant to herpes virus, and cells from donor No. 308 (family Vin) were resistant to herpes, adeno- and measles viruses, while cell lines obtained from their respective parents and from other siblings in the same families were susceptible to these viruses. This observation suggests that if susceptibility to viral infection in man is determined by a host cell genome, this control is likely to be of a multigenic nature.

Summary. In vitro tests of the susceptibility of 30 normal human fibroblast lines to infection by herpes hominis, adenovirus-7, echovirus-11 and measles viruses have shown significant variations in individual host susceptibility to infection by one or more of these agents. Resistance of an individual cell line to a given virus appears to be specific for that virus, and is not necessarily associated with resistance of the same cells to other viruses.

Family studies utilizing fibroblasts obtained from parents and from siblings genotyped for the HL-A system and other biological markers suggest that, if susceptibility to viral infection in man is determined by a host cell genome, this type of genetic control may be of a multigenic nature. No pattern of dominant inheritance has been observed in the limited studies reported here. The results suggest, however, the possibility that the host's HL-A genotype or genes closely linked to the HL-A chromosomal region may play a role in conditioning resistance to viral infection.

1. Hayflick, L., and Moorhead, P. S., Exp. Cell. Res. **25**, 585 (1961).

2. Brown, P. K., and Tyrrell, D. A. J., Brit. J. Exp. Pathol. **45**, 571 (1964).

3. Embil, J. A., and Faulkner, R. S., Can. J. Pub. Health **55**, 111 (1964).

4. Potter, C. W., Potter, A. M., and Oxford, J. S., J. Virol. **5**, 293 (1970).

5. Aaronson, S. A., J. Virol. **6**, 470 (1970).

6. Lilly, F., J. Exp. Med. **127**, 665 (1968).

7. Lilly, F., J. Nat. Cancer Inst. **45**, 1963 (1970).

8. Dausset, J., Colombani, J., Legrand, L., Feingold, N., and Rapaport, F. T., Blood **35**, 591 (1970).

9. Rhodes, A. J., and Van Rooein, C. E., "Textbook of Virology," 5th ed., Chap. 1, Table 1/1/8. Williams and Wilkins, Baltimore (1968).

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