7. R. M. Khaitov, Med. Ref. Zh., Section 21, No. 3, 9 (1976).
8. R. M. Khaitov and A. A. Batyrbekov, Byull. Éksp. Biol. Med., No. 5, 582 (1976).
9. B. Benacerraf and H. O. McDevitt, Science, 175, 273 (1972).
10. L. Lichtenberg, E. Mozes, G. M. Shearer, et al., Eur. J. Immunol., 430 (1974).
11. H. O. McDevitt and M. Sela, J. Exp. Med., 126, 969 (1967).
12. H. O. McDevitt, K. B. Bechtol, F. C. Grumet, et al., in: Progress in Immunology, ed. B. Amos, New York (1971), p. 495.
13. E. Mozes, G. M. Shearer, M. Sela, et al., J. Immunol., 3, 439 (1973).
14. A. J. Munro and M. J. Taussig, Nature, 256, 103 (1975).
15. M. J. Taussig, E. Mozes, and R. Isac, J. Exp. Med., 140, 301 (1974).

IMMUNE INTERFERON FORMATION AND BLAST TRANSFORMATION IN STIMULATED HUMAN LYMPHOCYTE CULTURES

L. S. Priimyagi and V. G. Reizenbuk

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KEY WORDS: immune interferon; blast transformation of lymphocytes.

There has been a steady rise in recent years in interest in the regulatory effect of interferon (IN) on the immune response, described by many workers [4, 8, 9, 12]. Immune IN, or type II IN, is nowadays regarded as a mediator secreted by lymphocytes in response to their stimulation [6, 14, 15].

The study of the individual features of immune IN production under the influence of mitogens and antigens by lymphocytes from people in different age groups is interesting. In the literature on this subject there are only a few, somewhat contradictory communications [5, 13].

The aim of the present investigation was to study production of IN and the dynamics of its formation in lymphocyte cultures (LC), obtained from children and adults and stimulated by phytohemagglutinin (PHA) and dried, purified tuberculin (PPD), and to study the relations between these processes and blast transformation of lymphocytes (BTL).

EXPERIMENTAL METHOD

Blood for LC was obtained from seven adults (aged 40-50 years) and 24 children (aged 5-7 years). IN formation was studied in 82 LC (52 from children and 30 from adults). The method of obtaining LC and of evaluating BTL was described in detail by the writers previously [2]. IN was determined in the supernatant of 3-day cultures stimulated by PHA (PHA-P, from Difco, USA) in a dose of 10 μg/ml, and 6-day LC stimulated by PPD (from Leningrad Institute of Vaccines and Sera) in a dose of 100 μg/ml. As the control, IN was determined in unstimulated LC from the children and adults under investigation, and also in nutrient medium with the addition of PHA and PPD, incubated for three and six days.

IN was determined by the method based on delay of the cytopathic effect produced in primary trypsinized human embryonic cultures by 100 CPD_{50} of vesicular stomatitis virus, Indiana strain.

EXPERIMENTAL RESULTS

IN production was studied in 35 LC stimulated by PHA and in 20 LC stimulated by PPD. During stimulation by PHA most indices of BTL in LC varied from 60 to 90% which, as was shown previously [2], is normal for clinically healthy persons. However, during a normal proliferative response to PHA, IN formation was absent in LC from 16 subjects (15 children and one adult). The negative response in six children was confirmed on retesting. This may be due both to low production of IN by children's leukocytes [3] or to the fact that the IN concentration was below the threshold of sensitivity of the method used to determine it. In the

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TABLE 1. Dynamics of Blast Transformation and IN Production in Human LC Stimulated by PHA and PPD

Stimulator	Donor	Time of incubation of culture, h	BTL index, %	Interferon titer
PHA	T.	24	0	<1:8
		48	38	1:64
		72	72	1:128
	V.	24	0	<1:8
		48	12	1:16
		72	42	1:32
		96	—	<1:8
		120	—	<1:8
PPD	T.	24	0	<1:8
		48	0	<1:8
		72	4	1:32
		96	7	1:128
		120	12	—
		144	16	<1:8
	M.	24	0	—
		48	0	—
		72	6	<1:8
		96	15	1:64
		120	18	1:64
		144	21	1:128

remaining LC stimulated by PHA, IN was found in titers of 1:8-1:128; positive correlation was not found between indices of BTL and titers of IN. Some LC with low BTL indices produced IN in high titers and, conversely, some with high BTL indices gave low IN titers. In the control LC interferon formation was never found.

During stimulation by PPD, a positive BTL phenomenon was found in 17 (BTL indices above 5%) of the 20 LC studied (obtained from 13 children and seven adults). Lymphocytes of three children did not respond to stimulation by PPD and no IN was produced in these cultures. Just as during stimulation by PHA, IN formation was not found in all LC with clearly expressed BTL. Of 17 positively responding LC, IN production was found in eight; IN was found in LC from children in low titers (1:8), but in cultures from adults the IN titers were 1:8-1:128, i.e., practically the same as the titers in LC stimulated by PHA.

Analysis of the data on interferon production led to the suggestion that IN production in some LD could take place at other times of growth of the culture, and would not be detected by a single fixation after 72 and 144 h (for PHA and PPD respectively). To shed light on this problem the dynamics of blast formation and IN production was studied in subcultures, stimulated by PHA and PPD, obtained from three subjects. The results showed (Table 1) that the peak of IN production in cultures stimulated by PHA in fact occurred at 72 h, although IN formation began as early as in 24-h subcultures, before any morphological changes could be seen (subcultures from donor V.). In response to stimulation by PPD both peaks (BTL and IN production) were shifted in time to 120-144 h. Data obtained by investigation of LC of donor T, when IN production was found only in 72- and 96-h subcultures and was not found at later stages of growth of the culture, are noteworthy. This is evidence that the test for IN formation must be carried out not only in cultures fixed at one time, but at different time intervals during their growth.

The results are interesting from at least two points of view. The first is assessment of the functional state of the T lymphocytes – the main IN producers in stimulated cultures. It will be evident that the proliferative activity of lymphocytes stimulated by a mitogen by no means completely reflects their functional integrity, but rather reflects the production of various lymphokines, among which IN plays a special role. Evidence that this is so is given by the suggestion that the study of IN formation in 3-day cultures stimulated by PHA should be used as a new function test for human lymphocytes [7]. However, there is as yet no general agreement regarding the interpretation of negative results and, as the data given above show, they are by no means rare. On the other hand, attention is drawn to the absence of correlation between IN titers and BTL indices, which was observed previously by the writers when studying relations between BTL indices and the production of another lymphokine – lymphotoxin [2]. We know that whereas practically all immunologically normal lymphocytes respond to stimulation by PHA, only those which are specifically sensitized respond to antigenic stimulation. In this case also, however, correlation did not exist between the intensity of blast transformation and interferon production. This evidently indicates that lymphokines are not produced by all transformed lymphocytes, but only by a certain population of them.

Pidot et al. [11] found wide individual variations between IN titers in leukocyte cultures obtained from different individuals and stimulated by Newcastle disease virus. Considerable variations in IN titers also were observed in the same subjects during a long (18 months) period of observation, possibly in connection with the functional state of the lymphocytes producing IN. The role of the genotypic characteristics of the individual in the manifestation of this process [1] has been confirmed in genetic studies on inbred mice with transplantation of bone marrow cells [10].

Taking into account the data in the literature and the results of the present experiments, a final conclusion on the value of the interferon formation test as a means of estimating functional activity of human lymphocytes can be drawn when the results of investigation of the same subjects at different time intervals are available. Work aimed at obtaining such results is now in progress.

LITERATURE CITED

1. M. S. Bektemirova and I. K. Karakuyumchan, Vopr. Virusol., No. 2, 186 (1969).
2. V. G. Reizenbuk, E. V. Aleksandrova, G. F. Kasesalu, et al., Zh. Mikrobiol., No. 8, 112 (1978).
3. V. D. Solov'ev, G. A. Bektemirov, N. V. Vorotyntseva, et al., Vopr. Virusol., No. 1, 19 (1969).
4. W. Braun and H. B. Levy, Proc. Soc. Exp. Biol. (New York), 141, 769 (1972).
5. E. Buimovici Klein, K. E. Weiss, and L. Z. Cooper, J. Infect. Dis., 135, 380 (1977).
6. L. B. Epstein, in: Effects of Interferon on Cells, Viruses and Immune System, A. Geraldes, ed., London (1975), pp. 394-408.
7. L. B. Epstein, H. W. Kreth, and L. A. Herzenberg, Cell. Immunol., 12, 407 (1974).
8. I. Heron, K. Berg, and K. Cantell, J. Immunol., 117, 1370 (1976).
9. M. Johnson and S. Baron, Cell. Immunol., 25, 106 (1976).
10. E. De Maeyer, J. Pierre, J. De Maeyer-Guignard, et al., Immunogenetics, 2, 151 (1975).
11. A. L. R. Pidot, G. O'Keefe, N. McManus, et al., Proc. Soc. Exp. Bio. (N.Y.), 140, 1263 (1972).
12. G. Sonnenfeld, A. D. Mandel, and T. C. Merigan, Cell. Immunol., 40, 285 (1978).
13. M. J. Valle, A. M. Bobrove, S. Strober, et al., J. Immunol., 114, 435 (1976).
14. M. J. Valle, G. W. Jordan, S. Haahr, et al., J. Immunol., 115, 230 (1975).
15. J. L. Virelizier, A. C. Allison, and E. Mayer, Infect. Immun., 17, 282 (1977).

AMOUNT OF EXOGENOUS LINEAR DNA TAKEN UP BY *Escherichia coli* CELLS TREATED WITH Ca^{++} CATIONS

M. I. Bukrinskii and A. G. Sabel'nikov

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Treatment of *Escherichia coli* cells with Ca^{++} cations, first used by Mandel and Higa [3], enables exogenous DNA to be introduced into this naturally untransformable microorganism. However, the frequency of chromosomal transformation obtained by what is currently the most widely used technique is extremely low, at best 10^{-7}-10^{-8} [1]. Meanwhile the quantity of irreversibly adsorbed DNA (i.e., not removed by washing out and resistant to the action of deoxyribonuclease) on Ca^{++}-treated cells is quite considerable [4]. It can thus be tentatively suggested that only a small proportion of this DNA penetrates into the cytoplasm. It is also possible that penetration of DNA into the cytoplasm takes place effectively, but the DNA is then quickly broken down by the action of intracellular enzymes and loses its transforming activity. The possible influence of various postrecombination processes on the appearance of the transformants likewise cannot be ruled out.

Laboratory of Molecular Microbiology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Department of Extrachromosomal Inheritance of Microorganisms, All-Union Research Institute of Protein Synthesis, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.)

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