BIOLOGICAL ROLE OF SPONTANEOUS HUMAN KILLER CELLS

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Spontaneous (natural) killers (SK) have recently been found in human blood [2]; without preliminary sensitization they develop a cytotoxic effect *in vitro* against tumor cell lines of allogeneic and xenogeneic nature [1, 4].

The biological role of SK is unknown. It has been suggested that *in vivo* they take part in the control of cell aberrations, in the elimination of cells of early tumor development, and in the regulation of homeostatis [1].

Developing the view that SK participate in the maintenance of homeostatis, the present writers suggested that SK activity ought to undergo fluctuations reflecting the intensity of immune processes in the body, in particular after transplantation of allogeneic tissue.

In the investigation described below this hypothesis was tested by the use of SK isolated from the peripheral blood of patients with a transplanted allogeneic unrelated kidney, in different phases of activity of transplantation immunity.

EXPERIMENTAL METHOD

Cells of continuous line K-562, generously provided by I. L. Leipunskaya and E. G. Slavina (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) was used as the "target." This line was grown in medium RPMI-1640, enriched with glutamine and antibiotics; inactivated bovine serum (BS) was used as additive; cells on the 2nd-3rd day of culture were used for the experiments.

The experiment was set up basically in accordance with method in [3]. The targets were labeled with ^{51}Cr (250 μCi ^{51}Cr, in the Na_2CrO_4 form, with a specific activity of 2-5 mCi/mmole per $1 \cdot 10^6$ tumor cells); after the label had been rinsed out the concentration of the targets was adjusted to $1 \cdot 10^5$/ml (RPMI + 10% BS).

The killers were isolated from peripheral blood, which was collected in 5% EDTA solution and fractionated in a Ficoll—Verografin density gradient (d = 1.077); the final cell concentration was adjusted to $5 \cdot 10^6$ cells/ml (RPMI + 10% BS).

The test was carried out in round-bottomed 96-well plates (from Cook), with the addition of $1 \cdot 10^4$ targets (0.1 ml) and $5 \cdot 10^5$ killers (0.1 ml) to each well. Incubation continued for 16 h at 37°C in air with the addition of 5% CO_2 and a relative humidity of 100%. The yield of radioactive label was counted in 0.1 ml supernatant by the equation suggested in [3]:

$$\%\ \text{lysis} = \frac{A - B}{C - B} \cdot 100,$$

where A is the mean yield of ^{51}Cr in three experimental wells (2 ×), B the same in three control wells, and C is 80% of the total incorporation of ^{51}Cr in $1 \cdot 10^4$ target cells. The spontaneous yield of ^{51}Cr (B) in this situation averaged 16% of the value of C (1% per hour).

Altogether 59 investigations of SK activity from cells isolated from seven recipients of an allogeneic kidney, 36 investigations on cells of patients with a chronic renal insufficiency (CRI) syndrome before transplantation, and 27 investigations of SK activity in healthy subjects were carried out.

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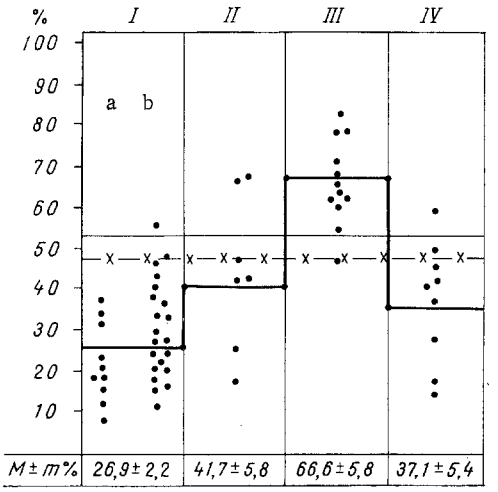

Fig. 1. SK activity in different phases of transplantation immunity. Dots denote concrete values of killing effect (in %) in recipients of allogeneic kidney. Continuous line indicates mean level of SK effect in healthy persons; broken line with crosses shows the same in patients with CRI syndrome. Numbers below show mean level of killing effect in each column.

EXPERIMENTAL RESULTS

The meak SK activity in healthy individuals was 53.6 ± 3.7% and in patients with a CRI syndrome before transplantation of an allogeneic kidney it was 48.8 ± 3.5%.

SK activity was studied after transplantation at times of different intensity of transplantation immunity.* The following phases of activity of the processes of transplantation immunity, named in accordance with clinical and biological criteria characterizing the function of the transplanted organ, were distinguished: 1) low activity of transplantation immunity (stable function of the graft); 2) precrisis activity of rejection reactions (condensation of the graft, depression of function); 3) high activity of transplantation immunity, expressed as manifestation of a rejection crisis; 4) "postcrisis" activity, arising in the case of termination of a rejection crisis.

All values of the killing effect, distributed in columns in accordance with the phases of activity of transplantation immunity, are shown in Fig. 1. Immediately after the operation SK activity (Fig. 1, Ia) was considerably depressed compared with that in healthy subjects and with the preoperative level in patients with CRI. The cause of the decrease is considered to have been the intensive immunodepressive therapy used on the first postoperative days. In the period of stable function of the graft (Fig. 1, Ib), in the precrisis period (Fig. 1, II), or on termination of the crisis (Fig. 1, IV) fluctuations were observed in the values of mean activity of SK, but differences between them were not statistically significant. On the whole in these periods SK activity was lower than in healthy subjects.

Determination of the significance of differences between the mean values of SK activity in the period of the crisis situation and in the phase of low activity of transplantation immunity, or in healthy subjects, using Student's test showed that the differences are statistically significant.

Attention was drawn to the fact that before a crisis situation SK activity rose above its level at the previous determination, outside a crisis. The coefficient of correlation

*All tests on recipients were carried out during immunodepressive therapy: imuran 3-4 mg/kg body weight and prednisolone 1.5 mg/kg body weight during the first 2 weeks after transplantation; later the doses were reduced to 50 and 30 mg daily respectively; in rejection crises methylprednisolone was given in doses of up to 1000 mg intravenously and imuran 250-300 mg daily.

TABLE 1. Correlation between Elevation of SK Activity and Graft Rejection Crisis (coefficient of correlation)

Rejection crisis	Increase in SK activity		
	+	−	
+ −	12 5	0 42	$r = 0,79$

TABLE 2. Quantitative Evaluation of Increase in SK activity in Crisis Situation

SK – mononuclear cells	SK activity before crisis, %	SK activity during crisis, %	Increase in activity, %
1	43,3 (12)	66 (14)	22,7
2	6 (6)	64,4 (15)	58,4
	58,5 (35)	77,3 (40)	19,5
		78,0 (44)	
3	58,5 (2)	83,4 (7)	24,0
4	42 (2)	63,7 (7)	21,7
5	35.6 (6)	78,5 (16)	42,9
6	47,1 (4)	65 (6))	17,9
7	26.4 (14)	45,5 (22	19,1

Legend. Number of days after transplantation when analysis was carried out shown in parentheses.

between these two processes — the rise in SK activity and onset of the rejection crisis — revealed the high degree of correlation between the phenomena (Table 1).

Differential analysis of the rise in SK activity before a crisis showed (Table 2) that the increase in the killing effect was approximately 20% if the previous analysis of SK activity had been carried out in the precrisis period (2-5 days before clinical manifestation of the crisis) and could reach 40% or more if the previous analysis had been carried out in a phase of low activity of transplantation immunity (9-10 days before the crisis).

The results thus show that nonspecific (unconnected with sensitization) SK activity may reflect the intensity of the specific immune process and, in particular, the intensity of reactions of transplantation immunity.

This phenomenon reveals a new aspect of the biological role of SK. The killing effect of SK can be regarded as the immanent reactivity of the mononuclear cell itself, for this cell possesses cytotoxic potential against a "target" with which it did not have contact *in vivo* (in this case the K-562 line). In the writers' view, immanent reactivity of SK both in the normal state and during intensification of transplantation immunity reflects the functional activity of the IR gene, whose presence in man has been postulated and whose fluctuations determine the phase of the immune process.

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