RELATIONS BETWEEN MYELOID AND STROMAL CELLS OF BONE MARROW IN ACUTE EXPERIMENTAL APLASIA

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Acute hypoplasia of the hematopoietic system was produced in guinea pigs by injecting vinblastin in a dose of 0.1 mg/100 g body weight intraperitoneally either once or twice at an interval of 7 days. The number of myelokaryocytes and the concentration (per 10^5 myelokaryocytes) and total number (per femur) of precursors of fibroblasts were determined in the femora by the fibroblast colony method. After injection of the cytostatic the decrease in number of myelokaryocytes was accompanied by a change in the concentration of stromal precursors per femur. It is postulated that the response of the bone marrow during hypoplasia is determined by interaction between the myeloid and stromal cells.

KEY WORDS: *hypoplasia of the hematopoietic system; bone marrow stroma; precursors of fibroblasts; fibroblast colony method.*

A definite role in the development of hypoplastic states of hematopoiesis is nowadays ascribed to a lesion of the bone marrow stroma [3-5]. In experiments in which repeated injections of Myleran were given to mice, the developing hypoplasia was accompanied by a lesion of the bone marrow precursor cells and, to a lesser degree, of its stromal elements. A single injection of vinblastin into guinea pigs [1] caused hypoplasia of the myeloid series, accompanied by a response of the bone marrow stroma in the form of some degree of hyperplasia.

The object of this investigation was to study the relations between the myeloid and stromal cells of the bone marrow after repeated administration of vinblastin, with the aim of causing death of the proliferating population of myeloid cells.

EXPERIMENTAL METHOD

Guinea pigs weighing 200-250 g were used. Vinblastin was injected intraperitoneally in a dose of 0.1 mg/100 g body weight once or twice at an interval of 7 days.

Altogether 140 animals, divided into three groups, were used: group 1) control animals (31), of the same weight as the experimental animals, were killed at the same time as the guinea pigs of the experimental group; group 2) 56 animals receiving a single injection of vinblastin; 19 guinea pigs of this group died on the 3rd-4th day after injection of vinblastin and 37 remained in the experiment; group 3) 53 guinea pigs receiving two injections of vinblastin; 32 guinea pigs of this group died on the 3rd-4th day after the second injection of vinblastin and 21 remained in the experiment.

The degree of hypoplasia of the bone marrow was estimated from the number of myelokaryocytes in the femora of the experimental animals. The state of the bone marrow stroma was studied by determining the concentration of fibroblast precursors in the bone marrow, i.e., the number of colony-forming units (CFU) per 10^5 bone marrow cells, and also by counting the total number of fibroblast precursors per femur (TNFP) [2]. Tests were carried out on the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 10th, and 14th days after injection of vinblastin.

The animals were deeply anesthetized with ether and both femora were removed; the bone marrow was flushed out into medium No. 199 (under standard conditions — always into 3 ml of

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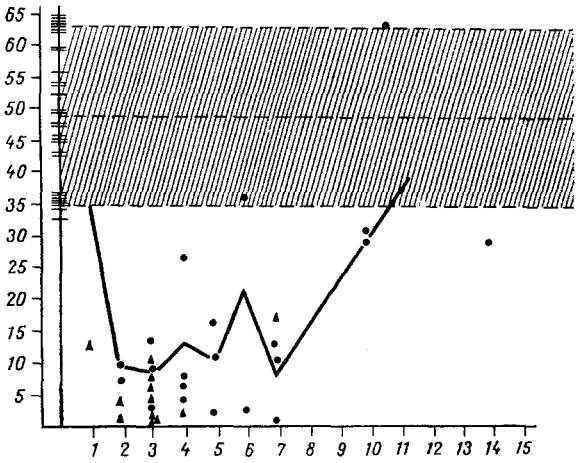

Fig. 1. Changes in number of myelokaryocytes after 1 (circles) and 2 (triangles) injections of vinblastin. Shaded area indicates limits of variations in number of myelokaryocytes in 1 mm^3 of suspensions of bone marrow cells of healthy animals ($M \pm \sigma$). Ordinate, number of myelokaryocytes (in thousands/mm^3 suspension of bone marrow cells); abscissa, days after injection of vinblastin.

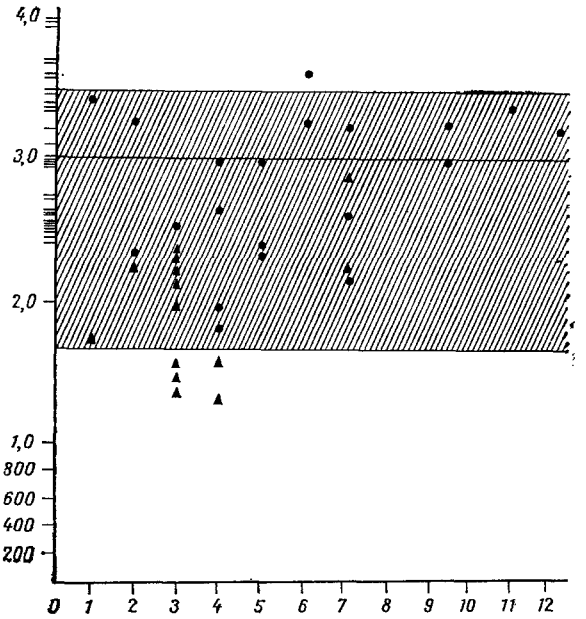

Fig. 2. Changes in TNFP after 1 (circles) and 2 (triangles) injections of vinblastin. Shaded area indicates limits of variations in number of fibroblast precursors per femur in healthy animals ($M \pm \sigma$). Ordinate, logarithms of values of TNFP; abscissa, days after injection of vinblastin.

medium). The suspension of myelokaryocytes was filtered through Kapron and their number in the suspension was counted. A monolayer culture of fibroblasts was grown in 100-ml Roux flasks. The nutrient medium consisted of medium No. 199 mixed with bovine serum (1:4). The number of growing colonies (CFU) was counted on the 11th day, after which the absolute number of fibroblast precursors per femur (TNFP) was calculated. In nearly every experiment two experimental and two control animals were used.

EXPERIMENTAL RESULTS

After a single injection of vinblastin the animals lost their appetite, ceased to gain weight, then started to lose weight, became apathetic, lost their hair, and then movement coordination was disturbed. These symptoms were found in all the guinea pigs which died and, as the subsequent investigation showed, there was a severe degree of hypoplasia of their bone marrow.

The results of the test on animals of all three groups are given in Figs. 1-3. The dynamics of the number of myelokaryocytes after one or two injections of vinblastin is illustrated in Fig. 1 (individual numbers of myelokaryocytes in healthy animals are plotted along the ordinate). The mean number of myelokaryocytes in the control guinea pigs was 49,300 ± 14,300/mm^3.

After a single injection of vinblastin all the animals developed hypoplasia of myelopiesis; the decrease in the number of myelokaryocytes was most marked on the 3rd-5th day. A tendency toward restoration of the normal number of myelokaryocytes was observed by the 10th-11th days.

The decrease in the number of myelokaryocytes in the bone marrow of the guinea pigs after two injections of vinblastin was more severe; in 12 of the 21 animals the number of myelokaryocytes did not exceed 5000/cm^3, reflecting the severe degree of hypoplasia of hematopoiesis. Since the animals of this group died as a rule on the third or fourth day after injection of the cytostatic, most of the tests were carried out on the third day and it is impossible to plot a complete curve of the change in the number of myelokaryocytes for this group of animals.

A semilogarithmic graph of the change in the absolute number of fibroblast precursors per femur at various times after injection of vinblastin is given in Fig. 2. Individual values of TNFP per femur for healthy animals are plotted along the ordinate. The mean value (M) of TNFP was 1600 ± 280. All values of TNFP for animals receiving a single dose of vinblastin lie within the normal scatter of $M \pm \sigma$, but there is a tendency for the absolute number of fibroblast precursors to decrease. After two injections of the cytostatic TNFP was considerably reduced: Most of the values lay below $M \pm \sigma$, i.e., hypoplasia of the stromal precursors was manifested to a significant degree ($P < 0.01$). The greatest decrease in the number of CFU occurred on the 4th day, i.e., at the time of greatest intensity of hypoplasia of myelopoiesis.

The decrease in TNFP which accompanied severe hypoplasia of the myeloid cells was used as the basis of an attempt to assess the degree of depression of the stroma in relation to the degree of hypoplasia of myelopoiesis (Fig. 3). After the first injection of vinblastin hypoplasia of the stroma was observed in cases of severe depression of myeloid hematopoiesis; with a decrease in the number of myelokaryocytes to 15,000/mm^3, no such dependence could be established. After two injections of vinblastin direct correlation was found between the number of fibroblast precursors and the number of myelokaryocytes ($r = 0.641$). The discovery of this correlation between myeloid and stromal cells during hypoplasia prompted its study under normal conditions (in healthy animals). It was found that in healthy guinea pigs, if the intensity of cloning was the same (the mean values of CFU were close to normal: 1-4/10^5 cells) the absolute number of stromal precursors was virtually independent of the number of myelokaryocytes ($r = 0.258$). Analysis of the results for the companion dynamics of CFU and TNFP (Table 1) shows that after a single injection of the cytostatic, while the number of myelokaryocytes in the bone marrow falls, the total number of fibroblast precursors per femur has a tendency to decrease (the considerable scatter of the normal values of TNFP makes it impossible to draw any more definite conclusions). The concentration of fibroblast precursors (number of CFU per 10^5 myelokaryocytes) was increased under these circumstances. This could reflect the relative integrity of the stroma after a single injection of the cytostatic. After two injections of vinblastin both TNFP and the number of CFU decreased. In that case absolute depression of the stromal precursors evidently took place.

It can be postulated on the basis of these results that the response to single and repeated injections of the cytostatic differs significantly. The response of the stroma to a single injection of vinblastin is ill-defined. Even in this case, however, there is a decrease in the total number of fibroblast precursors together with a decrease in the number of myelokaryocytes. Two injections of vinblastin separated by an interval of 7 days caused depression of both myelopoiesis and stromal cells. The mechanism of this response of the

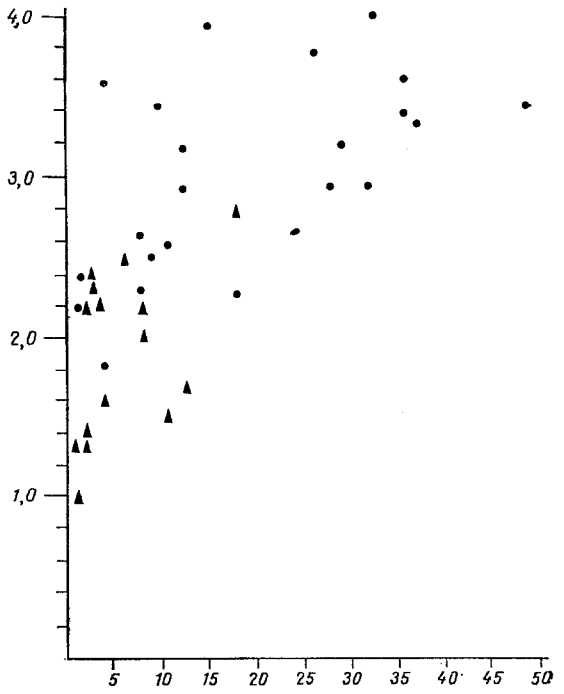

Fig. 3. Dependence of changes in TNFP on number of myelokaryocytes after 1 (circles) and 2 (triangles) injections of vinblastin. Ordinate logarithms of TNFP; abscissa, number of myelokaryocytes (in thousands/mm^3 suspension of bone marrow cells).

TABLE 1. Number of Myelokaryocytes and Concentration (per 10^5 myelokaryocytes) and Total Number (per femur) of Fibroblast Precursors in Control and Experimental Animals (M ± m)

Index studied	Group 1 (control)	Group 2	Group 3
Number of myelokaryocytes in 1 mm^3 suspension of bone marrow cells	49 830±2 487	20 739±4,050	5 269±657
TNFP per femur	1 600±280 (σ=1550)	948,5±171,0 (σ=772,0)	121±34,2 (σ=117,0)
Number of CFU per 10^5 myelokaryocytes	2,0±0,3	5,0±0,9	2,1±0,45

bone marrow is not yet clear. Depression of the stroma after injection of vinblastin can be explained either by the direct action of the compound on precursor cells of fibroblasts or by the action of the myeloid cells on the stroma. Against the first explanation are the observations that most of the stromal cells are in phase I_0 and are insensitive to the action of the cytostatic [6]. However, in the writer's opinion, the possibility that vinblastin may act on cells even in phase I_0 cannot be completely ruled out [7], and a certain number of fibroblast precursors sensitive to the action of the cytostatic may be present.

It can also be postulated that there is a definite interaction between myeloid and stromal cells as a result of which a marked decrease in the number of myelokaryocytes causes a decrease in the number of stromal precursors. This interaction possibly exerts its influence on the proliferative activity of the stromal precursors and makes them more sensitive to vinblastin. This may account for the sharp decrease in the total number of stromal precursors after the second injection of cytostatic.

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MOVEMENT OF HEPATOCYTES ALONG THE HEPATIC TRABECULA DURING PHYSIOLOGICAL REGENERATION

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The distribution of mitoses among hepatocytes and of dying cells along the hepatic trabecula was determined in rats. The relative rate of movement of the hepatocytes along the trabecula was calculated from these distributions. The direction and velocity of movement of the hepatocytes along the hepatic trabecula were obtained by recording the shift of the peak of labeled cells one month after giving the rats six injections of thymidine-^{3}H.

KEY WORDS: *hepatic trabecula*; *movement of hepatocytes*; *physiological regeneration*.

The direction and velocity of movement of cells from zones of mitosis into zones of their death can be calculated from the distribution of mitoses and dying cells in the hepatic trabecula. If the length of the trabecula is stable, the number of dividing cells must be equal to the number of dying cells, and the velocity of movement of the cells from the zone of mitosis into the zone of death will depend on the mutual position of these zones. The velocity of movement of the cells will be greatest if special zones of dividing and dying hepatocytes are present along the length of the trabecula, but if these zones coincide, movement of the cells will fluctuate within the limits of the diameter of one cell.

The object of this investigation was to determine the distribution of mitoses and dying cells, and the direction and velocity of movement of the hepatocytes along the hepatic trabecula.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male rats weighing 160-170 g. The plane along which the hepatocytes could move along the trabecula was determined from the arrangement of the metaphase plates relative to the axis of the trabecula, by means of an occular goniometer. These measurements were made on the liver of hepatectomized rats and the remaining investigations were performed on the liver of intact animals.

Complete karyolysis was used as the criterion of death of the hepatocytes [1, 2]. The distribution of mitoses and dying hepatocytes along the line (averaged for the number of

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