INTERACTION BETWEEN PERIPHERAL BLOOD MONONUCLEARS AND ISLET CELLS IN VITRO

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The ability of peripheral blood mononuclear cells (MNC) to inhibit insulin production by pancreatic islet cells (IC) during combined culture in vitro was described in 1981 by Boitard [2]. This model has been used to assess activity of anti-islet-cell immunity in Type I diabetics [4, 6]. However, we have obtained evidence that this phenomenon is not strictly specific and is observed also in the presence of normal human MNC.

EXPERIMENTAL METHOD

Pancreatic IC were isolated from Wistar rats with the aid of collagenase [5]. The Islets, purified by centrifugation in a Ficoll-Paque density gradient ("Pharmacia LKB," Sweden) were placed successively in 0.02% EDTA solution for 5 min (20°C) and then in 0.25% trypsin solution for 15 min (37°), after which they were carefully pipeted to convert them into a single-cell suspension. The suspension of IC was washed with Eagle's medium (MEM), containing a double set of amino acids and vitamins, L-glutamine (2 mM), gentamicin sulfate (80 mg/liter), 1% inactivated human AB (IV) serum, and HEPES (25 mM). The cell concentration was adjusted to 10^5/ml and 0.1 ml of suspension was added to each well of a 96-well planchet ("Costar," USA), after which the IC were cultured in a CO_2-incubator (5% CO_2, 37°C) for 18 h [1].

MNC from diabetics with a duration of the disease of between 2 and 20 years, and from healthy blood donors and Wistar rats were isolated by centrifugation in a Ficoll—Paque density gradient, and the cell concentration in MEM was adjusted to $4 \cdot 10^6$/ml; 0.1 ml of suspension was added to each well containing IC. Meanwhile, in some experiments 40 ng of *E. coli* lipopolysaccharide (LPS, from "Sigma," USA) was added to the wells. Combined culture was carried out for the next 18 h, after which 0.1 ml of medium was taken for determination of the tumor necrosis factor (TNF) concentration and the wells were washed with fresh serum. The IC were then incubated for 1 h in MEM containing 5.5 mM glucose, in order to determine basal insulin secretion (BS) or in the same medium, but with 16.5 mM glucose and 5 mM theophylline to determine stimulated secretion (SS). The insulin concentration in the medium was determined by radioimmunoassay, using the Soviet RIO-^{125}I-ins-M kit. The insulin concentration was expressed as the arithmetic mean of values obtained in six parallel tests. The coefficient of variation did not exceed 15%. The TNF concentration in the supernatants was determined with the aid of mouse fibroblasts of the L929 strain; the unit of activity was taken to be the TNF concentration causing 50% inhibition of cell growth [9]. The data were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

BS and SS in the control (IC cultured without MNC) varied from experiment to experiment within limits of 7.2-13.3 and 39.2-62.4 $\mu U/10^4$ cells/h respectively. Under these conditions we deemed it advisable to express the experimental results as percentages of the corresponding control values in each concrete experiment.

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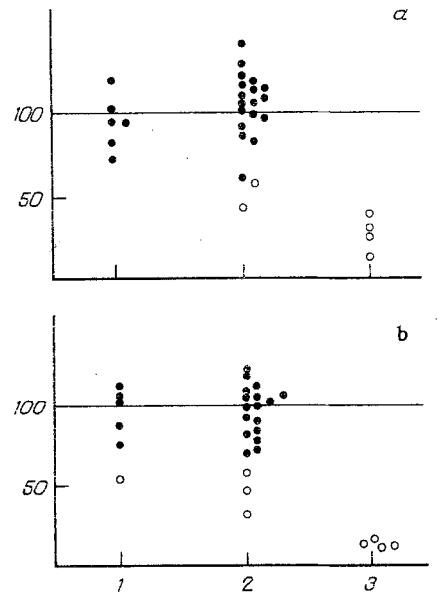

Fig. 1. BS (a) and SS (b) of insulin in IC after culture for 18 h in presence of mononuclears. Abscissa: 1) IC plus healthy human MNC, 2) IC plus MNC from diabetics, 3) IC plus rat MNC; ordinate, insulin secretion (in per cent of control). Here and in Fig. 2: filled circles denote $p > 0.05$; empty circles — $p < 0.05$.

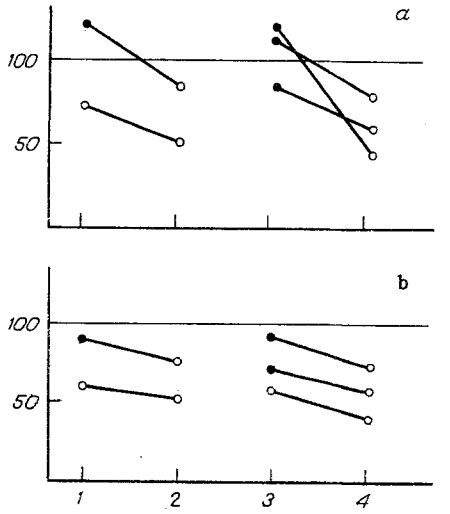

Fig. 2. Action of LPS on BS (a) and SS (b) of insulin in IC during culture with mononuclears. Abscissa: 1) IC plus healthy human MNC, 2) IC plus healthy human MNC plus LPS, 3) IC plus MNC from diabetics, 4) IC plus MNC from diabetics plus LPS; ordinate, insulin secretion (in per cent of control).

The results are given in Fig. 1. BS of insulin in the presence of healthy human ($n = 6$) MNC was 74-120% of that in the control ($p > 0.05$). SS in this group was 56-113%, the cells of one donor significantly depressing SS by 44% ($p < 0.05$). BS of insulin by β-cells cultured in the presence of MNC from diabetics ($n = 20$) was 42-140% of BS in the control; in two cases, inhibition of production by 58 and 42% respectively was significant. SS was 32-117%, and in three patients its reduction by

41.53% and 68% was statistically significant. We found no correlation between the level of inhibition of insulin production and the duration of the disease. Rat MNC inhibited BS by 61-87% and SS by 86-88%; in some experiments SS did not differ from BS.

We thus found no differences in the degree of inhibition of insulin production by IC in the presence of MNC from diabetics and from healthy blood donors, and we therefore postulated that the phenomenon is nonspecific and does not reflect activity of anti-islet-cell immunity.

In recent years evidence has been obtained that one mechanism of inhibition of β-cell function may be connected with cytokine production [3, 7, 8]. We studied this possibility and determined the TNF concentration in supernatants of the cultures. The TNF level in supernatants of MNC of diabetics and healthy blood donors was 0-5 U/ml, and its value did not correlate strictly with the degree of inhibition of insulin production. Supernatants of rat MNC contained 8-10 U/ml of TNF; rat MNC inhibited insulin production virtually completely. This indicates that cytokines are involved in inhibition of β-cell function in this particular model. At the same time we found that the TNF level in mixed IC plus MNC cultures did not differ from the control level (MNC alone). This suggested that the ability of MNC to inhibit insulin production is evidently the result of their initial activity and is not due to interaction between MNC and IC in vitro. Hence it ought to be expected that additional stimulation of MNC in mixed culture with IC would lead to an increase in TNF production and to stronger inhibition of the insulin-producing function of the β-cells. We found that addition of LPS to a mixed culture (200 ng/ml) led to a five-sevenfold increase in the TNF level, and under these circumstances more marked inhibition of insulin production was observed (Fig. 2). Incidentally, LPS did not affect insulin production by IC cultured without MNC.

The results confirm that MNC have the ability to inhibit the function of xenogeneic and allogeneic β-cells in vitro; this property does not reflect activity of cellular anti-islet-cell immunity, it includes the action of cytokines, and is probably determined by initial MNC activity.

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