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Effect of melphalan in vitro on induction of murine suppressor T cells by ConA

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Summary. The effect of treatment with melphalan in vitro on the activity of spleen cells from BALB/c mice was investigated. Incubation of spleen cells with 1.5–5 μg melphalan/1 × 10^7 inhibited subsequent mitogenic stimulation by ConA or PHA and the allogeneic response of BALB/c spleen cells against C57B1 target spleen cells. Incubation of spleen cells with ConA led to induction of suppressor T cells which when added to fresh cultures inhibited the allogeneic response. Preincubation of spleen cells with melphalan even at low concentrations (0.15–0.5 μg 1 × 10^7 cells) which do not directly affect mitogenic stimulation or allogeneic response partially inhibited the generation of suppressor T cells by ConA. Treatment with melphalan had no effect on already induced suppressor T cells as shown by incubation of spleen cells with melphalan (0.15–5 μg/1 × 10^7 cells) after incubation with ConA. Addition of cells treated with melphalan alone (without ConA) to fresh cultures led to an increase in the allogeneic response.

Introduction

We have shown [1] that treatment with a low nonimmunosuppressive dose of the alkylating drug melphalan (L-PAM: L-phenylalanine mustard) cured BALB/c mice from a large MOPC-315 plasmacytoma tumor, and eradication of the tumor was attributed to participation of the host's antitumor immunity. This assumption was strengthened by findings showing that low dose L-PAM therapy was not effective in immunosuppressed mice and that mice cured were highly resistant to challenge with an otherwise tumorigenic dose of MOPC-315 [1]. Recently [3] we have demonstrated that incubation of spleen cells from mice bearing large MOPC-315 tumors with L-PAM led to restoration of the potential for a cytotoxic response in vitro against target MOPC-315 cells. The conclusion drawn from the L-PAM-MOPC-315 experimental system is that L-PAM given at a low dose acts as an immunomodulator, thereby facilitating development of the host's antitumor immune response.

Inhibition of development of the antitumor response was assumed to be due in part to development of suppressor T cell population(s) in the tumor-bearing host [6, 11, 12]. Accordingly, the possibility should be considered that immunomodulation of the antitumor response by certain chemotherapeutic drugs is due to their selective effect on suppressor T cells. In this respect it has been shown that cyclophosphamide (CY) has a selective effect on human [9, 15] and murine [8, 10, 11] suppressor T cells and low dose therapy with CY was also effective in curing mice bearing a large MOPC-315 tumor [5, 7]. In a recent work [2] we have shown that L-PAM treatment of human peripheral blood lymphocytes prevented the induction of suppressor T cells by Concanavalin A (ConA).

The selective effect of drugs on suppressor T cells may play a role in the process of eradication of an established tumor (such as MOPC-315 in BALB/c mice) by chemotherapy. The purpose of this study was to determine the effect of L-PAM in vitro on the induction of suppressor T cells in spleen cell suspensions from BALB/c mice.

Materials and methods

Mice. Male BALB/c mice and male C57B1 mice (8–12 weeks old) were obtained from the breeding colony of the Hebrew University, Jerusalem.

Spleen cell suspensions. Spleen cells were pooled from at least three mice in each of the experiments. Single cell suspensions were prepared by mechanical disruption between glass slides in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mmole/ml glutamine. The spleen cells were washed three times in RPMI medium and resuspended to the desired concentration. The viability of the spleen cell suspensions was approximately 90% as estimated by trypan blue dye exclusion (0.1%).

Treatment of spleen cells with L-PAM. L-PAM powder (Burroughs-Wellcome Co., Research Triangle Park, NC, USA) was used. A concentrated solution of 20 mg/ml was prepared immediately before use in a solvent mixture composed of an alcohol : acid mixture (5 : 1 of 95% ethyl alcohol: 2N HCl). Further dilutions were made in serum-free RPMI medium. For treatment with L-PAM 1 × 10^7 spleen cells/ml were incubated for 1 h at 37 °C with various concentrations of L-PAM (0.15 μg to 5 μg/1 × 10^7 cells), washed three times in serum-free RPMI medium and resu-

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spended for setting in cultures in RPMI medium supplemented with fetal calf serum (FCS). Cells incubated for 1 h without L-PAM were used as controls.

Effect of L-PAM on mitogenic stimulation. Assays of lymphocyte stimulation were performed by a micromethod using an automatic harvester [13]. L-PAM-treated and untreated spleen cells were incubated for 3 days in RPMI medium supplemented with 10% FCS in flat bottom Linbro plates at 37 °C in a humidified atmosphere containing 5% CO_2. Phytonemagglutinin (PHA) (Wellcome, Beckenham, England; 0.1 μg/50 μl) or ConA (Calbiochem., San Diego, Calif. USA; 0.25 μg/50 μl) were added to spleen cell cultures containing 2×10^5 cells/100 μl at the beginning of the incubation time. Cultures incubated without mitogen were used as controls. ^{3}H-Thymidine (Nuclear Research Center, Negev, Israel; 1 μCi/50 μl per culture) was added for the last 6 h of incubation. Each culture was performed in quadruplicate. The quantities of PHA and ConA used were found to be optimal in preliminary experiments in which PHA was tested within the range of 0.05 μg to 0.5 μg/culture and ConA at doses of 0.25 to 16 μg/culture. The background of thymidine incorporation in the preliminary experiments was within the range of 140–260 cpm.

Effect of L-PAM on MLR (mixed lymphocyte reaction). L-PAM-treated and untreated BALB/c spleen cells were assayed in MLR cultures. Allogeneic stimulation of BALB/c splenocytes by C57Bl spleen cells was determined. C57Bl spleen cells were treated with mitomycin (MC, Sigma, St. Louis, USA; 50 μg MC/1×10^7 cells) for 30 min at 37 °C. Equal quantities of 4×10^5 BALB/c spleen cells and of MC-treated C57Bl spleen cells were mixed and cultured in 200 μl RPMI medium, with 10% FCS and mercaptoethanol at a final concentration of 5×10^{-5}M. The cultures were incubated for 4 days in Falcon flat bottom plates at 37 °C in a humidified atmosphere containing 5% CO_2. ^{3}H-Thymidine (1 μCi/50 μl per culture) was added for the last 6 h of incubation. Mixtures of BALB/c spleen cells and of MC-treated BALB/c spleen cells were set up as controls. Other controls were C57Bl spleen cells alone (mean cpm 600±73), MC-treated C57Bl spleen cells only (mean cpm 388±97), and mixtures of MC-BALB/c×MC-C57Bl spleen cells (mean cpm 955±105). Each combination culture was performed in quadruplicate.

Induction of suppressor T cells by ConA [16]. A quantity of 1×10^7 BALB/c spleen cells was incubated with 15 μg ConA for 48 h in culture medium containing 5% FCS and 5×10^{-5}M mercaptoethanol. Cells were harvested and washed three times. The first washing was done in medium containing 0.05 mM α-methylmannoside. The cells were treated with MC for 30 min at 37 °C (50 μg MC/1×10^7 cells) before addition to fresh MLR cultures.

Effect of L-PAM on suppressor T cell activity. The effect of L-PAM was examined in the following combinations: prior treatment with L-PAM followed by incubation for 48 h with ConA; prior treatment with L-PAM followed by incubation without ConA; incubation with ConA followed by treatment with L-PAM; and, incubation without ConA followed by treatment with L-PAM. Cells not treated with L-PAM and incubated without ConA were used as controls. MC-treated cells of the various combinations were assayed for their suppressive activity on MLR by adding various amounts (0.25×10^5, 0.5×10^5 or 1×10^5 cells/culture) at the beginning of the incubation period.

Results

Inhibition of T cell mitogenic response by L-PAM. Spleen cells were treated with five different concentrations of L-PAM ranging from 0.15 μg to 5 μg/1×10^7 cells and then cultured in the presence of ConA, PHA or without mitogen. Treatment with either 0.15 μg or 0.5 μg L-PAM had no appreciable effect on the mitogenic response to ConA or PHA. A marked decrease in ^{3}H-thymidine uptake in mitogen-stimulated cultures was observed when cells were preincubated with at least 1.5 μg L-PAM/1×10^7 cells (Table 1).

Inhibition of allogeneic response by L-PAM. Treatment with 0.5 μg L-PAM/1×10^7 cells had only a slight effect on the allogeneic response of BALB/c spleen cells toward MC-treated C57Bl spleen cells. A marked decrease in thymidine incorporation in MLR cultures was observed in cultures containing responding BALB/c spleen cells pretreated with at least 1.5 μg L-PAM/1×10^7 cells. Concentrations of 1.5 to 5 μg L-PAM/1×10^7 cells also reduced the "background" of thymidine incorporation in cultures containing BALB/c responder spleen cells and MC-treated BALB/c spleen cells. The results are presented in Table 2.

Effect of treatment with L-PAM on ConA-induced T cell suppression. Addition of untreated cells reduced ^{3}H-thymidine incorporation by comparison with incorporation in MLR mixtures cultured without added cells. Addition of various amounts of BALB/c spleen cells incubated for 48 h with ConA, to MLR cultures, led to a marked suppression of the allogeneic response of BALB/c spleen cells against MC-treated C57Bl spleen cells as compared with the allogenic response in cultures supplemented with cells incubated without ConA. Treatment with L-PAM (0.15 μg–3.0 μg/1×10^7 cells) before incubation with ConA

Table 1. The in vitro effect of melphalan (L-PAM) on mitogenic stimulation of BALB/c spleen cells

L-PAM treatment[a]	^{3}H-thymidine incorporation (cpm ± SE)[b]		
μg/1×10^7 cells	ConA added	PHA added	Without mitogen
None	1,487 ± 177	1,379 ± 141	176 ± 27
Diluent only	1,102 ± 180	1,044 ± 175	194 ± 37
0.15	1,463 ± 112	1,591 ± 122	185 ± 21
0.5	1,281 ± 114	1,448 ± 95	262 ± 35
1.5	628 ± 53	718 ± 64	228 ± 37
3.0	409 ± 62	438 ± 36	197 ± 55
5.0	212 ± 59	285 ± 28	143 ± 41

[a] Spleen cells were incubated for 1 h with L-PAM at 37°C and washed before culturing; spleen cells incubated alone or with diluent used for preparation of the high concentration of L-PAM (5 μg/1×10^7 cells) were used as controls. The experiment was repeated twice with similar results.

[b] Spleen cells were cultured for 72 h with or without mitogen; 2×10^5 cells/culture; ConA: 0.25 μg/culture; PHA: 0.1 μg/culture; cpm ± SE: means of four parallel samples; ^{3}H-thymidine (1 μCi/culture) was added for the last 6 h of incubation

Table 2. The in vitro effect of melphalan (L-PAM) on allogeneic stimulation (MLR)

L-PAM treatment[a]	^{3}H-thymidine incorporation[b]			
μg/1 × 10^7 cells	BALB/cxMC-C57B1		BALB/cxMC-BALB/c	
	cpm ± SE	Inhibition[c] %	cpm ± SE	Inhibition %
None	42,067 ± 1,487		7,878 ± 772	
0.5	35,205 ± 2,561	16	7,995 ± 690	-1
1.5	13,133 ± 734	69	3,759 ± 197	52
3.0	6,007 ± 367	86	1,818 ± 176	77
5.0	1,408 ± 87	97	545 ± 66	93

[a] Spleen cells were incubated for 1 h with L-PAM at 37°C and washed before culturing; spleen cells incubated without L-PAM were taken as controls; the experiment was repeated three times with similar results

[b] 4 × 10^5 BALB/c spleen cells previously incubated with or without L-PAM were mixed with 4 × 10^5 MC-treated C57B1 or BALB/c spleen cells and cultured for 4 days; ^{3}H-thymidine (1 μCi/culture) was added for the last 6 h of incubation; cpm represent means of four parallel cultures ± SE

[c] % Inhibition: % inhibition was calculated with reference to MLR cultures containing BALB/c spleen cells not treated with L-PAM

markedly prevented the induction of suppressor cells by ConA. The effect of pretreatment with L-PAM was observed even at doses of 0.15 or 0.5 μg L-PAM/1 × 10^7 cells, i.e., at doses which did not directly affect mitogenic stimulation or the allogeneic response. Addition of cells treated with low doses of L-PAM (0.15 μg to 3.0 μg/1 × 10^7) to MLR cultures and incubated for 48 h without ConA, slightly increased the allogeneic response of BALB/c spleen cells against C57Bl cells, but this increase was found to be insignificant. The results are presented in Table 3. Pretreatment with doses of 0.05 or 0.015 μg L-PAM/1 × 10^7 cells did not affect induction of suppressor cells by ConA (results not included in Table).

In another series of experiments the treatment of spleen cells with L-PAM was performed after induction of suppressor cells by ConA. Spleen cells first incubated with ConA and treated with L-PAM afterwards retained their suppressive activity on the allogeneic response in MLR cultures, even when the dose of L-PAM used was up to 5 μg/1 × 10^7 cells (Table 4). Addition of spleen cells incubated without ConA and subsequently treated with 0.15 μg to 5 μg L-PAM/1 × 10^7 cells (before addition to MLR cul-

Table 3. Effect of pretreatment with melphalan (L-PAM) on ConA-induced T cell suppression of allogeneic response (MLR)

Treatment of spleen cells added to MLR[a]		^{3}H-thymidine incorporation in presence of treated cells[b]					
		1/4		1/8		1/16	
L-PAM μg/1x10^7	ConA 15 μg/1x10^7	cpm ± SE	% of control[c]	cpm ± SE	% of control	cpm ± SE	% of control
Control of medium alone (no cells added)		34,416 ± 1,559					
None	No	18,970 ± 079		22,170 ± 2,191		25,551 ± 3,653	
None	Yes	2,781 ± 54	15	6,559 ± 530	30	16,808 ± 1,998	66
0.15	Yes	7,074 ± 210	37	17,949 ± 2,777	81	29,613 ± 1,718	116
0.5	Yes	7,287 ± 719	38	9,580 ± 300	43	32,885 ± 2,834	129
3.0	Yes	7,594 ± 962	40	19,734 ± 1,694	89	35,155 ± 1,439	138
0.15	No	23,276 ± 1,121	123[d]	25,977 ± 1,625	117	20,685 ± 1,178	81
0.5	No	22,176 ± 2,136	117	27,220 ± 2,184	123	31,006 ± 3,019	121
3.0	No	20,263 ± 641	107	27,128 ± 1,065	122	33,630 ± 1,859	132

[a] Cells added to MLR cultures were first incubated for 1 h at 37°C with or without *L*-PAM, washed and incubated for 48 h with or without Con A

[b] 4 × 10^5 BALB/c spleen cells were cultured with 4 × 10^5 MC-treated C57B1 spleen cells; treated spleen cells were inactivated by MC (50 μg/1 × 10^7 cells for 30 min at 37°C) and then added to MLR cultures in quantities of 1 × 10^5 cells/culture (1/4), 0.5 × 10^5 cells/culture (1/8), or 0.25 × 10^5 cells culture (1/16); ^{3}H-thymidine (1 μCi/culture) was added for the last 6 h of incubation; all cultures were performed in quadruplicate; mean cpm ± SE in BALB/c × [MC] BALB/c mixture: 3,658 ± 220; the experiment was repeated twice with similar results

[c] % of control was calculated with reference to cultures supplemented with cells not treated with *L*-PAM and incubated in medium alone (without ConA)

[d] Augmentation of thymidine incorporation in cultures supplemented with cells incubated with *L*-PAM alone was found to be not significant by Student's *t*-test

Table 4. Effect of treatment with melphalan (*L*-PAM) after ConA incubation on suppression of allogeneic response (MLR)

Treatment of spleen cells added to MLR [a]		^{3}H-thymidine incorporation in presence of added cells 1/4		1/8		1/16	
ConA 15 μg/1x10^7	*L*-PAM 15 μg/1x10^7	cpm ± SE	% of control	cpm ± SE	% of control	cpm ± SE	% of control
Control of medium alone (no cells added)		59,410 ± 3,645					
No	None	9,534 ± 725		24,556 ± 2,270		37,452 ± 1,813	
Yes	None	759 ± 96	8	1,529 ± 270	6	2,777 ± 1,338	7
Yes	0.15	636 ± 196	7	1,834 ± 90	7	4,806 ± 266	13
Yes	0.5	769 ± 108	8	2,680 ± 272	11	6,780 ± 1,136	18
Yes	1.5	951 ± 59	10	2,653 ± 271	11	6,710 ± 340	18
Yes	3.0	700 ± 177	7	2,194 ± 81	9	7,069 ± 329	19
Yes	5.0	842 ± 63	9	1,652 ± 61	7	5,562 ± 236	15
No	0.15	13,505 ± 1,908	142 [b]	33,123 ± 4,128	135	43,991 ± 2,201	117
No	0.5	8,362 ± 1,866	88	22,779 ± 1,303	93	43,950 ± 1,745	117
No	1.5	18,308 ± 2,300	192	34,135 ± 3,513	139	52,479 ± 1,277	140
No	3.0	16,617 ± 1,768	174	28,697 ± 4,867	117	43,411 ± 4,434	116
No	5.0	17,743 ± 1,812	186	35,618 ± 2,168	145	43,767 ± 2,441	117

[a] See Table 3 for details: mean cpm ± SE in BALB/cX(MC) BALB/c mixture 17,430 ± 1647; the experiment was repeated twice with similar results

[b] Augmentation of thymidine incorporation in cultures supplemented with cells incubated with *L*-PAM alone was found to be not significant by Student's *t*-test with the exception of comparisons between "1/8" and "1/16" mixtures supplemented with untreated cells (24,556 ± 2,270 and 37,452 ± 1,813, respectively) vs cultures supplemented with *L*-PAM treated cells (35,618 ± 2,168; $P < 0.02$ and 52,479 ± 1,277; $P < 0.001$)

tures) slightly increased the allogeneic response, but this increase was found to be insignificant in most cases (Table 4).

Discussion

As shown previously [16], incubation of murine spleen cells with ConA induced the appearance of suppressor T cells. We report here that preincubation of BALB/c spleen cells with various doses of L-PAM partially inhibited the induction of suppressor T cells by ConA. This effect was shown in an experimental model of cell-mediated immune reactivity based on the allogeneic response of BALB/c spleen cells toward C57Bl splenocytes. Inhibition of induction of suppressor cells by preincubation with L-PAM was observed with doses of L-PAM lower than those required for direct inhibition of mitogenic stimulation or the allogeneic response by the drug. Thus, pretreatment with L-PAM inhibited the mitogenic response by ConA and PHA and the allogeneic response of BALB/c spleen cells against C57Bl spleen cells at doses equal or greater than 1.5 μg L-PAM/1 × 10^7 cells, whereas even a minimal dose of 0.15 μg L-PAM/1 × 10^7 cells markedly inhibitied the induction of suppressor cells by ConA. This finding indicates the selectivity of the L-PAM effect on the induction of suppressor cells by ConA. The lack of dose response for the L-PAM effect within the range of 0.15–3.0 μg L-PAM/1 × 10^7 cells may be due to the fact that a quantity of 0.15 μg L-PAM/1 × 10^7 cells is sufficient to eliminate a certain proportion of precursors of suppressor T cells and that a higher quantity of L-PAM (up to 3.0 μg) is unable to remove more precursors of suppressor T cells or other types of cells.

Treatment with various doses of L-PAM ranging from 0.15 μg to 5.0 μg/1 × 10^7 cells after incubation with ConA, did not affect the suppressive activity of ConA-incubated cells. It seems therefore, that L-PAM is effective against precursors of suppressor T cells but has no effect on ConA-induced suppressor cells.

It has been reported [4] that treatment of murine spleen cells with an active derivate in vitro of CY namely 4-hydroperoxycyclophosphamide (4 HPCy) has a selective toxic effect on suppressor T cells. In this work no mention was made of whether the effect of 4 HPCy is exerted on precursors of suppressor T cells or on mature populations of suppressor T cells. As far as we are aware the effect of 4 HPCy and L-PAM on the induction of murine suppressor T cells by ConA has not yet been examined. On the other hand, it has been reported [9, 15] that 4 HPCy selectively affects the induction of human suppressor T cells by ConA and it was suggested that 4 PHCy affects a precursor population of suppressor T cells and not a mature one [9, 15]. We found recently [2] that treatment in vitro with L-PAM has a similar effect on the induction of human suppressor T cells by ConA.

The data presented in this report deal with the effect of L-PAM on suppressor T cell activity in an allogeneic response system. We have found previously [2] that L-PAM has a similar effect on suppressor T cells acting on PHA stimulation of human lymphocytes. However, it is possible that suppressor T cells acting in various systems differ one from another and that their suppressors are not affected in a similar way by L-PAM. This possibility is now under investigation.

It has been reported [14] that incubation with ConA induced interleukin-2 (IL-2) receptor expression on T lymphoblasts. Accordingly, it might be that ConA-incubated cells added to fresh cultures adsorb the IL-2 released and in this way suppress the allogeneic response, and that pretreatment with L-PAM affects the induction of IL-2 recep-

tor expression by ConA. This possibility has yet to be investigated.

The purpose of the present study was to use the system of ConA induction of suppressor T cells by ConA as a tool for investigating the effect of L-PAM on the function of a certain subset of T cells. It has been reported that low dose therapy with either CY [8] or L-PAM [1] was highly effective in the eradication of an established MOPC-315 plasmacytoma tumor in BALB/c mice and that this was due to promotion of the host's antitumor T cell response by the drugs. It was also claimed that elimination of suppressor T cells by drugs may play a role in the determination of the effectiveness of antitumor chemotherapy [6, 11, 12]. Therefore, it seems plausible that determination of the effect of chemotherapeutic drugs on suppressor T cells may lead to a better understanding of the mechanism of their activity as antitumor agents.

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References

1. Ben-Efraim S, Bocian RC, Mokyr MB, Dray S (1983) Increase in the effectiveness of melphalan therapy with progression of MOPC-315 plasmacytomas tumor growth. Cancer Immunol Immunother 15: 101
2. Ben-Efraim S, Komlos L, Notmann J, Hart J, Halbrecht I (1985) In vitro selective effect of melphalan on human T-cell populations. Cancer Immunol Immunother 19: 53
3. Bocian RC, Ben-Efraim S, Dray S, Mokyr MB (1984) Melphalan-mediated potentiation of antitumor responsiveness of immunosuppressed spleen cells from mice bearing a large MOPC-315 tumor. Cancer Immunol Immunother 18: 41
4. Diamanstein T, Klos M, Hahn H, Kaufman SHE (1981) Direct in vitro evidence for different susceptibilities to 4-hydroxyperoxycyclophosphamide of antigen-primed T cells regulating humoral and cell-mediated immune response to sheep erythrocytes: a possible explanation for the inverse action of cyclophosphamide on humoral and cell-mediated immune resonse. J Immunol 126: 1717
5. Dray S, Mokyr MB (1985) Immunomodulation by cyclophosphamide and its effect on eradication of established tumors. In: Fudenberg H, Ambrogy F (eds) Frontiers in Immunomodulation. Plenum Press New York, (in press)
6. Glaser M (1979) Augmentation of specific immune response against syngeneic SV-40-induced sarcoma in mice by depeletion of suppressor T cells with cyclophosphamide. Cell Immunol 48: 339
7. Hengst JCD, Mokyr MB, Dray S (1980) Importance of timing in cyclophosphamide therapy of MOPC-315 tumor-bearing mice. Cancer Res 40: 2135
8. Hengst JCD, Mokyr MB, Dray S (1981) Cooperation between cyclophosphamide tumoricidal activity and host antitumor immunity in the cure of mice bearing large MOPC-315 tumors. Cancer Res 41: 2163
9. Klayman A, Drucker I, Manor J, Ben-Efraim S (1984) In vitro effects of 4-hydroperoxycyclophosphamide on concanavalin A induced human suppressor T cells. Cancer Immunol Immunother 18: 113
10. L'Age-Stehr J, Diamantstein T (1978) Induction of autoreactive T lymphocytes and their suppressor cells by cylophosphamide. Nature (Lond) 271: 663
11. North RJ (1977) Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J Exp Med 55: 1063
12. North RJ (1984) Adoptive immunotherapy. Adv Immunol 35: 89
13. Oppenheim JJ, Rosenstreich DL (1976) Lymphocyte transformation: Utilization of automatic harvesters. In: Bloom BR, David JR (eds) In vitro methods in cell-mediated and tumor immunity. Academic Press New York, San Francisco, London, p. 593
14. Osawa H, Diamantstein T (1984) A rat monoclonal antibody that binds pecifically to mouse T lymphoblasts and inhibits IL-2-receptor functions: A putative anti-IL-2-receptor antibody. J Immunol 132: 2445
15. Ozer H, Cowens JW, Colvin M, Nussbaum-Blumenson A, Sheedy D (1982) In vitro effects of 4-hydroperoxycyclophosphamide on human immunoregulatory T subset function. I. Selective effects on lymphocyte function in T-B cell collaboration. J Exp Med 155: 276
16. Shand FL, Orme IM, Ivanyi J (1980) The induction of suppressor T cells by concanavalin A is independent of cellular proliferation and protein synthesis. Scand J Immunol 12: 223

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