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Concanavalin A-activated suppressor cells in normal human peripheral blood lymphocytes

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SUMMARY

A 24-hr preincubation of human peripheral blood lymphocytes in the presence of Con A renders them suppressive for the response of untreated cells to soluble antigens, allogeneic cells and Con A.

INTRODUCTION

The role of regulatory cells, both enhancing and suppressive, on immune response is well documented in animals. Suppressor cells have been demonstrated which control antibody synthesis (Dutton, 1975; Baker, 1975) and cell-mediated immune reactions such as skin test delayed hypersensitivity (Zembala & Asherson, 1974; Katz, Parker & Turk, 1974; Neta & Salvin, 1974; Phanu Phak, Moorehead & Claman, 1974) graft-versus-host disease (Gershon, Liebhaber & Ryu, 1974; Hardin, Chused & Steinberg, 1973) mixed lymphocyte culture (MLC) (Rich & Rich, 1975; Folch & Waksman, 1974), cell mediated cytotoxicity (Hodes & Hathcock, 1976; Eggers & Wunderlich, 1975) and *in vitro* response to T-cell mitogens (Folch & Waksman, 1973). Such an active suppression has been attributed to several cell classes, including T-cell subpopulations (Zembala & Asherson, 1974; Folch & Waksman, 1973; Ha, Waksman & Treffers, 1974; Burns *et al.*, 1975; Kilburn, Smith & Gorczynski, 1974) and B cells (Neta & Salvin, 1974; Katz, Parker & Turk, 1974). In man, suppressor cells have been identified in the spleen of uraemic patients at the time of kidney grafting (Kauffman, 1975). It was observed that spleen cells, precultured during 3 days in the presence of Concanavalin A (Con A), were suppressive for MLC performed with peripheral blood lymphocytes (PBL). Recent observations further indicate that suppressor T lymphocytes are involved in the pathogeny of some cases of acquired hypogammaglobulinaemia and selective IgA deficiency (Waldmann *et al.*, 1974; Delespesse *et al.*, 1976). In the present study we demonstrate that preculturing normal PBL with Con A renders them suppressive for the response of untreated lymphocytes to a soluble antigen (Candidin), allogeneic cells and Con A.

MATERIALS AND METHODS

Blood was obtained from healthy volunteers under 45 years of age. The methods of lymphocyte purification and culture have been described elsewhere (Delespesse *et al.*, 1976). Briefly, PBL were purified by centrifugation on Ficoll metrizoate (d:1077) and finally resuspended in RPMI 1640 (Flow Lab.) containing 10% heat-inactivated AB serum, 2mM glutamine, 50 μg/ml streptomycin and 50 μg/ml penicillin. Aliquots of 8×10^6 cells suspended in 4 ml were incubated for 24 hr at 37°C in screw-capped glass tubes (Kimax, U.S.A.). These 'precultures' were performed either in culture medium alone (AC cells) or in the presence of 10 μg/ml Con A (AS cells) (Concanavalin A, Calbiochem Lab.). After 24 hr, AC and AS cells were washed three times in Hanks's BSS (Gibco Lab.) and resuspended at the concentration of 2×10^6 cells/ml in fresh culture medium. In some cases AC and AS have been treated with mitomycin C (40 μg/ml, during 30 min at 37°C) before use in the next step of the experiment. After this 24-hr incubation, fresh blood was drawn from the same donor and

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the PBL were purified as described above (A cells). Cultures were comprised of a mixture of either A+AC or A+AS cells, with 2×10^5 cells of each type. Quadruplicate cultures were performed in micro culture plates (3040 Microtest II, Falcon U.S.A.) in a volume of 0·2 ml.

The cultures were supplemented with one of the following stimulants: 10 μg/ml Con A, 500 μg/ml Candidin or 2×10^5 mitomycin treated allogeneic lymphocytes. Other controls consisted in separate cultures of A, AC and AS. Mitogen containing cultures were harvested after 3 days while MLC and Candidin containing cultures were harvested after 7 days. 18 hr before harvesting, 4 μCi of [^{3}H]thymidine (Institut des Radioéléments, Fleurus, Sp.act.: 10 C/mM) was added to each culture. Finally the cells were collected and washed over glass filter discs using a MASH II cell harvester (Microbiological Associated, Bethesda). The discs were dried and immersed in 10 ml Bray's solution for counting. Results are expressed in ct/min.

RESULTS

A Con-A activation of suppressor cells was first suspected when we found that AS were less responsive to Con A than AC (Table 1).

Direct confirmation of this view was provided by comparing the response of A+AC to that of A+AS (Table 2). Adding AC to A resulted in an additive effect while adding AS to A induced a suppressive effect. As AC and AS were not treated with mitomycin the suppressive effect of the latter was partly masked by their own tritiated thymidine uptake. Con A-activated suppressor cells were able to inhibit the lymphocyte response to Candidin (Table 2) and to allogeneic cells (Table 4). In these experiments AC and AS have been treated with mitomycin, so that a direct inhibition on the response of A cells could be more clearly observed.

TABLE 1. Influence of a preincubation in the presence or in the absence of Con A on the lymphocyte response to Con A

Cells in culture	Expt 1	Expt 2
A	151,782±14,762	257,499±26,573
AC	191,099±9,815	203,067±17,693
AS	89,084±9299	108,507±4754

Ac correspond to lymphocytes preincubated in culture medium alone, As to lymphocytes preincubated in the presence of Con A and A to lymphocytes not preincubated from the same donor. 3 days cultures in the presence of 10 μg/ml Con A.

Ct/min, mean of four replicates±s.d.

TABLE 2. Influence of cells preincubated with Con A on the lymphocyte response to Con A

Cells in culture	Expt 1	Expt 2
A	47,252±3452	50,399±4951
A+AC	151,018±2981	100,645±6374
A+AS	17,078±1439	36,513±3775
AS	22,611±2819	27,810±3271
AC	89,462±7891	42,404±2574

3 days cultures in the presence of 10 μg/ml of Con A. A, AS and AC cultures contain 2×10^5 cells; A+AS and A+AC contain 2×10^5 cells of each type.

Ct/min, mean of four replicates±s.d.

TABLE 3. Influence of cells preincubated with Con A on the lymphocyte response to candidin

Cells in culture	Expt 1	Expt 2	Expt 3
A	17,497±340	14,547±717	22,143±1521
AC_M	2705±1825	1570±559	6020±1217
AS_M	2370±923	1421±728	3797±642
$A+AC_M$	17,869±2145	19,978±3944	22,788±894
$A+AS_M$	6397±2800	8845±516	8111±1271

7 days cultures in the presence of 500 μg/ml Candidin. AC_m and AS_m correspond to mitomycin treated AC and AS.

Same comments as for Table 2.

TABLE 4. Influence of cells preincubated with Con A on the lymphocyte response in MLC

Cells in culture	Expt 1	Expt 2
$A+B_M$	31,986±6236	27,198±6382
AC_M+B_M	2786±1452	1332±2351
AS_M+B_M	5167±2129	2893±1862
$A+B_M+AC_M$	26,543±1727	20,547±2872
$A+B_M+AS_M$	5060±215	16,739±1505

A and B correspond to the lymphocytes of two unrelated subjects. B_m, AC_m and AS_m correspond to mitomycin treated cells.

Same comments as for Table 2.

DISCUSSION

The present data clearly demonstrate that PBL from healthy subjects become suppressive after 24 hr incubation in the presence of Con A. These Con A-activated cells inhibit the lymphocyte response to mitogens, soluble antigens and allogeneic cells. Further characterization of the nature and properties of Con A-induced suppressor cells are actually in progress in our laboratory. Assessment of the functional integrity of these suppressor cells is warranted in various clinical situations such as autoimmunity, ageing and neoplasia. Indeed a decline of suppressor T-cell activity has been observed in similar conditions on experimental animals (Folch & Waksman, 1973; Kilburn, Smith, Gorczynski, 1974; Kirchner *et al.*, 1974; Barthold, Kysela & Steinberg, 1974).

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