INCREASED CELLULAR IMMUNE RESPONSE DURING IMMUNIZATION OF MICE WITH CELLS PREINCUBATED IN INTERFERON

S. V. Skurkovich, N. V. Ol'shanskaya, and E. I. Eremkina

UDC 612.014.3.017.1:576.858
095.383

Mice of the strain (CBA × C57BL/6)F_1 were immunized by a single intraperitoneal injection of L-1210 cells previously treated with interferon or with a "dummy" preparation. Mice of the strain CBA were injected with MKh-11 cells, previously treated in the same way. Injection of cells treated with interferon was shown to result in a stronger cellular immune response.
KEY WORDS: interferon; cellular immunity.

The exceptional importance of interferon in the formation and manifestation of the immune response is now becoming increasingly evident. In the writers' earlier investigations the injection of small doses of interferon against the background of antigenic stimulation was shown to lead to strengthening of humoral and transplantation immunity in mice [1, 4, 5, 11, 12].

The object of this investigation was to study the effect of interferon on the cytotoxic activity of lymphocytes of mice immunized with cells preincubated in interferon.

EXPERIMENTAL METHOD

Inbred DBA/2, CBA, and C57BL/6 mice and (CBA × C57BL/6)F_1 hybrids, male and female, weighing 18 and 20 g were used. Cells of L-1210 ascites leukemia, transplanted each week into DBA/2 mice, and cells of an MKh-11 tumor, maintained in the ascites form in C57BL/6 mice were used for immunization. Interferon was induced in a culture of L cells by Newcastle disease virus [12]. The control for interferon was medium 199 and also a dummy preparation of "pseudointerferon," obtained in the same way as the true preparation, except that allantoic fluid of uninfected chick embryos was used instead of the inducing virus. Interferon was titrated by the method of inhibition of the cytopathic action of vesicular stomatitis virus in a culture of L cells [12]. Interferon with a titer of 500 units/ml was used. The control preparations had no antiviral activity. The cells were treated with interferon and the "dummy" preparation in the following way. To $5 \cdot 10^7$ L-1210 or

TABLE 1. Effect of Treatment of L-1210 Cells with Interferon before Immunization on Cytotoxic Activity of Lymphocytes of (CBA × C57BL/6)F_1 Mice against L-1210 Target Cells (M ± m)

Substance used for treating L-1210 cells	Washing cells	Cytotoxic index, %			
		experiment 1	experiment 2	experiment 3	experiment 4
Medium 199	—	26,2±0,9	13,8±1,1	21,1±3,6	26,3±2,0
Pseudointerferon	+	23,9±5,5	7,8±2,6	25,6±5,1	26,3±2,6
Interferon	+	33,5±4,4	17,2±0,4	36,3±1,2	36,9±1,2
Pseudointerferon	—	23,6±1,7	9,0±2,2	11,7±6,9	34,2±1,4
Interferon	—	53,0±5,9	21,5±0,3	27,7±2,1	48,4±0,9

Laboratory of Immunology of Leukemias, Central Institute of Hematology and Blood Transfusion, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1230-1232, October, 1976. Original article submitted January 16, 1976.

TABLE 2. Effect of Treatment of MKh-11 Cells with Interferon before Immunization on Cytotoxic Activity of Lymphocytes of CBA Mice against MKh-11 Cells (M ± m)

Substance used for treating MKh-11 cells	Washing cells	Cytotoxic index, %				
		experiment 1	experiment 2	experiment 3	experiment 4	experiment 5
Medium 199	—	14,6±1,7	2,7±0,5	6,4±0,8	5,0±0,3	6,5±0,6
Pseudointerferon	+	16,2±1,4	10,3±1,5	12,4±0,9	9,6±0,7	5,1±0,5
Interferon	+	19,4±1,5	18,8±1,3	17,5±0,9	13,8±0,2	11,8±0,3
Pseudointerferon	—	18,4±1,6	5,8±0,5	8,9±0,6	6,8±0,1	6,4±0,5
Interferon	—	25,4±1,1	17,0±2,2	11,9±0,3	10,1±0,1	13,6±0,7

MKh-11 cells 1 ml interferon or of the control preparations was added and, after suspension, the sample was incubated at 4°C for 18 h. The supernatant was then poured off and medium 199 was layered above the cells, or the cells were washed twice and covered with a layer of medium 199. The (CBA × C57BL/6)F_1 mice were immunized by a single intraperitoneal injection of L-1210 cells in a dose of $5 \cdot 10^7$, treated with interferon or the "dummy" preparation. MKh-11 cells treated with interferon or the control preparation were injected into CBA mice in the same dose and by the same scheme. On the 10th-11th day the animals were killed and the cytotoxic activity of the splenocytes of mice of the experimental and control groups was determined. The cytotoxic action of the immune lymphocytes was estimated by a radiocytotoxic method using target cells labeled with ^{51}Cr [7]. The maximal liberation of label from the target cells was determined by treating them with 2% Triton X-100 (West Germany) solution. The duration of the reaction was 4-6 h. Lymphocytes were added to the target cells in the optimal ratio of 100 : 1. The number of pulses was counted on an LKB (Sweden) γ-ray counter. The specific liberation of label from the target cells was estimated in per cent by the formula:

$$\frac{\left(\begin{array}{c}\text{Liberation of } ^{51}Cr \text{ in presence} \\ \text{of immune lymphocytes}\end{array}\right) - \left(\begin{array}{c}\text{Liberation of } ^{51}Cr \text{ in presence} \\ \text{of normal lymphocytes}\end{array}\right)}{(\text{Maximal liberation of } ^{51}Cr) - \left(\begin{array}{c}\text{Liberation of } ^{51}Cr \text{ in presence} \\ \text{of normal lymphocytes}\end{array}\right)} \cdot 100.$$

EXPERIMENTAL RESULTS

The results of determination of the cytotoxic activity of immune lymphocytes obtained from mice immunized with L-1210 cells, previously treated with interferon or pseudointerferon, are given in Table 1. As the results show, injection of cells incubated in interferon caused a more marked immune response in the animals: their lymphocytes exhibited higher cytotoxic action on ^{51}Cr-labeled L-1210 cells than lymphocytes of mice immunized with cells treated by the control preparation. Similar results were obtained by the use of MKh-11 cells, previously treated with interferon or pseudointerferon, for immunization (Table 2). It is interesting to note that washing the L-1210 and MKh-11 cells twice to remove interferon after incubation did not abolish the potentiation effect.

The results of these experiments showing potentiation of the antigenic properties of the cells by interferon, although original in character, nevertheless are in good agreement with the writers' previous views on the exceptional role of interferon in the formation and manifestation of the immune response [4-6, 13]. Cells with interferon on their surface evidently carry it to the lymphoid system, where it can lead to potentiation of the functions of the lymphocytes or to the recruiting of additional numbers of lymphoid cells into the immune process. In the light of recent observations [8, 9] showing that the expression of antigens of the H-2 locus on the surface of lymphocytes is strengthened by the action of interferon, the possibility likewise cannot be ruled out that under its influence additional new antigenic determinants are formed or are exposed on the surface of the cells, thereby leading to an increase in the immune response. The fact that washing the cells to remove interferon after incubation with it did not abolish the potentiation effect deserves attention. Bearing in mind the experimental data described above, there is evidently a good case for using interferon to stimulate immunity against leukemia and tumors in man. Since extensive investigations into active immunization of patients with acute leukemia by means of allogeneic leukemic cells are in progress at the present time [2, 3, 10], the desirability of preincubating the cells with interferon is something which ought to be considered.

LITERATURE CITED

1. S. V. Skurkovich and I. M. Aleksandrovskaya, Prob. Gematol., No. 3, 43 (1973).
2. S. V. Skurkovich, T. I. Bulycheva, N. S. Kislyak, et al., in: Material on the Immunotherapy of Acute Leukemia (Russian Language Summaries of Proceedings of the Second National Congress of Oncology) [in Bulgarian], Varna (1974), p. 77.

3. S. V. Skurkovich, T. I. Bulycheva, L. G. Kovaleva, et al., Probl. Gematol., No. 5, 32 (1970).
4. S. V. Skurkovich, E. I. Eremkina, and É. G. Klinova, in: Proceedings of the All-Union Conference on General and Applied Immunology [in Russian], Vol. 2, Moscow (1974), p. 41.
5. S. V. Skurkovich, É. G. Klinova, I. M. Aleksandrovskaya, et al., Byull. Éksp. Biol. Med., No. 4, 66 (1973).
6. S. V. Skurkovich, É. G. Klinova, E. I. Eremkina, et al., Byull. Éksp. Biol. Med., No. 12, 48 (1974).
7. K. T. Brunner, J. Mauel, J.-C. Cerottini, et al., Immunology, 14, 181 (1968).
8. P. Lindahl, P. Leary, and J. Gresser, Proc. Nat. Acad. Sci. USA, 70, 2785 (1973).
9. P. Lindahl, P. Leary, and J. Gresser, Europ. J. Immunol., 4, 779 (1974).
10. S. V. Skurkovich et al., in: Abstracts of the 11th International Cancer Congress, Vol. 4, Florence (1974), p. 174.
11. S. V. Skurkovich et al., in: Proceedings of the 13th Congress of the International Society of Blood Transfusion, Washington (1972), p. 39.
12. S. V. Skurkovich et al., Immunology, 25, 317 (1973).
13. S. V. Skurkovich et al., Nature, 247, 551 (1974).

LOCALIZATION OF SPECIFIC ANTIGEN IN ORGANS OF ANIMALS VACCINATED WITH LIVE MEASLES VACCINE

V. A. Romanov, L. P. Gorshunova, and R. V. Berezhkova

UDC 615.371:576.858.5].015.46+612.017.1.014.46:615.371:576.858.5

Temporary localization of attenuated measles virus antigen in neurons and blood vessels of the brain of newborn mice and hamsters vaccinated subcutaneously with live measles vaccine was demonstrated by an immunofluorescence method. Virus-specific antigen was found in adult animals in the lymphoid system only. Signs of serous meningitis and also vascular disorders were observed for a short time in the brain tissue of any animal by biological testing in tissue culture.
KEY WORDS: attenuated measles virus; immunofluorescence.

As a result of the wide use of live measles vaccine in preventive medical practice the incidence of measles in children has been greatly reduced. However, reports have been published [1, 2, 5] indicating that measles vaccination may lead to the development of vaccinal reactions and sometimes of postvaccinal lesions of the nervous system. The pathogenesis of these complications is not clear and the few attempts that have been made to study their mechanism experimentally have yielded contradictory results [7, 8]. Other problems still awaiting solution are the ability of vaccinal strain L-16 to penetrate into the structures of human and animal nerve tissue, the dynamics of its accumulation in the organs, and the nature of the course of the vaccinal reaction and the histological changes following injection of measles vaccine into animals with altered reactivity, and so on.

The object of this investigation was to study the distribution of attenuated measles virus or its antigen and the histopathological changes in the brain tissue of newborn albino mice and Syrian hamsters, and also in intact guinea pigs and guinea pigs sensitized with AMDT vaccine, vaccinated with measles vaccine.

Department of Microbiology, Yaroslavl' Medical Institute. Laboratory of Immunopathology, D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1232-1234, October, 1976. Original article submitted March 9, 1976.