EFFECT OF POLYINOSINIC ACID - POLYCYTIDYLIC ACID ON COLONY-FORMING CAPACITY OF THE HEMATOPOIETIC STEM CELL FRACTION DURING ALLOGENEIC INHIBITION

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Partial restoration of colony-forming ability was found in a graft of parental (C57BL/6) bone marrow in a (CBA × C57BL/6)F_1 hybrid recipient irradiated in a dose of 800 rad, after injection of a polyI–polyC preparation. Colony formation was increased to the same extent as after addition of thymus cells of the same parent. In both cases the number of colonies per spleen was more than doubled compared with the control. In the syngeneic system thymus cells and the polyI–polyC preparation had no effect on the number of splenic colonies formed. If the number of transplanted bone marrow cells remained constant ($5 \cdot 10^5$), an increase in the dose of polyI–polyC (from 50 to 100 μg) and of thymus cells (from $4 \cdot 10^6$ to $8 \cdot 10^6$) did not lead to any increase in the efficiency of colony formation.

KEY WORDS: allogeneic inhibition; colony-forming units; thymus; polyinosinic–polycytidylic acid.

There is now no question about the important role of the thymus in immunogenesis [1, 3]. Cells of the thymus are also evidently necessary for the maintenance of hematopoiesis. Information has appeared in recent years that the efficiency of colony formation in the spleen of irradiated mice is determined, possibly more than by anything else, by processes of cooperation between a certain part of the population of thymus cells and pluripotent cells of the hematopoietic stem fraction [6]. Partial recovery of ability to form colonies has been found in parental bone marrow grafted into an irradiated F_1 hybrid recipient after the addition of thymus cells from the same parent to it, i.e., partial abolition of the phenomenon of allogeneic inhibition [8]. This phenomenon is manifested as defective growth of the bone marrow cells of one parent in the spleen of the lethally irradiated F_1 hybrid recipient.

In immunogenesis synthetic polyribonucleotides can have an action identical to that of thymus cells, such as, for example, in the restoration of the immune response in thymectomized mice [4].

It is therefore interesting to study the possibility of replacing the population of thymus cells by synthetic polyribonucleotides in the maintenance of hematopoiesis.

In the investigation described below the possibility of partial abolition of the phenomenon of allogeneic inhibition by means of a synthetic double-stranded polyribonucleotide – polyinosinic–polycytidylic acid (polyI–polyC) – was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male C57BL/6 mice and 250 (CBA × C57BL/6)F_1 hybrids weighing 18-20 g. The donors of bone marrow and thymus cells C57BL/6 mice and the (CBA × C57BL/6)F_1 hy-

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TABLE 1. Results of Experiments to Study Effect of Mouse Thymus Cells, PolyI–PolyC, or Both Factors Together on Number of Exogenous Splenic Colonies in an Allogeneic System

Group No.	Factor	No. of colonies per spleen (M ± m)	P compared with group 4	P compared with group 3
1	Thymus cells	14,9±0,8 n=10	<0,01	>0,05
2	PolyI – polyC	13,7±1,0 n=11	<0,01	>0,05
3	Thymus cells + polyI – polyC	14,8±1,3 n=11	<0,01	—
4	Control	5,8±1,0 n=11	—	<0,01

Legend. n) Number of animals.

TABLE 2. Effect of Thymus Cells and PolyI–PolyC on Number of Exogenous Splenic Colonies in a Syngeneic System[1]

Group No.	Factor	No. of mice in group	No. of colonies per spleen (M ± m)[2]
1	Control	10	6,6±0,4
2	Thymus cells	10	6,2±0,5
3	PolyI – polyC	10	5,7±0,5

[1]Both donors and recipients were CBA × C57BL/6) F_1 hybrids.
[2]Mean number of colonies per spleen calculated for $1 \cdot 10^5$ bone marrow cells injected.

brids were recipients. The donors were subjected to γ-ray irradiation in a dose of 200 rad on the "Luch-1" apparatus with a dose rate of about 40 rad/min 72 h before removal of the bone marrow, as recommended by Salinas and Goodman [8]. The recipients were irradiated in a dose of 800 rad on the "Gammacell" apparatus with a dose rate of about 1800 rad/min 18-24 h before transplantation of bone marrow.

The recipients received intravenous injections of $5 \cdot 10^5$ bone marrow cells and $4 \cdot 10^6$ thymus cells from C57BL/6 mice in a volume of 0.5 ml with an interval of 40-60 min between the first and second injections.

Intravenous injections of polyI–polyC (Calbiochem, USA) in a dose of 50 μg per mouse in 0.25 ml of physiological saline were made at the same times after injection of the bone marrow. To prevent embolism, the mice received 50 units heparin by intraperitoneal injection 10-20 min before injection of the thymus cells.

The animals were killed 8 days later and the total number of colonies in their spleens was counted; the number of endogenous colonies per spleen under these circumstances was less than 0.2. The number of endogenous colonies does not rise significantly after injection of polyI–polyC or of thymocytes ($\leq$1).

Altogether four series of experiments were carried out in which the effect of thymus cells, polyI–polyC, and both agents together was studied on colony formation in the spleens of the irradiated mice.

The numerical results were subjected to the usual methods of analysis of variance and dispersion [2].

EXPERIMENTAL RESULTS

The results of a typical series of experiments are given in Table 1. The polyI–polyC preparation, injected together with the suspension of donor's bone marrow, effectively increased colony formation in the F_1 hybrid recipients by almost the same degree as thymus cells. In both cases the number of colonies per spleen was more than doubled compared with the control. After combined injection of thymus cells and polyI–polyC into the recipient mice, no further increase in the number of splenic exocolonies were observed. Dispersion analysis of the experimental results showed that the effect of each agent separately was highly significant when the two were used together. Meanwhile, differences between the effects of combined administration of thymus cells and polyI–polyC and the effect of each used separately was not statistically significant. Similar results were obtained in the other series of experiments.

In a special series of experiments a relationship was found between the number of splenic colonies and the dose of thymus cells and polyI–polyC given. If the number of transplanted bone marrow cells remained unchanged ($5 \cdot 10^5$) an increase in the dose of thymus cells to $8 \cdot 10^6$ and of polyI–polyC to 100 μg did not lead to an increase in the efficiency of colony formation compared with doses of thymus cells of $4 \cdot 10^4$ and of polyI–polyC of 50 μg.

This suggests that the observed absence of additiveness of the effects of polyI–polyC and thymus cells on the number of splenic colonies formed reflects identical mechanisms of exertion of their action on hematopoietic stem cells. The presence of a "saturation" phenomenon during an increase in the dose of thymus cells and polyI–polyC evidently indicates that the bond marrow of animals contains a limited population of thymus-dependent cells [7].

In experiments in a syngeneic system thymus cells and polyI–polyC had no effect on the production of splenic colonies (Table 2). Thymus cells are evidently necessary only in case of their possible damage (transplantation into a nonsyngeneic recipient, and so on).

The mechanism of allogeneic inhibition, manifested as defective growth of bone-marrow grafts, is unknown but, according to Till and McCulloch's hypothesis [10], it could be absence of a control mechanism which, under normal conditions, regulates cellular proliferation by means of a gene product which is evidently a component of the cell membrane.

The addition of thymus cells to the bone marrow suspension of the parent donor evidently enables normal proliferation of stem cells in the hematopoietic system to take place. Regulation of the proliferative activity of the cells is evidently secured by production of a hormonal factor by the thymus cells or by a direct effect on interaction between cell surfaces [5].

This suggests that synthetic polyribonucleotides are either analogous in their action to thymus hormones or they can replace the effect of thymus manifested at the level of interaction between cell surfaces. Data showing that thymus cells have a stronger negative charge than other lymphocytes [9] are an example in support of the second explanation.

The two nonalternative possibilities indicated above can be achieved through a change in the production of cyclic AMP [11].

Interferon, formed as the result of administration of polyI–polyC, could also be the cause of such a change. The preliminary data now obtained show that the exogenous interferon can abolish allogeneic inhibition to the same extent as polyI–polyC.

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