Influence of Polyinosinic:Polycytidylic Acid on the Circulating White Blood Cells in Mice (37182)

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Alteration of the peripheral white blood cell count (WBC) is a characteristic manifestation in most infectious diseases. High WBC, mainly granulocytic with a shift to the left is regularly seen during bacterial infection, whereas normal or low WBC is generally considered a feature of viral infection. In fact, a wide variation of WBC response, both increase and reduction can be registered in connection with viral diseases (1, 2). Leukocytosis during viral infection is probably mainly due to tissue destruction and subsequent release of products of this destruction, and to secondary bacterial infection (2, 3). Leukopenia is generally considered as a direct result of the introduction of viral agent. It has been reported that leukopenia follows not only naturally occurring measles (4) but also introduction of live measles vaccine (5). It has been proposed that the disappearance of leukocytes is a direct result of viral destruction, or alternatively due to a movement of leukocytes into the stationary reticuloendothelial system. The exact mechanism of this process remains to be determined.

Recently it has been reported by several authors, that interferon (IF) and IF inducers inhibit the growth of a number of different cell types. Besides inhibition of virus-induced tumors, inhibition was also seen of transplantable tumors, not induced by introduction of virus (6–8), and of tumor cells cultivated *in vitro* (9–11). An inhibition of mouse hemopoietic colonies was induced by the synthetic double-stranded polynucleotide, polyinosinic–polycytidylic acid (poly I:C), or influenza virus, and this activity was directly proportional to the IF titer (12, 13). Recently it has been reported that mouse IF inhibits multiplication of normal mouse embryo and mouse kidney cells in primary cultures (14). These results may suggest that IF exerts a regulatory effect on at least certain types of cells.

In the light of these results it seemed of interest to determine the *in vivo* effect of IF and IF inducers on the peripheral WBC. The effect of poly I:C on mouse WBC is reported herein.

Materials and Methods. Polyinosinic (poly I) and polycytidylic (poly C) acid were obtained as individual homopolymers (Miles Laboratories, Elkhart, Indiana). Equimolar solutions were complexed as indicated by Field *et al.* (15). Hypochromic effect (ca. 30%) proved the complex formation. Both complex poly I:C and the individual homopolymers were kept in stock solutions at −20°.They were thawed immediately before employment.

Experimental design. Young albino mice of either sex, weighing 20–23 g were employed. They were kept in plastic cages, 4–6 per cage and were fed commercial mouse pellets and water *ad libitum*.

The synthetic polynucleotides were administered intraperitoneally (ip). Blood samples were obtained from the tail tip at various intervals after the injection. The samples were diluted in counting fluid, containing Cetrimid bromide (0.5 g/1000 ml), in order to hemolyze the red blood cells. Total WBC was determined by Celloscope 101 (AB L. Ljungberg, Sweden). All samples were counted three times and the mean count was registered. Differential counts were made on Wright or Giemsa stained smears. At least 100 cells were counted in each preparation.

Interferon test. Interferon titers were assayed in blood samples obtained from the

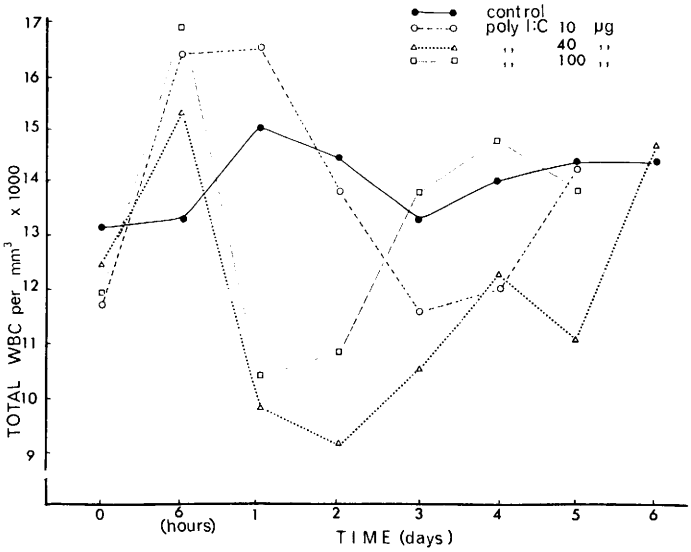

FIG. 1. Peripheral white blood cell count of mice after intraperitoneal injection of poly I:C. Each line represents the mean count from 4–6 mice.

axillary vein at 4 hr after ip administration of the polynucleotides. Serum was separated after clotting, then pH was adjusted to 2 by 1 *N* HCl. After two days pH was readjusted to 7 by 1 *N* NaOH. IF titers were determined by means of a micro test for infectivity inhibition as described in detail elsewhere (16). IF titers were calculated by the method of Reed and Muench as the dilution which inhibited the cytopathogenic effect of Vesicular stomatitis virus in 50% of the cups containing L-F_1 cell monolayers.

Results. Alteration of the WBC after administration of polynucleotides. Randomly selected mice, 4–6 per group, were injected with 10, 40, or 100 μg poly I:C. A fourth group received identical volume, 0.2 ml saline. Before the injection and at various intervals afterwards blood samples were obtained from the tail tip and the total WBC was determined. The mean count in the control group varied ± 10% at the different sampling times (Fig. 1). Variation of the same magnitude but with a different profile was observed in a repeated experiment. This variation was therefore ascribed to random variation independent of the physical strain of the injection and blood sampling. A two-phase response of WBC was seen in all poly I:C treated mice (Fig. 1). An initial increase of cell count was followed by a reduction from 1 to 5 days after the injection. The alterations were not strictly dose dependent, although the development was slower and the depression was less pronounced following the administration of the smallest dose, 10 μg poly I:C. The initial increase was not dose dependent. No significant variation was seen after the 6th day.

For analysis of the response among the individual cell types blood smears from 6 mice were differential counted at different times after poly I:C injection, and the total numbers of the two dominating cell types, lymphocytes and granulocytes were calculated (Fig. 2). The data indicate that the total WBC increase immediately after poly I:C is entirely due to an increase of the neutrophil granulocytes. As for the total WBC no dose dependency could be demonstrated. In a repeated experiment administration of 10 and 100 μg poly I:C resulted in nearly identical increase of the granulocytes. The depression during Days 1–5 is mainly a function of lymphocyte reduction, although also the granulocytes are slightly reduced in number. The number of circulating immature forms of granulocytes (juveniles and stabs) increased initially, parallel to the general granulocyte increase. These forms were virtually absent during the granulocyte depression on Days 2

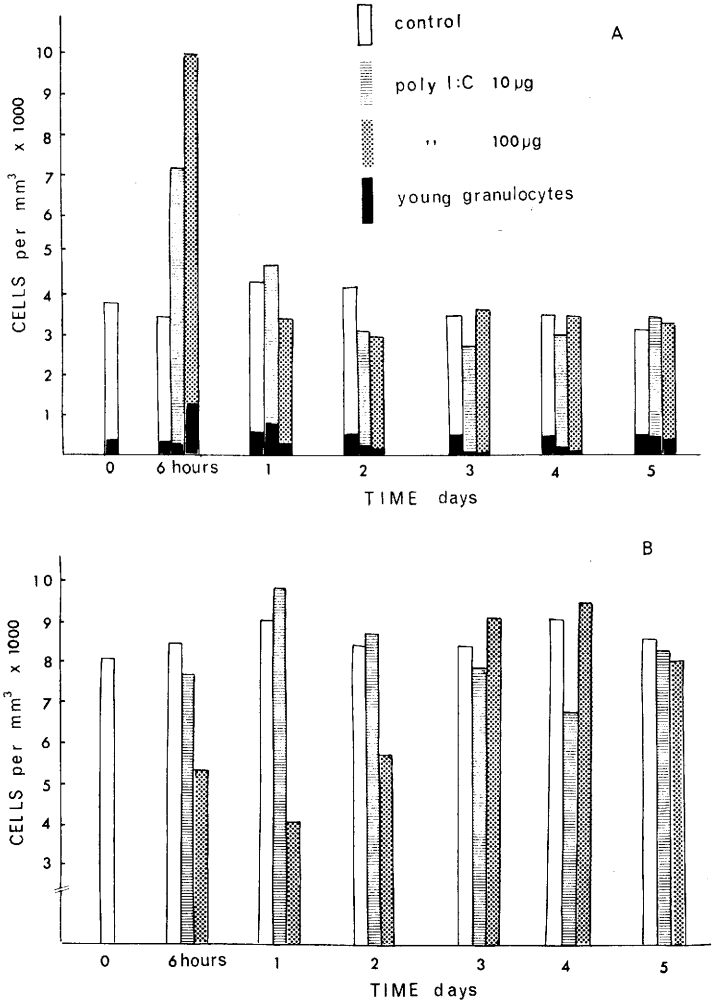

FIG. 2. Effect of poly I:C on the number of circulating granulocytes (A) and lymphocytes (B) in mice. Each column represents the mean count from 6 mice.

and 3. Alteration during this period depended on the dose of injected poly I:C for both main cell types.

Injection of single-stranded poly I and poly C by the same route and the same dose as poly I:C did not result in significant variation of the total WBC, nor did it affect the distribution of lymphocytes and granulocytes.

IF titer and WBC count. Groups of mice, 4 mice per group, were given 10, 40, or 100 μg poly I:C, 100 μg poly I or poly C or saline. Four hours later all mice were exsanguinated, blood samples were pooled for each group, and IF titers were determined. Slightly increasing titers of IF were found with increasing doses of poly I:C (Table I). There is a linear relationship between IF titers and the dose of poly I:C. There is a certain correlation between the IF titers and maximal reduction of total WBC, thus the lowest IF titer parallels the smallest extent of reduction. No IF could be detected 4 hours after injection of the single stranded polynucleotides or saline.

Discussion. The experimental results presented herein demonstrate that injections of the double-stranded polynucleotide, poly I:C do influence the peripheral WBC in the mouse, while single-stranded poly I and poly C are devoid of such an effect. The various

TABLE I. Interferon Production and Alteration of White Blood Cell Count After Intraperitoneal Injection of Single-Stranded and Double-Stranded Polynucleotides.

	poly I:C				
	10 μg	40 μg	100 μg	poly I	poly C
Interferon titer per 0.1 ml 4 hr after injection	133	224	316	<10	<10
Maximal increase of WBC (day/hour after injection)	23% (6 hr)	13% (6 hr)	31% (6 hr)	13% (24 hr)	0
Maximal reduction of WBC (day/hour after injection)	15% (4 d)	38% (2 d)	32% (1 d)	4% (6 hr)	12% (1 d)

cell types responded differently, the difference being not only quantitative but also qualitative. Lymphocytes were clearly reduced in number for a period of about two days. The extent of reduction was dose dependent, and with the highest dose employed it exceeded 50% compared to the controls. In contrast granulocytes showed a two-phase response, an initial leukocytosis immediately after injection, including numerous immature forms, followed by a moderate depression for a period of about two days.

At the present stage of this study it is difficult to define the mechanism by which the poly I:C influences the mouse peripheral WBC. The results may suggest that IF production is involved since the single stranded polynucleotides neither produced IF nor influenced the WBC. The different effect of poly I:C on the different cell types is not surprising. It is in accordance with findings of several authors of widely varying results about the effect of IF and IF inducers on different cell types (9–11, 14, 17–24). Both stimulating and inhibiting factors of the hemopoietic colony forming cells were demonstrated in the serum of poly I:C treated mice (12, 13).

The demonstrated WBC changes are similar to those observed during experimental infection of mouse with Sendai virus (3). Certain types of human viral infections, including vaccination with live measles vaccine (5), are accompanied by comparable WBC variations. It is tempting to recall that the paramyxoviruses, which produce these variations, are both good IF inducers. Circulating IF has been demonstrated after primary measles vaccination in 17 of 18 children (25). IF induction is also a prominent feature of poly I:C action. This common factor might be involved in the production of leukopenia.

The kinetics of the alterations indicate that not only the cell supply to the circulation is reduced, *e.g.*, by inhibition of multiplication of precursor cells, but also there is a disappearance of mature cells from the circulation. Such process may be a result of a migration of cells into the stationary reticuloendothelial system, or alternatively by a direct lethal effect on the cells. It has been demonstrated (26) that IF exerts both a depressing effect on the growth rate of L-929 cells and a direct lethal effect.

Studies are in progress to delineate the nature of the poly I:C action on the peripheral WBC.

Summary. Intraperitoneally injected poly I:C resulted in a significant alteration of the peripheral WBC in mice. The effect was different on the various cell types: the number of circulating lymphocytes was reduced substantially for a period of up to two days. The number of granulocytes, especially the immature forms in the circulation, initially increased, followed by a moderate depression. The reduction was dose dependent, and to some extent corresponded to the titer of IF produced. Single-stranded poly I and poly C were without effect on the WBC, and no IF production could be detected.

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