IS THE REGULATORY ACTION OF DIUCIPHON-TREATED MONONUCLEARS DEPENDENT ON THE MEMBRANE-ASSOCIATED FORM OF INTERLEUKIN-2

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Key words: mononuclear cells; diuciphon; synthesis; secretion; interleukin-2.

The immunostimulator diuciphon has been shown to induce production of interleukin-2 (IL-2) by immunocompetent cells [3]. It has also been discovered that when such cells are treated with diuciphon they enhance the proliferative response of lymphocytes to mitogens in vitro [4] and the immune response to sheep's red blood cells in vivo [2]. On the basis of these results, a method of immunocorrection, based on the in vivo administration of autologous IL-2 producers, stimulated in vitro, has been developed and used in clinical practice to treat patients with immunodeficiency states [1]. Meanwhile, we have obtained results showing the absence of any direct dependence between the level of IL-2 production by diuciphon-stimulated cells and the manifestation of their helper effect in vitro. One possible explanation of this phenomenon may be the suggestion that functional activity of regular cells may be linked with their membrane structures and, in particular, with the membrane-associated form of IL-2. Under these circumstances, the signal from the regulator cell with mediator molecules expressed on the membrane is transmitted to the acceptor cell in the process of intercellular interaction. The existence of the membrane-associated form of IL-2 has been demonstrated [5]. It can be tentatively suggested that in the case of regulatory cells stimulated by diuciphon, structures expressed on the membrane can participate in the mechanism of action of such cells.

With this aim, human mononuclear cells (HMC) were stimulated to produce IL-2 by diuciphon and fixed with glutaraldehyde (GA) to prevent synthesis and secretion of soluble products by them; later, the fixed cells were added to a culture of intact, mitogen-stimulated autologous HMC and their immunoregulatory action was assessed.

EXPERIMENTAL METHOD

For the experiments we used HMC obtained from clinically healthy donors, male and female, aged 28-42 years. The HMC were isolated on a Ficoll—Verografin density gradient, as described previously [3]; the cells thus obtained were incubated for 3 h in medium 199 in the presence or absence of diuciphon in a dose of 50 μg/ml, at 37°C, in 96% humidity, and with 5% CO_2, washed 3 times with medium 199, and either used in the experiments as described previously [4], or fixed with 0.125% glutaraldehyde ("Serva," West Germany) for 30 min. After thorough washing 3 times with medium 199 the fixed HMC were counted and added in the necessary number to a culture of autologous or allogeneic (depending on the experimental conditions), freshly isolated HMC. PHA ("Sigma," USA) was used as the mitogen. In separate experiments, before incubation with diuciphon or fixation with GA, the HMC were treated for 30 min with 100 μg/ml of trypsin (SPOFA, Czechoslovakia). Further culture of the HMC was carried out in 96-well planchets (NUNC, Denmark), in medium RPMI-1640 ("Flow," U. K.) with the addition of 10 μg/ml of gentamicin ("Farmakhim," Bulgaria), 10 mM HEPES ("Sigma," USA), 2 mM glutamine ("Serva," West Germany), and 10% fetal calf serum ("Sigma," USA) at 37°C, in 96% humidity and with 5% CO_2 for 72 h. The level of the proliferative response was assessed by measuring incorporation of ^{3}H-thymidine, added at the rate of 1 μCi per well, into HMC. Radioactivity was counted after transfer of the cell cultures to

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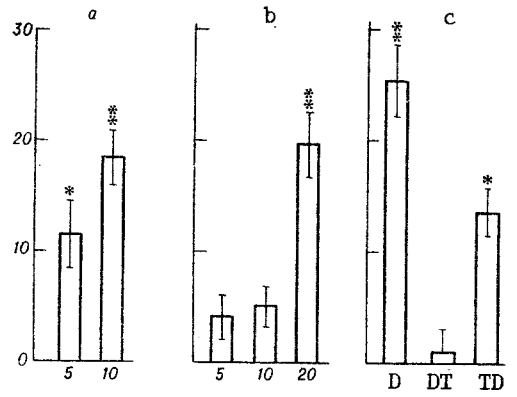

Fig. 1. Effect of treatment of diuciphon-stimulated MNC with GA and trypsin on manifestation of their modulating activity. Ordinate, enhancement ratio of proliferative response of MNC culture in the presence of diuciphon-treated MNC compared with that of MNC cultures in the presence of cells cultured without diuciphon, expressed in per cent. a) MNC preincubated in the presence or absence of diuciphon were added to the culture without fixation with GA; b) the same cells were added to the culture after fixation with a 0.125% solution of GA (a, b: number of activated cells in mixture amounted to 5, 10, and 20%); c) MNC cultures containing 5% of cells fixed with GA treated beforehand with diuciphon (D) or by diuciphon and trypsin (DT) or trypsin and diuciphon (TD), in different order.

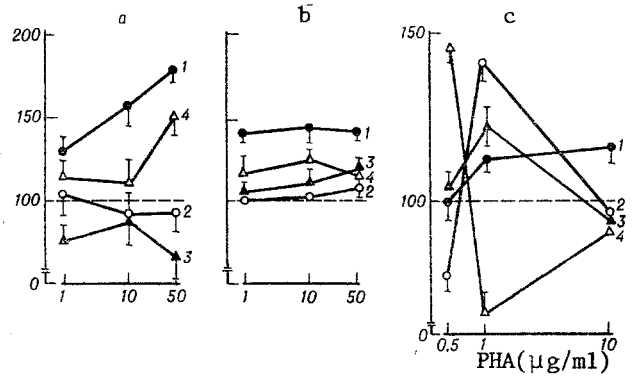

Fig. 2. Action of mononuclear cells treated with diuciphon and fixed with GA on proliferative response of intact MNC in presence of monoclonal antibodies to Tac antigen. Abscissa, diuciphon concentration (in μg/ml); ordinate, level of proliferative response of MNC cultures after addition of 5% of diuciphon-stimulated cells (in % of corresponding value for cultures containing 5% MNC preincubated without diuciphon; a) cultures stimulated by 1 μg/ml PHA, b) 10 μg/ml PHA; A and B: control cultures without addition of anti-Tac antibodies (1), cultures with addition of anti-Tac monoclonal antibodies in dilutions of 1:20 (2), 1:400 (3), and 1:800 (4); c) MNC stimulated by diuciphon and fixed were added to autologous (1) or allogeneic (2-4) cultures and stimulated by PHA in a concentration of 1 μg/ml.

filters. The numerical results were subjected to statistical analysis by Student's test. Monoclonal antibodies to TAC-antigen were generously provided by Dr. T. A. Waldmann (National Institutes of Health, Bethesda, USA).

EXPERIMENTAL RESULTS

The results of a parallel investigation of the helper activity of 5% HMC, not treated (Fig. 1a) and treated (Fig. 1b) with 0.25% GA (subsequently described as HMC-D and HMC-DGA, respectively) in an autologous system are described. The level of helper activity of HMC—D was estimated by the equation suggested previously:

$$ER = 1 - \frac{(HMC + HMC\text{-}B) - (HMC\text{-}MtmC + HMC\text{-}B)}{(HMC + HMC\text{-}D) - (HMC\text{-}MtmC + HMC\text{-}D)} \cdot 100,$$

where (HMC + HMC-B). The helper activity of HMC—DGA was estimated by a simplified equation, which is used in the case of inability of the GA-treated cells to incorporate the radioactive label:

$$ER = 1 - \frac{(HMC + HMC\text{-}BGA)}{(HMC + HMC\text{-}DGA)} \cdot 100,$$

where ER denotes the enhancement ratio, expressed in per cent, (MNC + MNC—WGA) the level of the proliferative response of a mixture of freshly isolated MNC with 5% MNC preincubated in the absence of diuciphon and treated with GA, and (MNC + MNC—DGA) denotes the level of the proliferative response of a mixture of freshly isolated MNC with 5% MNC stimulated by diuciphon and fixed with GA.

It follows from Fig. 1a, b that both living MNC and MNC fixed with GA can exert a significant stimulating action on the proliferative response of intact PHA-stimulated MNC. The evident difference is that in the case of MNC—DGA this effect was exhibited on the addition of a relatively larger number of cells (20%). Consequently, MNC—DGA, which cannot secrete soluble mediators, do nevertheless modify the proliferative response. The mechanism of their action is probably linked with membrane structures of protein nature expressed on such cells. Evidence of this is given by data in Fig. 1c. In these experiments, the MNC were treated with 100 μg/ml trypsin before or after incubation with diuciphon, fixed with GA just like the control nontrypsinized MNC—D, and were added in a proportion of 5% to a culture of freshly isolated autologous MNC, stimulated by PHA. Clearly, trypsinization of the cells after diuciphon stimulation completely abolished their potentiating action, whereas trypsinization before incubation with the preparation simply reduced this effect.

Further investigations showed with a high degree of probability that the stimulating action of MNC—DGA is effected through interaction of these cells with intact MNC through a receptor for IL-2. As will be clear from Fig. 2, addition of monoclonal anti-Tac antibodies, directed against the receptor for IL-2, to the cell culture led to dose-dependent abolition of the potentiating action of MNC—DGA. It must be noted that these antibodies, in the concentrations used, had no action on the proliferative response of the MNC themselves, or on that response in the presence of control MNC, fixed with GA. It is a very important fact that, as Fig. 2c shows, the action of MNC—DGA was not genetically restricted. MNC—DGA possessed stimulating activity on both autologous and allogeneic MNC, which, however, was manifested in different donors within a different range of concentrations of the mitogen, possibly due to differences in individual sensitivity of the MNC.

Our results are in agreement with the view that the mechanism of the immunoregulatory action of cells treated with diuciphon may be due not only to secretion of IL-2, as has been suggested [4], but also to expression of an associated form of IL-2 in the process of incubation with the membrane preparation [5]. Perhaps it is this method of signal transmission (through membrane-associated forms of mediators), and not mediator secretion into the external medium that is the latest and most perfect, specific form of regulation. Accurate signal transmission from cell to cell does not affect any other systems, organs, or cells. This state of affairs can partly explain the multiplicity of functions of mediators of the immune system, revealed by testing their soluble forms in experimental models in vivo and in vitro.

It can be tentatively suggested that corpuscular structures, carrying one or several types of biologically active molecules, which may open up wide prospects for the creation of a new generation of immunomodulators with a high degree of targeting of the effect, may play the role of regulatory cells of the immune system.

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COMPARATIVE ANALYSIS OF THE ACTION OF UNPURIFIED MOUSE ERYTHROPOIETIN AND HUMAN RECOMBINANT ERYTHROPOIETIN ON ERYTHROID AND GRANULOCYTIC-MACROPHAGAL PRECURSOR CELLS IN SEMISOLID MOUSE BONE MARROW CULTURES

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Erythropoietin is a circulating polypeptide hormone which regulates proliferation and differentiation of the erythroid branch of hematopoiesis. In recent years the gene of human erythropoietin has been isolated and a recombinant erythropoietin, comparable with natural erythropoietin in its biological and immune properties, has been obtained [3, 8]. This has opened up new prospects not only for the study of erythropoiesis, but also for the treatment of erythropoietin-deficiency anemias.

A comparative study of the action of recombinant and natural erythropoietin on human hematopoietic precursor cells has shown that recombinant erythropoietin, in equal doses, has a stronger stimulating action on erythroid and polypotent precursors than natural erythropoietin [4].

Another source of erythropoietic activity is mouse serum (plasma), enriched with erythropoietin [9]. This readily available preparation of erythropoietin stimulates colony formation from late erythroid precursor cells in mouse and human bone marrow culture [7]. However, the presence of inhibitors of hematopoiesis of different nature in plasma [2] and also the presence of growth factors make the use of such preparations for the study of erythropoiesis in culture more difficult.

The aim of this investigation was to study the action of unpurified preparations of murine erythropoietin and human recombinant erythropoietin on committed precursor cells of mouse bone marrow.

EXPERIMENTAL METHOD

Experiments were carried out on bone marrow from female (CBA × C57BL)F_1 mice weighing 18-20 g. A cell suspension was prepared in α-MEM medium with 20% fetal calf serum (FCS). Bone marrow cells in a concentration of $2 \cdot 10^5$/ml were cultured by Iscove's method [5] in a modification. The culture medium contained methylcellulose ("Fluka") in α-MEM medium in a final concentration of 0.9%, with 30% FCS ("Flow"), 1% L-glutamine, 1% bovine serum albumin (7.5% of fraction V, from "Gibco"). 10^{-4} M 2-mercaptoethanol (2-ME), 2% HEPES buffer, and antibiotics. To stimulate colony formation a conditioning medium was used in a final concentration of 10%; it was obtained during culture of mouse splenocytes ($2 \cdot 10^6$/ml) in medium RPMI-1640, enriched with glutamine, 2-ME, FCS, and inactivated human plasma, each in a proportion of 2.5%; pokeweed mitogen ("Flow") was added in a concentration of 0.75-1%. The conditioning medium obtained after culture for 7 days was centrifuged and filtered (pore diameter 0.45 μ).

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