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INTERFERON PRODUCTION DURING INTERACTION BETWEEN LYMPHOCYTES AND TARGET L-CELLS IN DIFFERENT PHASES OF THE MITOTIC CYCLE

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Interferon is found in the culture medium after interaction between target L-cells synchronized (in the G_1 and S) and unsynchronized with lymphocytes (intact, immune, and nonimmune, treated with actinomycin D). On interaction between immune lymphocytes and L-cells in the G_1 phase, interferon production visibly commences after 30 min (25 units/ml), reaches a maximum after 10 h (75 units/ml), and then falls slightly to 48 h (65 units/ml). Interferon production during interaction between immune lymphocytes and L-cells in the G_1 phase correlates with the cytotoxic action of the lymphocytes on these cells.

KEY WORDS: interferon; lymphocytes; target cells; cell cycle.

During interaction between immune lymphocytes and target cells, interferon is found in the culture medium [7, 10]. Interferon production *in vivo* was demonstrated previously in the writers' laboratory in mice in response to injection of antigenically foreign materials [5]. It was suggested that interferon production during interaction between immune lymphocytes and target cells is connected with recognition of histocompatibility antigens by the lymphocytes. In that case, the intensity and rate of interferon production may differ depending on the phase of the cell cycle in which the target cells are found, for histocompatibility antigens are known to be expressed to different degrees on the cell surface: maximally in the G_1 phase and minimally in the S phase [8, 9].

This paper gives data on the investigation of interferon production during interaction between synchronized and unsynchronized target L-cells with immune, intact, and actinomycin D-treated lymphocytes.

EXPERIMENTAL METHOD

Synchronized and unsynchronized transplantable mouse L fibroblasts from C3H ($H\text{-}2^k$) mice were used as target cells. Synchronization was carried out by the addition of an excess of thymidine (2 mg/ml) [12]. The mean degree of synchronization was 80-85%. Parameters of the mitotic cycle of the L-cells were determined from the curve of labeled mitoses at the 50%

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TABLE 1. Interferon Production and Cytotoxic Effect During Interaction Between Intact, Immune, and Actinomycin D-Treated Lymphocytes and L-Cells (Unsynchronized and Synchronized)

L-cells	Lymphocytes	Interferon level in culture medium and cytotoxicity			
		30 min	5 h	10 h	48 h
Unsynchronized	Intact	<5/6,8	<5/5,7	5/1,5	<5/15,6
	Immune	≤5/5,5	5/43,2	20/55,4	55/73,7
	Treated with actinomycin D	5/0	5/4	35/18,6	5/63,8
In G_1 phase	Intact	5/3,2	5/17,2	18/0	≤5/3,8
	Immune	25/38	45/46,1	75/59,4	65/68,2
	Treated with actinomycin D	10/0	40/13,2	80/35	30/69,3
In S phase	Intact	—	5/11,1	25/5,5	40/5,5
	Immune	—	—	40/4,2	—
	Treated with actinomycin D	—	20/4,5	75/31,6	75/55,4

Legend. Numerator — interferon activity (in units/ml), denominator — cytotoxic index (in %).

level [11], and were as follows: generation time (T) 19 h, phase of synthesis (S) 6 h, presynthetic phase (G_1) 10 h, postsynthetic phase (G_2) 3 h, phase of mitosis (M) 30 min. An unsynchronized culture of L-cells was used as the control. Lymphocytes were obtained from the lymph nodes of C57BL/6 ($H\text{-}2^b$) mice. To obtain immune lymphocytes the mice were immunized once with L-cells in 11 places: subcutaneously (into the four paws, forearm, thigh, back, and abdomen) and intraperitoneally — in a dose of 1 ml of a suspension containing $5 \cdot 10^7$ cells per mouse. Lymphocytes treated with actinomycin D in a dose of 1.5 μg/ml also were used. Immune, intact, or actinomycin D-treated lymphocytes, in a concentration of $4 \cdot 10^6$ cells/ml and in a volume of 1 ml, were added to tubes containing $1 \cdot 10^5$ target L-cells in 1 ml at the beginning of each phase of the cell cycle studied. The cytotoxic effect of the lymph node cells was determined in the same tubes from the number of surviving L-cells, by staining with trypan blue and eosin [2], and was expressed as a cytotoxic index (CTI). Interferon was determined in the fluid taken 30 min and 5, 10, and 48 h after injection of the lymphocytes. Interferon activity was determined by the standard method [4]. The test samples, in the corresponding dilutions in medium No. 199, were layered in a dose of 1 ml above 48-h transplantable cultures of L-cells in tubes (the initial dose of cells was 60,000 in 1 ml medium No. 199). After exposure for 24 h at 37°C the contents were poured off and vesicular stomatitis virus (100 tissue cytopathic doses) was added to each tube. After incubation for 24 h at 37°C the contents were poured off and vesicular stomatitis virus (100 tissue cytopathic doses) was added to each tube. After incubation for 24 h at 37°C the result was read on the basis of 50% inhibition of the cytopathic action of the virus. The numerical results were subjected to statistical analysis by the Fisher–Student method [1].

EXPERIMENTAL RESULTS

The experimental results are summarized in Table 1. Clearly on interaction between the immune lymphocytes and L-cells in the G_1 phase interferon production could be detected after only 30 min, it reached a maximum by 10 h, and had fallena littleby 48h. During incubation of the immune lymphocytes with unsynchronized L-cells, interferon production was much less at all times of investigation. Comparison of the interferon titers with the degree of cytotoxicity (CTI) showed that during interaction between immune lymphocytes and L-cells in the G_1 phase, when there was a statistically significant cytotoxic effect after 30 min (CTI = 38%), interferon production was significantly greater than in the control (25 units/ml). By 10 h, when CTI had risen to 59.4%, the interferon titer reached 75 units/ml, and by 48 h CTI and the interferon titer still remained high (68.2% and 65 units/ml respectively). Interferon production was less intensive during interaction between immune lymphocytes and L-cells in the S phase: By 10 h it reached 40 units/ml. The parallel between interferon formation and manifestation of the cytotoxic effect also was observed on interaction between nonimmune lymph node cells treated with actinomycin D and L-cells in the G_1 phase, when a statistically significant cytotoxic effect appeared after 10 h (CTI = 35%), rising to 69.3% after 48 h. Interferon formation reached 80 units/ml after 10 h, and fell to 48 h. A similar pattern was observed during interaction between lymph node cells treated with actinomycin D and L-cells in the S phase, i.e., the peak of interferon production preceded the peak of cytotoxic

effect. This was probably due to the fact that after incubation for 10 h the quantity of interferon reaches a considerable level, capable of increasing the sensitivity of the L-cells to the cytotoxic action of nonimmune lymphocytes, in agreement with available data [6]. Neither the L-cells themselves (synchronized or unsynchronized), nor lymphocytes themselves (immune, intact, or treated with actinomycin D) without the target cells produced interferon when cultured for 48 h: Its level did not exceed 5-10 units/ml (1 unit is equivalent to 3 standard N.I.H. units). The substance found in the culture medium was in fact interferon, for it had all the principal biological and physicochemical properties of interferon [3]: 1) It possessed antiviral activity, 2) it possessed species specificity (did not protect human Fl-cells against the cytopathic action of the virus), 3) it was stable at pH 2.0 for 24 h, 4) it preserved its activity after heating for 30 min to 56°C, 5) it was sensitive to the action of proteolytic enzymes (inactivated by trypsin), and 6) it preserved its activity at 4°C for not less than 4-5 months.

The results show that during interaction between synchronized and unsynchronized L-cells and lymphocytes (immune, intact, and nonimmune, treated with actinomycin D), parallel with the cytotoxic effect, interferon can be found in the culture medium. Interferon production and the magnitude of the cytotoxic effect during interaction between immune lymphocytes and target cells are clearly dependent on the phase of the cell cycle of the latter. During activation of nonimmune lymphocytes by actinomycin D, this dependence is much less marked.

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