Sensitivity of Human Cell Strains to Interferon[1] (34650)

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Viral infections constitute a serious threat to the embryo-fetus and neonatal human being. The more mature host apparently has the capacity to limit the effect of viral infections whereas disseminated viral disease is an unfortunately frequent phenomenon in immature individuals (1). Interferon (IF) of endogenous origin may play an important part in limiting viral disease (2). Several investigators have shown that embryonic, fetal, and neonatal tissue can be induced *in vitro* to produce IF (3, 4), and others have studied the interrelationships between viral disease, interferon, and immaturity (2, 5, 6). The basis of the experiments to be reported herein was the speculation that immature human tissues might be inadequately protected by amounts of IF which would suppress viral replication in more mature tissues. To test this hypothesis, cell cultures derived from human products of conception of various gestational age were exposed to reference human IF preparations prior to viral challenge. Differences in the IF sensitivity of the various cell strains were observed. The purpose of this paper is to report the details of these experiments and to discuss their possible significance.

Materials and Methods. Cell strains. Four embryonic, three fetal and three neonatal human fibroblast-like cell strains were tested. Their origin and characteristics are presented in Table I. They were established by the method described by Hayflick and Moorhead (7), or obtained from commercial sources. The primary cultures of cell strains designated C999, Cum laude, Git lung, and LY HEL were derived from trypsinized prenatal tissues, while the cultures of neonatal tissues were obtained by mincing parts of the human organ with small scissors without the use of trypsin. All cell lines were established in minimum essential medium (Eagle) with Hanks' balanced salt solution[5] enriched with 2× L-essential amino acids, 2× vitamins, 10% fetal bovine serum[6] and antibiotics (penicillin 800 U/ml, streptomycin 800 μg, kanamycin 80 μg, and nystatin 80 U/ml). Cultures were passaged twice a week by trypsinizing and splitting in a 1:2 ratio whenever growth was sufficient. All experiments were carried out between the tenth and fiftieth passages of these cell strains.

Cell strains were prepared for assays of IF sensitivity and virus titer in 125-mm roller tubes and for IF induction in 32-oz prescription bottles. The general appearance and the number of cells per tube or bottle allowed valid comparisons of IF sensitivity and yield. For IF induction or assay experiments, the cell lines remained in growth medium; all virus titration experiments were carried out in maintenance medium: MEM (Eagle) with Earle's BSS with 10% fetal bovine serum and antibiotics.

Viruses. The C_G strain of Newcastle Disease virus (NDV) was used for the production of all IF preparations. The virus had

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TABLE I. Characteristics of Human Cell Strains.

Designation of tissue	Source of cells	Producer	Age of embryo-fetus or neonate	Chromatin bodies	Reason for availability of tissue
C999	Skin and muscle	Authors	8 weeks	Negative	Therapeutic abortion
Cum laude	Skin and muscle	Authors	9 weeks	Negative	Therapeutic abortion
Git lung	Lung	Authors	11 weeks	Positive	Therapeutic abortion
WI-38	Lung	MBA	13 weeks	Positive	Therapeutic abortion
GIBCO A1	Lung	GIBCO	16 weeks	Negative	Complete hysterectomy
LY HEL	Lung	Authors	22 weeks	Negative	Premature delivery with neonatal death
GIBCO A3	Lung	GIBCO	32 weeks	Negative	Premature delivery with neonatal death
D 55	Lung	Authors	2 days following term birth	Positive	Autopsy (ventricular hemorrhage)
Foreskin II	Foreskin	Authors	4 days following term birth	Negative	Bell circumcision
MA-184	Foreskin	MBA	7 days following term birth	Negative	Ritual circumcision

been passed five times in the allantoic fluid of 11-day old embryonated hens' eggs since its receipt from Drs. F. B. Bang and M. Foard in 1964. The virus pool had a titer of $10^{8.5}$ tissue culture doses (TCD_{50})/0.1 ml when tested on baby hamster kidney (BHK-21) tissue obtained from MBA. Sindbis virus and vesicular stomatitis virus (VSV) were used as challenge viruses. The strain of vesicular stomatitis virus (VSV) was received by this laboratory in 1964 from Dr. Allan H. Levy. It had been passed once in primary mouse embryo cell culture and once in primary chick embryo cell culture. The Sindbis virus strain, kindly given by Dr. Lowell A. Glasgow in 1966, had been passed once in WI-38 tissue (MBA) prior to its use as challenge virus.

All three viral agents were stored in their harvest fluids at —80° without further purification. The same pool of each virus was used throughout these experiments.

Interferon preparation. Two sets of IF preparations were employed in this study. The first was comprised of fluids harvested from six human leukocyte cultures after exposure to NDV essentially as described by Wheelock (8). These leukocyte interferons were used as reference IF's in each experiment in which the IF sensitivity of any of the cell strains was tested. The second set was comprised of 10 pairs of IF preparations induced by NDV in those human fibroblast cell strains described above. These IF preparations were assayed to compare the IF yield from various cell strains; they were not used as standard or reference IF preparations.

Leukocytes were obtained from heparinized venous blood from healthy adults or from cord blood samples which were kept upright in the syringe at room temperature for 15 min after the addition of two parts of 3% dextran (mol wt 200,000–300,000) to each specimen. After separation from the red blood cells, the leukocyte-rich plasma–dextran mixture was spun at 300*g* for 10 min. The cell button was resuspended in a small amount of medium 199 with 20% agamma-calf serum. Total and differential leukocyte counts were done, and the cultures were set up using 3×10^6 mononuclear cells/ml of medium. The cultures were inoculated immediately with 3×10^7 TCD_{50} of NDV/ml (NDV: lymphocyte multiplicity, 10:1) and incubated rolling in plastic screw cap tubes at 37°. Twenty-four hr later the cultures were centrifuged at 900*g* for 10 min and the supernatant IF fluids were harvested. IF was

stored at 4° until the NDV was inactivated by lowering the pH to 2.2 for 48 hr. After the pH had been restored to normal, the specimens were kept in small aliquants at −80°.

Fibroblast IF preparations were produced by inoculating complete, confluent monolayers of each cell line in 32-oz prescription bottles with 21×10^6 TCD_{50} of NDV. Each bottle contained 30 ml of medium. Eighteen hr later the IF-containing fluids were decanted, acidified, and 48 hr later the pH was returned to 7. They were then stored in the same manner as the leukocyte IF preparations.

Assay of sensitivity of cell strains to IF. IF assays were carried out according to a modification of a method described by Wheelock (8). In each experiment, two or three cell strains were compared. Twenty-four-hr-old monolayers of each strain were treated with 1.0 ml/tube of serial twofold dilutions of three of the six reference IF preparations diluted in fibroblast growth medium. After stationary incubation at 37° for 2 hr, 5000 TCD_{50} of Sindbis virus or 2500 TCD_{50} of VSV in 1.0 ml of medium were added to each tube. The tubes were then incubated in a rotation rack at 37°. Cell cultures without IF but with an equal quantity of medium were used as controls. The IF titer was expressed as the reciprocal of the highest IF dilution which limited cytopathic effect (CPE) to less than 25% at a time when the virus control tubes showed more than 75% but less than 100% CPE.

Originally it had been planned to use the commercial MA-184 cell strain as the comparison standard for both VSV and Sindbis IF titrations. However, it was found that MA-184 cells were not sufficiently sensitive to Sindbis virus cytopathic effect (CPE) to permit their use. Therefore, the Git lung cell strain was substituted in all comparative IF assays in which Sindbis virus was the challenge virus, whereas MA-184 did serve as the standard in all comparative IF experiments in which VSV was the challenge virus.

Sensitivity of cell strains to Sindbis and VSV. In each experiment one or two cell strains in addition to the reference tissue were tested. The Cum laude cell strain was chosen arbitrarily to be the reference tissue for all comparative Sindbis virus and VSV titrations. Serial tenfold dilutions of viral suspensions in Hanks' balanced salt solution were inoculated, 0.1 ml/tube, in sets of 3 tubes/dilution. The tubes were incubated rolling at 37° for 12 days. They were inspected for CPE twice a week and fed. The titer given was the reciprocal of the highest dilution of the viral inoculum which produced cytopathic effect (CPE) and the TCD_{50} was calculated by the method of Reed and Muench (9).

Sex chromatin determinations. Cells were removed from the glass surface by scraping and fixed in 95% ethyl alcohol. Concentrated cell suspensions were transferred to microscope slides and ignited. After HCl hydrolization, the cell preparations were stained with Thionin and inspected for Barr bodies after brief rinses in ethyl alcohol and xylol.

Results. Sensitivity of cell strains to IF. The extent to which standard IF preparations could be diluted and still protect cell strains varied greatly (Tables II and III). When VSV was used as challenge, the IF titers observed in three of the six cell lines (C999, Git lung, LY HEL) from embryos less than 22-weeks gestation were consistently one-eighth that titer obtained in neonatal reference tissue. All such lines were always less sensitive than cell strains derived from neonatal tissues. Less difference was observed when IF sensitivity was tested by Sindbis

TABLE II. Sensitivity of Human Cell Strains to Interferon.

Cell strain	IF titer[a]			IF titer in MA-184 in same exp.		
C999	40	40	160	640	640	2560
Cum laude	40	160	320	640	1280	2560
Git lung	160	160	160	1280	2560	2560
WI-38	160	320	320	640	640	2560
GIBCO A1	80	320	320	640	2560	2560
LY HEL	80	320	320	640	2560	2560
GIBCO A3	80	320	320	640	2560	2560
D 55	160	160	640	640	640	1280
Foreskin II	320	320	1280	640	640	1280

[a] Titer of standard IF preparations in human cell strains using VSV as challenge virus and MA-184 as standard cell strain.

TABLE III. Sensitivity of Human Cell Strains to Interferon.

Cell strain	IF titer[a]			IF titer in Git lung in same exp.		
C999	320	320	1280	320	320	1280
Cum laude	160	1280	1280	80	320	320
WI-38	320	1280	1280	160	640	640
GIBCO A1		ND[b]			ND	
LY HEL	320	1280	1280	320	1280	1280
GIBCO A3	640	2560	2560	320	1280	1280
D 55	640	1280	2560	320	320	1280
Foreskin II	1280	1280	5120	320	320	1280

[a] Titer of standard IF preparations in human cell strains with Sindbis as challenge virus and Git lung as standard cell strain.

[b] Not done.

virus challenge, but the same general relationship obtained. Thus, in general, cell strains derived from the least mature human tissues were the least sensitive to human interferon.

Sensitivity of cell strains to viral CPE. Table IV presents the results of VSV and Sindbis virus titrations on the various cell strains using CPE as the end point. The only significant difference in the susceptibility of these cell lines to the test viruses was that MA-184 was insensitive to the cytopathic potential of Sindbis virus.

IF production by fibroblast cell strains. The titer of IF produced in the different cell lines varied from 10 to 160 (Table V). The gestational age of the strains source did not seem to be related to the amount of IF produced.

TABLE IV. Sensitivity of Human Cell Strains to Virus.

Cell strain	Virus	
	VSV	Sindbis
C999	5.5[a]	5.5
Cum laude	5.5, 6.5, 6.3	6.3, 5.5, 5.5
Git lung	6.5	5.5
WI-38	5.8	6.5
GIBCO A1	5.8	5.5
LY HEL	5.8, 6.3	5.5, 5.5
GIBCO A3	6.5	5.5
D 55	6.5	6.5
Foreskin II	6.8	6.5
MA-184	5.5	1.5

[a] Log_{10} TCD_{50}/ml.

TABLE V. Interferon Production by Human Cell Strains.

Cell strain	IF titers (units/ml)	
	First test	Second test
C999	(10)[a]	(10)[a]
Cum laude	20	80
Git lung	160	160
WI-38	160	80
GIBCO A1	80	80
LY HEL	80	80
GIBCO A3	40	80
D 55	160	80
Foreskin II	160	160
MA-184	40	80

[a] Tissue not fully satisfactory at time of testing.

Reproducibility of IF and virus assays. In 25 instances, different vials of several of the leukocyte IF harvests were retitrated on the same cell strain using either VSV or Sindbis virus. The repeat titers were identical to the original ones in 18 instances, in 6, a twofold increase or decrease was found. In one experiment the titer was one fourth that first observed. Table VI shows the IF titers found in 4 instances in which a particular IF specimen was retitrated at least 5 times on the same cell strain with the same challenge virus.

TABLE VI. Reproducibility of IF Sensitivity Assay.

Cell strain and challenge virus	Titer of leukocyte IF preparation	
	Preparation 1	Preparation 2
Git lung, Sindbis virus	320	160
	640	320
	1280	320
	1280	320
	1280	320
	1280	
MA-184, VSV	1280	320
	2560	640
	2560	640
	2560	640
	2560	640
		640

Discussion. The concept that the sensitivity of a tissue to IF might be a determinant of its resistance to virus invasion is not new; it was investigated by Isaacs and Baron in 1960 (3). When chorioallantoic membranes from chick embryos of different ages were treated with chick IF of various concentrations prior to infection with influenza virus, those from 15-day old embryos were more than 16 times more sensitive than those from 6-day embryos. The same type of experiment carried out on minced tissue from mouse embryos of different ages gave similar results (3).

Cantell's experiments (4) are more comparable to the method employed in our investigations. Like Isaacs, Cantell worked with a chick system, but instead of using intact pieces of chorioallantoic membranes he trypsinized chick embryos of various ages and then assayed standard IF's on primary cell cultures derived from those embryos. His results demonstrated that the IF sensitivity of chick cell cultures depended on the age of the embryos from which the cells were derived.

Our data using a human system are consistent with but do not conclusively confirm these results. When VSV was used as a challenge virus, the neonatal cell lines as a group were more IF sensitive than the prenatal tissues; however, there was no direct relationship between the age of the source embryo and the IF sensitivity of the cell strain derived from it.

The differences observed may have been due to factors other than gestational age of the source tissue. DeMaeyer and DeMaeyer-Guignard (10) have studied the influence of genotype and age on the amount of circulating IF induced in mice. They used three different strains of mice and observed as much as sevenfold variations in IF yield in mice of the same age and strain. The extent of variation increased to fortyfold when mice of the same age, but from different strains, were compared. They did not study IF sensitivity. Although the information in regard to the racial background of our cell lines is incomplete, one cell line was derived from a negro fetus, and of the six cell lines known to be of white origin one is of Jewish extraction and another one was obtained in Denmark. No conclusions can be drawn about this aspect of the matter.

In addition, the cell strains studied in our experiments were derived from several different tissues; skin and muscle, lung, and foreskin. To our knowledge it is not known whether the organ source of human fibroblasts affects their interaction with IF. Fibroblast cultures of human embryo pharyngeal mucosa differed from fibroblast cultures derived from the lung of the same embryo in their ability to divide when infected experimentally with rubella (11), a function which might be a result of such interaction.

The most critical question concerning our experimental approach is whether there is a difference between the IF sensitivity of a cell strain and the *in vivo* IF sensitivity of tissues from which it was derived. The small amount of tissue available from each human embryo and the large amount of tissue required for each experiment precluded testing the IF sensitivity of cell strains prior to their eighth passage. There was very little difference in these experiments when the same IF preparation was tested on the same tissue between the tenth and fiftieth passage. Thus, the relationship between the results of these *in vitro* and those of *in vivo* studies which theoretically might be done is unknown.

The question of whether insensitivity of IF might decrease a tissue's resistance to viral infection and virus-induced destruction was not answered by the virus titrations done on these cell strains: cell strains which differed markedly in their sensitivity to IF did not differ in their suscepibility to the CPE of the viruses used.

Summary. These studies revealed that cell strains derived from the least mature human embryos were the least sensitive to interferon and those derived from neonatal tissues were the most sensitive to IF. The significance of these observations was not demonstrated; the possible implications of the findings are obvious and were briefly discussed.

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