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RELATIONSHIP BETWEEN IN VIVO AND IN VITRO  
PRODUCTION OF INTERFERON

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Previous determinations of interferon in mouse blood serum [1] showed that different strains of mice after intravenous injection of virus produce unequal amounts of interferon, i.e., the level varies with the genotypic characteristics of the animals. It seemed of interest, therefore, to compare the ability of different strains of mice and their leukocytes to produce interferon in vitro. The capacity of leukocytes from human beings of different ages to produce interferon was also determined.

Material and Methods

Newcastle disease virus was used in mice as an interferonogen. The mode of administration and determination of interferon in the serum are described in an earlier report of ours [1].

Chikungunya virus, grown in chick embryos by inoculation into the allantoic cavity, was used as an interferonogen in suspensions of mouse and human leukocytes. The amount of virus in the allantoic fluid was determined by titrating it on a monolayer culture of chick fibroblasts.

The leukocyte suspensions were prepared as follows. Blood was drawn into test tubes with heparin in the proportion of 1 drop of dilute 1:10 liquid heparin per ml of blood and placed in a

refrigerator at 4° for 18 to 20 hours. The leukocyte film was then removed and suspended in medium 199 at the rate of 500,000 leukocytes per ml of suspension. Chikungunya virus was added at the rate of 1 CPD<sub>50</sub> per leukocyte. After the leukocytes were kept at 37° for 24 hours, they were removed by centrifugation and the supernatant fluid was acidified to a pH of 2.4, which was brought to 7.4 after 18 to 20 hours.

The interferon content of the supernatant fluid was determined by testing it in successive double dilutions on a monolayer culture of human embryonal musculocutaneous tissue. Smallpox virus (100 CPD<sub>50</sub> in a volume of 0.1 ml), used as the test virus, was introduced into the human tissue culture 20 hours after the addition of interferon. The results of titration were calculated 3 to 4 days from the cytopathic effect of the virus.

### Results and Discussion

Experiments on inbred mice revealed a correlation between the amount of interferon in serum following intravenous injection of the animals and the amount of interferon produced in vitro by the leukocyte suspensions obtained from the same strains. The results are summarized in Table 1.

Table 1. Correlation Between Interferon Production by Different Strains of Mice and Their Leukocytes.

| (1)<br>Линия | (2) Титр интерферона                            |                                                                                |
|--------------|-------------------------------------------------|--------------------------------------------------------------------------------|
|              | (3)<br>в крови при<br>внутривенном<br>заражении | (4)<br>в культураль-<br>ной жидкости<br>зараженной<br>лейкоцитарной<br>взвесью |
| C3H          | 1:16                                            | 1:4                                                                            |
| CC57Br       | 1:128                                           | 1:32                                                                           |
| A            | 1:128                                           | 1:32                                                                           |

**Key:** 1 - Strain; 2- Interferon titer;  
3 - in blood after intravenous  
injection; 4 - in cultural fluid  
of infected leukocyte suspension

The conclusion to be drawn from the results is that the capacity of leukocytes to produce interferon in vitro is fully correlated with that of the integral organism. Consequently, by withdrawing leukocytes we can evaluate the organism's capacity to produce interferon. Since interferon is regarded as a factor in resistance to virus infection, it is reasonable to assume that interferon production varies with the state of the physiological development of the organism. It is common knowledge, for example, that infants are highly susceptible to many virus infections, probably because of their low level of interferon production. The data presented in Table 2 completely support this assumption.

Table 2. Interferon Production by Leukocytes from Adults and Children

| (1)<br>Всего<br>исследо-<br>ванных | (2)<br>Возраст<br>обследо-<br>ванных | (3) Титр интерферона |     |     |      |      |
|------------------------------------|--------------------------------------|----------------------|-----|-----|------|------|
|                                    |                                      | <1:4                 | 1:4 | 1:8 | 1:16 | 1:32 |
| 48                                 | 20—50 лет (4)                        | 0                    | 3   | 22  | 15   | 8    |
| 25                                 | 1 месяц—<br>1 1/2 года (5)           | 22                   | 3   | 0   | 0    | 0    |

Key: 1 - Total number of persons examined; 2 - Age of those examined; 3 - Interferon titer; 4 - 20 to 50 years; 5 - 1 month to 1 1/2 years

The data presented in Table 2 indicate that there is a marked difference between the leukocytes of adults and children in interferon production. The leukocytes of all the adults produced interferon, whereas the leukocytes of the great majority of infants were unable to do so. It is obvious, then, that the immaturity or inadequacy of this defense mechanism is one of the reasons for the high susceptibility of infants to virus infections. This conclusion is indirectly confirmed by the results of examination of leukocytes in leukemic patients. The latter suffer acutely from such diseases as measles and chickenpox, and severe complications often follow the use of vaccine. The meager production of interferon may be another factor in the severity of the course of these infections (Table 3).

Table 3. In Vitro Production by Leukocytes of Leukemic Patients

| Фамилия<br>больного (1) | Титр интерферона<br>(2) |
|-------------------------|-------------------------|
| Г.                      | <1:4                    |
| Р.                      | <1:4                    |
| З.                      | <1:4                    |
| С.                      | <1:4                    |
| Ц.                      | <1:4                    |
| Н.                      | 1:16                    |

Key: 1 - Patient; 2- Interferon titer

It is evident from Table 3 that the capacity of leukocytes for interferon production was markedly suppressed in 5 of the 6 patients examined. Similar results were obtained by Lee et al. [2].

The foregoing data suggest that in vitro production by leukocytes can be used as a test for general reactivity of the body.

Interferon production may also depend on temperature and other conditions. This, in turn, may affect the susceptibility to some virus infections, influenza in particular. Our earlier experiments on mice [1] showed that keeping animals at 4° depresses their capacity to produce interferon. In view of the foregoing data and the differences in susceptibility to the influenza virus (high in winter and low in summer) shown by volunteers, we joined L. A. Porubel' and R. I. Rappoport in determining the amount of interferon in the contents of the upper respiratory tract. Volunteers were injected intranasally with influenza virus inactivated by ultraviolet rays. The results of this study warrant special discussion, but we shall merely note at this time that interferon was detected in smears more often and in higher titers in summer, when the interferon level was three times than in winter. We regard this as one of the reasons for the differences in human susceptibility to respiratory virus infections from season to season.

Thus, determination of interferon production can serve as a test of reactivity to viruses. This test can probably be used to judge immunoreactivity in healthy persons and in patients during stages of disease and convalescence. We are continuing our research in this field.

### Conclusions

1. In vitro interferon production is correlated with in vivo production.
2. Children's leukocytes produce little or no interferon. Leukemic patients also seem to be weak and irregular producers of interferon.
3. The capacity of leukocytes to produce interferon can be used as a test of immunological reactivity.

### Bibliography

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2. Lee, S. H. S., Ozere, K. L., and Van Rooyen, C. E. Proc. Soc. Exp. Biol. (New York), 1966, Vol. 122, p. 32.