FUNCTIONAL PROPERTIES OF A LYMPHOCYTE POPULATION RESPONDING TO LOW SUBOPTIMAL DOSES OF CONCANAVALIN A

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The proliferative response of lymphocytes in cultures stimulated by mitogens is characterized by marked dependence of the intensity of proliferation on the dose of mitogen. It has been shown recently that populations of T lymphocytes differing in function differ in their sensitivity to concanavalin A (con A), a widely used nonspecific T-cell stimulator. For instance, activation of killer T lymphocytes is effected by optimal (i.e., leading to maximal proliferation in the cultures) doses of con A [2]. For suppressor cell generation higher doses of the mitogen are used [3, 4]. The T-cell subpopulation responding by proliferation in cultures containing suboptimal low con A concentrations has not been characterized from the functional point of view, but, as was shown previously [1], compared with T lymphocytes responding to optimal doses of mitogen, it has higher sensitivity to the proliferation-stimulating effect of non-T cells and also to the inhibitory action of nonspecific spontaneous suppressor cells.

This paper describes the results of a study of the properties of human lymphocytes responding by proliferation to a low suboptimal con A concentration.

EXPERIMENTAL METHOD

Human mononuclear cells (MC) were isolated from peripheral blood in a Ficoll-Verografin density gradient and cultures *in vitro* by standard methods described by the writer previously [1]. The culture medium was the same in all experiments and consisted of 80% medium 199, 20% inactivated group IV (AB) human serum, and antibiotics [penicillin (100 units/ml) and streptomycin (100 μg/ml)]. The con A used in the experiments was from Sigma (USA), Grade III (concentrations calculated in terms of pure protein). The intensity of the proliferative response of the lymphocytes was estimated from incorporation of [^{3}H]thymidine into nucleoprotein fractions of the cells.

EXPERIMENTAL RESULTS

Preliminary incubation of MC with 50 μg/ml con A (the concentration inducing suppressor activity [1, 3]) was accompanied by a marked decrease in the response in reactivated cell cultures of four of the six donors, and also on average (Table 1). Differences, however, were not statistically significant. The intensity of proliferation of cells preincubated with 0.5 μg/ml con A ($MC_{conA\ 0.5}$) in reactivated cultures did not differ from the response of the control cells incubated for 42 h without con A (MC_{conA_0}). However, $MC_{conA\ 0.5}$, like MC activated with 50 μg/ml of con A ($MC_{conA\ 50.0}$), could inhibit proliferation of autologous intact lymphocytes in cultures stimulated with an optimal dose (5 μg/ml) of the mitogen. It must be pointed out that $MC_{conA\ 50.0}$ effectively suppressed proliferation of autologous lymphocytes also in cultures stimulated by 0.5 μg/ml con A [1], whereas $MC_{conA\ 0.5}$ was unable to do so (Table 2). In connection with these results the following facts are very interesting: Heijnen et al. [5] and Lydyard and Hayward [8], under rather different experimental conditions, demonstrated that differentiation of precursor cells into effector T suppressors in man takes place under the influence of activated T-T-helper lymphocytes. It is also known

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TABLE 1. Effect of Preliminary Activation of Cells with con A on Subsequent Proliferative Response

Type of cells responding to con A	Response	Number of observations	Con A concentration in culture, μg/ml	
			0	5,0
0.5×10^6 $MC_{conA_{0.5}}$		4	2765±648,1	20 501±4071,3
0.5×10^6 MC_{conA_0}		4	2460±651,9	19 537±5372,8
0.5×10^6 $MC_{conA_{0.5}}$ + 0.5×10^6 MC_{conA_0}	True*	4	7665±1429,1	25 895±8701,0
	Expected	4	5225±1090,3	40 038±8180,7 $P<0,05$†
0.5×10^6 $MC_{conA_{50.0}}$		6	1972±426,6	5 090±553,8
0.5×10^6 MC_{conA_0}		6	882±304,9	76,14±1844,0
0.5×10^6 $MC_{conA_{50.0}}$ + 0.5×10^6 MC_{conA_0}	True	6	3379±802,9	5 315±2305,3
	Expected	6	2854±641,7	12 704±1905,2 $P<0,05$

*Here and in Table 2: 0.5×10^6 MC incubated for 42 h with 0.5 μg/ml con A ($MC_{conA_{0.5}}$) or with 50 μg/ml con A ($MC_{conA_{50.0}}$) were mixed with 0.5×10^6 intact incubated autologous cells (MC_{conA_0}). The proliferative response of this cell mixture was regarded as true. The expected response is the sum of the responses of $MC_{conA_{0.5}}$ (or $MC_{conA_{50.0}}$) and of MC_{conA_0}, cultured separately.

†Values of P are given when there are statistically significant differences between the true and expected responses (Student's t test).

TABLE 2. Proliferation of $MC_{conA_{0.5}}$ in Reactivated Cultures and Their Effect on Proliferative Response of Autologous Lymphocytes Stimulated by 0.5 μg/ml con A

Donor	Intensity of proliferation, cpm			
	type of "responding" cells			
	0.5×10^6 MC_{conA_0}	0.5×10^6 $MC_{conA_{0.5}}$	0.5×10^6 MC_{conA_0} + 0.5×10^6 $MC_{conA_{0.5}}$	
			expected response of mixture	true response of mixture
1	16 006	13 312	29 318	20 319
2	3 389	9 885	13 274	13 969
3	19 817	14 110	33 927	23 868
4	6 190	8 827	15 017	20 242
$M\pm m$	11 351±3 909	11 534±1286,0	22 885±5144,5	19 600±2053,6

that among lymphocytes which respond *in vitro* to low doses of con A there are T cells secreting a mitogenic factor, whereas acquisition of sensitivity to its action is induced by higher doses of mitogen [7]. It can thus be postulated that suppression of the proliferative response in cultures stimulated by 5 μg/ml of con A is the result of cooperative interactions between $MC_{conA_{0.5}}$ and lymphocytes responding to 5.0 μg/ml of con A. Correspondingly, among $MC_{conA_{50.0}}$ there are functionally active effector suppressor cells, and it is this which determines their inhibitory effect on the proliferative response stimulated by both 5.0 μg/ml and 0.5 μg/ml of con A.

It will be clear from Table 3 that preincubation of MC for 18 h before the first activation by con A did not lead to changes in suppressor activity of $MC_{conA_{0.5}}$ and $MC_{conA_{50.0}}$. Meanwhile preincubation caused a decrease in the proliferative response of $MC_{conA_{0.5}}$ and $MC_{conA_{50.0}}$ in cultures reactivated by 5.0 g/ml of mitogen. It follows from these results that the observed decrease in proliferation could not be due to a change in activity (or the appearance of new activity) of the regulatory cells. Possibly the effect discovered was due to elimination, in the course of 18 h of incubation, of cells participating in the proliferative response stimulated by 5.0 μg/ml con A. The presence of 0.5 μg/ml of mitogen in the

TABLE 3. Changes in Functional Properties of Cells Responding to con A after Brief Preincubation

Type of "responding" cells in culture	Number of cells in culture flask	Preincubation for 18 h before stimulation by con A for 42 h	Proliferative response, cpm, dose of con A, μg/ml	
			0	5
$MC_{conA_{0.5}}$	$1{,}0 \cdot 10^6$	—	4 591±1492,2	29 410±3333,6
		+	4 912±882,4	18 099±2745,0 ($P<0{,}05$*)
Mixture: $MC_{conA_{0.5}} + MC_{conA_0}$	up to $0{,}5 \cdot 10^6$	—	11 633±3154,1	13 845±4530,6
		+	5 367±460,9 ($P<0{,}05$)	12 087±1064,9
$MC_{conA_{50.0}}$	$0{,}5 \cdot 10^6$	—	1 972±426,2	5 090±553,8
		+	2 078±364,7	2 533±831,1 ($P<0{,}05$)
Mixture: $MC_{conA_{50.0}} + MC_{conA_0}$	up to $0{,}5 \cdot 10^6$	—	3 379±802,9	5 315±2305,3
		+	4 932±1287,8	6 937±1501,1

*Values of B given if statistically significant differences exist in the response of cells preincubated for 18 h before the first activation by con A and non-preincubated MC (Student's t test or Wilcoxon—Mann—Whitney U test).

culture during preincubation prevented their elimination. The absence of differences in the response of MC_{conA_0} and $MC_{conA_{0.5}}$ to 5 μg/ml of con A, already mentioned above (Table 2), was evidently due to compensation population.

The effect of preincubation for 18 h on the ability of $MC_{conA_{0.5}}$ to induce proliferation in a mixture with autologous intact cells in the absence of mitogen also deserves attention (Tables 1 and 2) [6, 9]. Possibly the reduction in the ability of $MC_{conA_{0.5}}$ to develop a proliferative response when mixed with autologous MC_{conA_0} is due to elimination of some of the lymphocytes capable of self-recognition during preincubation.

The experiments thus showed that among lymphocytes responding to low suboptimal doses of con A there are cells which participate in the formation of mitogen-stimulated suppressors. These are probably T-T-helper lymphocytes, inducing the formation of effector suppressor T lymphocytes from precursors activated by higher concentrations of the mitogen. Furthermore, low doses of con A prevent the elimination of short-living lymphocytes in the culture that participate in the proliferative response to optimal con A concentrations and also in the response to autologous lymphocytes.

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LITERATURE CITED

1. G. Z. Shubinskii and V. P. Lozovoi, Immunologiya, No. 2, 24 (1981).
2. B. J. Bonavida, J. Exp. Med., 145, 293 (1977).
3. S. M. Fineman, F. B. Mudawwar, and R. S. Geha, Cell. Immunol., 45, 120 (1979).
4. H. M. Hallgren and E. J. Yunis, J. Immunol., 118, 2004 (1977).
5. C. J. Heijnen, F. UytdeHaag, C. H. Pot, et al., Nature, 280, 589 (1979).
6. N. E. Goeken, J. S. Thompson, L. Klassen, et al., Hum. Immunol., 1, 65 (1981).
7. E.-L. Larsson and A. Coutinho, Eur. J. Immunol., 10, 93 (1980).
8. P. M. Lydyard and A. R. Hayward, Clin. Exp. Immunol., 39, 496 (1980).
9. M. E. Weksler, J. Exp. Med., 137, 799 (1973).