INCREASED REACTIVITY OF MOUSE SPLEEN CELLS SENSITIZED IN VITRO AGAINST SYNGENEIC TUMOR CELLS IN THE PRESENCE OF A THYMIC HUMORAL FACTOR*

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The central role of the thymus and of thymus-derived lymphocytes in cell-mediated immunity has been well established (1). Besides the production by the thymus of lymphocytes endowed with specific immunological properties, there is an increasing body of evidence in favor of the existence of a hormonal mechanism in thymic function. This concept is based on the specific capacity of thymus extracts to partially repair the immunological damage induced by thymectomy (2–4). Moreover, it has been shown that thymic extracts play an essential role in the differentiation processes that lead immature lymphoid elements towards acquisition of immunocompetence (5, 6). Previous observations from this laboratory have suggested that a thymic humoral factor (THF)[1] acts upon young T lymphocytes and transforms them into immunologically reactive cells, when their capacity to induce a graft-vs.-host reaction (GVHR) and to cooperate with B lymphocytes for antibody synthesis was measured (7, 8).

Mouse lymphoid cells sensitized in vitro against allogeneic fibroblasts were shown to mediate specific in vitro cytotoxicity against these cells (9), as well as in vivo rejection of tumors genetically related to the sensitizing fibroblasts used (10). We have recently described a similar in vitro cell-mediated reaction of mouse lymphocytes against syngeneic tumor cells (11). In this particular system, spleen cells of C57BL strain origin were cultured on 3LL Lewis tumor monolayers and tested for cytotoxicity according to the microassay described by Takasugi and Klein (12). It was found that these lymphoid cells exerted, under the experimental conditions used, specific cytotoxicity against 3LL Lewis tumor target cells.

It was, therefore, interesting to investigate to what extent THF participates in the process that leads uncommitted lymphoid cells to the level of cells specifically sensitized against cell targets, by using the in vitro model of cell-

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; EM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; GVHR, graft-vs.-host reaction; THF, thymic humoral factor.

mediated response mentioned above. Since this reaction can be separated into two main phases, namely, sensitization and cytotoxicity, we have tested the effect of THF on each of these phases. In addition, experiments were aimed at characterizing the type of lymphocyte involved in this cell-mediated reaction and defining the cell population activated by THF.

Materials and Methods

Mice.—2-mo old inbred male and female C57BL/6J mice were used as the source of lymphoid cells. In one experiment, mice of the same strain, thymectomized at 1 day of age according to the technique of Miller (13), were used as donors of spleen cells. In a series of experiments involving the use of T or B lymphocytes, the donors were previously thymectomized at the age of 6–8 wk by the technique described by Gross (14).

Irradiation.—Mice were submitted 7 days after adult thymectomy to a total body exposure of 750 R from a ^{60}Co source (gamma beam 150 *A*, Atomic Energy of Canada Ltd., Ottawa, Ontario, Canada) 60 R/min, focal skin distance 34 inches.

Preparation of Lymphoid Cells.—Spleens and thymuses were gently pressed through a fine stainless steel mesh. The cells, suspended in Dulbecco's modified Eagle's medium (EM), were filtered through a metal sieve and washed twice in EM. Bone marrow cell suspensions were obtained from pooled tibiae and femora. The marrow was discharged by forcing a solution of EM through the bone cavity. The cells obtained were separated by passage through a 27 gauge needle, filtered on a metal sieve, and washed twice by centrifugation. All the cell suspensions prepared were submitted to the trypan blue exclusion test for evaluation of viability.

Preparation of Sensitizing Cell Monolayers.—The 3LL Lewis tumor was used as the target for sensitization. This is a lung carcinoma, which arose spontaneously in a C57BL mouse (15). This tumor has been maintained in our laboratory by serial passage in syngeneic C57BL/6J male mice. 2 wk after subcutaneous inoculation, the local tumor was aseptically removed, minced with scissors, and trypsinized for 30 min in a 0.25% solution of trypsin. 5×10^6 3LL tumor cells were resuspended in 4 ml of Waymouth's medium supplemented with 5% calf serum and plated on 60-mm plastic Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). In one experiment, rat BN fibroblasts were prepared as previously described (16) and seeded onto 60-mm Petri dishes in Waymouth's medium, plus 5% calf serum.

Spleen Cell Sensitization.—Tumor cell and fibroblast monolayers were irradiated before sensitization with 8,000 and 2,000 R, respectively. 24 h later, the medium was removed and 30×10^6 viable spleen cells, suspended in 4 ml of EM supplemented with fetal calf serum (FCS) (Armour Pharmaceutical Co., Chicago, Ill.), were poured onto the sensitizing monolayers and incubated at 37°C in humidified air plus 10% CO_2. After 20 h of incubation, the culture medium was changed, and fresh medium added. On the 4th day, the sensitized lymphoid cells were removed by gently pipetting, washed twice in EM, and counted. From 10 to 20% of the initial spleen cell inoculum was usually recovered, 60 to 80% of which was viable according to the trypan blue exclusion test.

In Vitro Assay of Cytoxicity.—The technique described by Takasugi and Klein (12), with some modifications, has been presently used. 1,000–2,000 trypsinized 3LL tumor cells from a freshly explanted tumor were pipetted with a 10 μl microdispenser (Oxford Laboratories, Foster City, Calif.) into the wells of microplates (Falcon microtest tissue culture plate 3034). 24 h later, approximately 10% of the tumor cells attached to the plastic bottom of the wells and appeared as typical flattened spindle-shaped cells. The medium was removed from each well by suction, and spleen cells were dispensed into the holes of the microplate and incubated for 20–40 h. The number of surviving target cells was recorded in the following way: the wells were flooded with Waymouth's medium plus 5% calf serum, and the plates were

tilted in order to remove the nonadherent cells. The excess of medium was then poured out and the microplates were examined under an inverted phase-contrast microscope. Only flattened spindle-shaped target cells were recorded as viable cells, since it was found previously that the majority of the rounded tumor cells stains positively with trypan blue and eosin Y (11). The accuracy of the assay was increased by precounting the number of tumor spindle cells before the addition of spleen cells. The number of surviving tumor cells divided by the precount of the same well gave a ratio that reduced the effect of varied tumor cell plating on the experimental data. A cytotoxicity index was calculated as follows:

$$\frac{\text{no. of target cells in control group} - \text{no. of target cells in test group}}{\text{no. of target cells in control group}} \times 100.$$

Statistical significance between groups representing the mean of the number of cells counted in 6–10 wells was determined by Student's t test.

Titration of Cells Bearing θ-Antigen and Lymphocyte Activation by Concanavalin A (Con A).—Anti-θ serum was obtained from AKR/J mice hyperimmunized with C3H/eb thymocytes. The reaction was performed in an excess of antiserum plus complement and read with the addition of trypan blue. Con A stimulation was performed as follows: 5×10^6 spleen cells in 1 ml of EM plus 10% FCS were incubated for 72 h in the presence of 2 μg of Con A (Miles-Yeda, Rehovot, Israel). 2 h before the end of the reaction, 2 μCi of [^{3}H]thymidine (Amersham, England) were added to the cultures, which were gently shaken in a 37°C water bath. The cells were then poured onto fiberglass filters (Wathman, England) and rinsed successively with saline, a cold solution of 5% TCA, and absolute ethanol. The filters were dried and counted in 2,5-diphenyloxazole (PPO)–1,4-bis[2-(4-methyl-5-phenyloxazolyl]benzene (POPOP) toluene. Each experimental result is the average of four different cultures.

Preparation of THF.—Extracts from calf thymus were prepared and dialyzed according to the procedure previously described (17). The preparations used were diluted at a standard concentration of 1 mg of protein per ml. Extracts of calf lymph node and calf spleen were prepared by similar procedures and were used at the same protein concentration.

RESULTS

Effect of THF on the Two Phases of In Vitro Sensitization of Lymphoid Cells against Syngeneic Tumor Cells.—In the first experiments we tested the effect of THF on in vitro sensitization of C57BL spleen cells against 3LL syngeneic tumor cells. For this purpose spleen cells were exposed to tumor cell monolayers for a 4 day period. These lymphoid cells were then recovered, washed by repeated centrifugation, and dispensed at a concentration of 2,000 or 5,000 living cells per 10 μl into the wells of the standard microplates used. The wells were seeded 24 h earlier with 3LL tumor cells, and by the time of their exposure to lymphocytes, contained from 50 to 100 target cells, according to the different experiments performed. 24 h after exposure, the number of tumor cells remaining in each well was recorded, and the cytotoxicity index calculated on the basis of the respective number of target cells counted in the experimental group compared with that in the control group exposed to unsensitized spleen cells.

THF was added at a 2% concentration to the culture medium, since this has been found adequate to restore the immunological competence of lymphoid cells in previous experiments when the in vitro GVHR was measured (18).

Each standard experiment included spleen cells both sensitized and assayed in the presence of THF, spleen cells sensitized only or assayed only in the presence of THF, and spleen cells sensitized and assayed in the absence of THF. Table I summarizes the results of four consecutive experiments. It can be seen that: (*a*) THF present during both phases of the reaction increased the cytotoxicity mediated by sensitized lymphoid cells; (*b*) similarly, THF, added during the sensitization phase only, promoted further cytotoxicity of the spleen cells; (*c*) no increased cytotoxicity was detected when THF was added during the effector phase only; and (*d*) THF was more effective when present during both phases of the reaction than when added during sensitization only.

TABLE I

Effect of THF on the Two Phases of In Vitro Sensitization of C57BL Spleen Cells against 3LL Syngeneic Tumor Cells

Exp.	no. of C57BL spleen cells	Sensitizing cells	THF during sensitization	THF during effector phase	Mean ± SE of 3LL target cells*	Cytotoxicity of 3LL target cells	P‡
						%	
1	5,000	3LL	+	+	7.3 ± 1.3	73	+++
	5,000	"	+	−	15.3 ± 2.6	43	+
	5,000	"	−	+	20.7 ± 5.5	22	NS
	5,000	"	−	−	25.3 ± 5.1	5	
2	2,000	3LL	+	+	135.0 ± 20.4	42	++
	2,000	"	+	−	190.1 ± 13.7	18	+
	2,000	"	−	−	239.6 ± 15.8	−3	
3	5,000	3LL	+	+	83.1 ± 5.1	56	++
	5,000	"	+	−	94.6 ± 4.0	50	+
	5,000	"	−	+	98.2 ± 4.8	48	NS
	5,000	"	−	−	107.7 ± 5.9	43	
4	5,000	3LL	+	−	37.0 ± 3.2	24	+
	5,000	"	−	+	48.1 ± 4.9	1	NS
	5,000	"	−	−	47.1 ± 4.8	3	

+, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$; NS, $P > 0.05$.

* SE, standard error of the mean number of surviving 3LL target cells.

‡ Probability computed by Student's *t* test compared with percent cytotoxicity of spleen cells sensitized and assayed without THF.

It seems, therefore, that THF acts more predominantly upon the steps leading to production of specifically sensitized lymphocytes rather than on the expression of cytotoxicity itself. Moreover, as can be seen from exps. 1, 2, and 4 (Table I), THF discloses more striking effects when added to cell cultures relatively poorly reactive by themselves, than when added to cell cultures with a high cytotoxicity index (exp. 3, Table I). This suggests the possible

existence of a ceiling level for in vitro reactivity of normal spleen cells, which once reached, cannot be exceeded by further addition of THF.

Finally, it is interesting to mention that the presence of THF does not modify the viability and number of lymphoid cells maintained in culture nor does it increase the percentage of large blastoid cells found (Table II). It can be seen that the number of viable lymphoid cells and the percentage of large blastoid cells recovered per plate remained unaffected, whether or not THF had been added to the cultures.

Lack of Effect of THF on Unsensitized Lymphocytes or on Lymphocytes Sensitized against Transplantation Antigens Unrelated to Those of the Target Cells.—In the light of the above results it was critical to test whether THF induces or increases cell killing when added to lymphoid cells not previously sensitized against 3LL tumor target cells. As shown in Table III, exps. 1, 2, and 3, the

TABLE II

Number of Viable Lymphoid Cells and Percentage of Large Blastoid Cells Recovered after Sensitization with THF

Exp.	Sensitizing cells	THF during sensitization	no. of live lymphoid cells recovered per plate	Percentage of large blastoid cells
				%
1	3LL	+	3.8×10^6	20
	"	−	3.4×10^6	25
2	3LL	+	4.2×10^6	16
	"	−	5.2×10^6	14
3	3LL	+	2.5×10^6	26
	"	−	3.1×10^6	28

addition of 2% THF to unsensitized spleen cells did not modify the number of target cells counted in the respective wells. In exp. 4, Table III, C57BL spleen cells were sensitized either against 3LL tumor cells or against rat fibroblasts and assayed for cytotoxicity against 3LL target cells. It can be seen that the selective addition of THF in the reaction against xenogeneic antigens did not affect the nonspecific cytotoxicity, which is usually found under these conditions of sensitization (11).

Specificity of the Effect Mediated by THF.—It was important, at this stage of the investigation, to test whether the increased cytotoxicity mediated by in vitro sensitized lymphocytes in the presence of THF was or was not exclusive to thymic extracts. With this aim in view, spleen and lymph node extracts of calf origin were prepared by a procedure similar to that described for THF and tested in parallel with THF. As seen in Table IV, in contrast to thymus extracts, neither spleen extract tested on lymphoid cells from normal mice (exps. 1 and 2), nor lymph node extract added to spleen cells from neo-

natally thymectomized donors (Table IV, exp. 3), had any significant effect on the in vitro cell-mediated reaction. These results clearly indicate that THF activity upon in vitro sensitization of lymphoid cells is organ specific.

Identification of the Lymphoid Cell Population Activated by THF.—The experimental conditions described above did not provide information about the type of lymphocyte population responsible for in vitro sensitization and cytotoxicity, and, therefore, did not permit identification of the cell specifically activated by THF in this reaction. Accordingly, instead of normal spleen cells,

TABLE III

Lack of Effect of THF on Unsensitized Spleen Cells and on Spleen Cells Sensitized against Rat BN Fibroblasts

Exp.	no. of C57BL spleen cells	Sensitizing cells	THF during sensitization	THF during effector phase	Mean ± SE of 3LL target cells	Cytotoxicity of 3LL target cells	P*
						%	
1	2,000	None	−	+	232.0 ± 14.3		
	2,000	"	−	−	232.5 ± 20.4		
2	5,000	None	−	+	20.5 ± 2.0		
	5,000	"	−	−	20.4 ± 3.4		
3	5,000	None	−	+	46.8 ± 5.1		
	5,000	"	−	−	48.6 ± 4.2		
4	5,000	3LL	−	−	107.7 ± 5.9	43	++
	5,000	Rat BN	+	+	137.5 ± 8.0	27	NS
	5,000	" "	+	−	139.8 ± 15.2	26	NS
	5,000	" "	−	+	135.7 ± 14.8	28	NS
	5,000	" "	−	−	138.3 ± 9.5	27	

++, $P < 0.01$; NS, $P > 0.05$.

* Probability computed by Student's t test compared with percent cytotoxicity of spleen cells sensitized against rat BN fibroblasts on the absence of THF.

which contain a mixture of T and B lymphocytes, we used more defined populations—either T or B cells produced according to accepted procedures (19). For this purpose, adult thymectomized, lethally irradiated C57BL mice were injected with either syngeneic bone marrow cells or syngeneic thymocytes. 8–10 days later, spleen cells from these animals were recovered and tested for in vitro sensitization. The homogeneity of these lymphoid cell populations was previously checked by their sensitivity or resistance to anti-θ serum and by their reactivity to Con A stimulation. As seen in Table V, 91% of the spleen cells recovered from animals restored with thymocytes were θ-positive and showed normal reactivity to Con A. On the other hand, spleen cells from mice reconstituted with bone marrow cells disclosed a residual T-cell presence of only 4% and did not react to Con A. These respective T and B cells were then

TABLE IV

Effect of Thymus (THF), Spleen (SE), and Lymph Node (LNE) Extracts on In Vitro Sensitization

Exp.	no. of C57BL spleen cells	Sensitizing monolayer	Sensitization phase	Effector phase	Mean ± SE of 3LL target cells	Cytotoxicity of 3LL target cells	P*
						%	
1	5,000	3LL	THF	THF	27.7 ± 2.7	62	++
	5,000	"	SE	SE	37.3 ± 3.3	49	NS
	5,000	"	—	—	40.0 ± 2.5	46	
	5,000	None	—	THF	69.0 ± 5.8	7	+++
	5,000	"	—	SE	63.3 ± 7.7	14	
	5,000	"	—	—	73.8 ± 7.0	0	
2	5,000	3LL	THF	THF	19.8 ± 2.0	38	++
	5,000	"	SE	SE	40.2 ± 10.4	−27	NS
	5,000	"	THF	—	24.8 ± 3.3	22	+
	5,000	"	SE	—	35.2 ± 3.7	−10	NS
	5,000	"	—	—	33.1 ± 3.8	−3	
3‡	5,000	3LL	THF	THF	7.2 ± 4.4	82	++
	5,000	"	LNE	LNE	26.6 ± 2.8	35	NS
	5,000	"	—	—	24.1 ± 3.6	34	

+, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$; NS, $P > 0.05$.

* Probability computed by Student's t test compared with percent cytotoxicity of spleen cells sensitized and assayed without extract.

‡ Experiment performed with spleen cells from neonatally thymectomized C57BL mice.

TABLE V

Sensitivity of Spleen Cells from Animals Restored with Thymocytes or Bone Marrow Cells to Anti-θ Serum and Con A

Spleen cells*	Percentage of θ-positive cells	cpm without Con A	cpm with Con A
	%		
T	91	1,478 ± 96	50,578 ± 5,801
B	4	901 ± 157	2,822 ± 423

* Spleen cells recovered from adult thymectomized, lethally irradiated (750 R) mice restored with either 50×10^6 thymocytes (T) or 30×10^6 bone marrow cells (B).

cultured separately on 3LL tumor monolayers and assayed for cytotoxicity. The results shown in Table VI indicate that while T cells were responsible for in vitro sensitization and cytotoxicity, B cells were totally inactive, at both concentrations of lymphoid cells tested. Another indication suggesting T-cell involvement in this reaction was that by the end of sensitization, 33% of the T lymphoid cells tested were transformed into large blastoid cells, whereas an increase of only 4% was found when B cells were tested.

TABLE VI

Reactivity of T and B Spleen Cells Sensitized In Vitro against 3LL Tumor Cells

Spleen cells*	Sensitizing cells	Large blastoid cells	2×10^3 spleen cells/well		10^4 spleen cells/well	
			Mean ± SE 3LL target cells	Cytotoxicity‡	Mean ± SE 3LL target cells	Cytotoxicity‡
		%		%		%
T	3LL	37	87.3 ± 10.5	22 +	28.0 ± 3.5	49 ++
T	None	4	112.3 ± 8.5		55.1 ± 4.7	
B	3LL	14	125.8 ± 10.4	−6 NS	47.9 ± 2.8	10 NS
B	None	10	118.8 ± 14.2		53.5 ± 5.5	

++, $P < 0.01$; +, $P < 0.05$; NS, $P > 0.05$.

* See footnote Table V.

‡ Probability computed by Student's t test compared with percent cytotoxicity of unsensitized spleen cells.

The next step in this investigation consisted of testing THF activity directly, either on T or on B cells sensitized in vitro. As seen in Table VII, the addition of THF to T cells or to thymocytes (exps. 1 and 2, respectively) resulted in a significant increase in the reactivity of these cells against 3LL tumor targets. In contrast to these findings, the lack of B-cell reactivity remained unmodified in the presence of THF.

In conclusion, these experiments clearly indicate that (*a*) T cells are the responsible instrument of sensitization in vitro, and (*b*) THF can directly potentiate the reactivity of these cells, when measured by their degree of cytotoxicity.

DISCUSSION

The use of a complete in vitro model of cell-mediated immunity provides an adequate tool to study the processes by which unprimed lymphocytes become sensitized and give rise to a population of effector cells specifically committed. We had previously shown that contact between C57BL spleen cells and 3LL tumor cell monolayers generated specifically sensitized lymphocytes, able to mediate cytotoxicity against target cells identical with the sensitizing cells (11). This model was, therefore, considered adequate to study the influence of THF on lymphocytes involved in cell-mediated reactions. In the present experiments, spleen cells sensitized and assayed for cytotoxicity in culture medium supplemented with THF were found to be significantly more cytotoxic than spleen cells cultured in normal medium. Moreover, when THF was selectively added, either during the phase of generation of sensitized lymphocytes, or during the effector phase, it was observed that THF exerts its activity during the sensitization phase only. Still, in almost every experiment performed, the cytotoxicity index of spleen cells cultured with THF during both phases of the reaction was higher than that of spleen cells activated by THF during the

TABLE VII

Effect of THF on T and B Lymphoid Cells or on Thymocytes Sensitized In Vitro against 3LL Syngeneic Tumor Cells

Exp.	Lymphoid cells	Sensitizing cells	THF during sensitization	THF during effector phase	no. of lymphoid cells/well	Mean ± SE 3LL target cells	Cytotoxicity of 3LL target cells	*P*
							%	
1	T*	3LL	+	−	5,000	218 ± 23	51	+++
	T	"	−	−	5,000	300 ± 19	33	+++
	T	None	−	−	5,000	449 ± 24	0	
	B*	3LL	+	−	5,000	359 ± 47	−28	NS
	B	"	−	−	5,000	318 ± 60	−14	NS
	B	None	−	−	5,000	279 ± 45		
2	Thymocytes	3LL	+	+	15,000	125.9 ± 10.8	42	+
	"	"	−	−	15,000	180.5 ± 23.7	16	NS
	"	None	−	+	15,000	243.1 ± 37.9	−13	
	"	"	−	−	15,000	215.4 ± 31.7	0	
	B‡	3LL	+	+	15,000	151.5 ± 12.0	−24	
	B	"	−	−	15,000	131.7 ± 11.3	−8	
	B	None	−	+	15,000	132.3 ± 6.1	−9	
	B	None	−	−	15,000	121.8 ± 6.8	0	

* Spleen cells recovered from adult thymectomized, lethally irradiated (750 R) mice restored with either 50×10^6 thymocytes or 30×10^6 bone marrow cells.

‡ Spleen cells recovered from adult thymectomized, lethally irradiated (950 R) mice restored with 30×10^6 bone marrow cells.

sensitization phase only. A possible explanation for this finding is that by the end of the 4 day culture period with THF (sensitization phase), there were still lymphocytes in the process of sensitization, requiring the further presence of THF in order to become effector cells for cytotoxicity. Conversely, in the absence of THF, the process of sensitization is probably completed by the end of the 4 day culture period, and the number of effector cells already determined. Thus, the addition of THF during the cytotoxicity assay only may occur too late or be too short in time to affect the outcome of the test.

We also found that THF specifically increases cytotoxicity against target cells bearing transplantation antigens identical with those of the sensitizing cells. Indeed, when THF was added to spleen cells sensitized against nonrelated antigens, or to unsensitized spleen cells, no increased cytotoxicity was found. This indicates that THF does not increase indiscriminate killing by sensitized lymphocytes, nor does it trigger an unprimed spleen cell population to potential cytotoxic capacity. As controls to thymic extracts, we have tested several extracts from calf lymphoid organs prepared by a procedure similar to that used for THF. No activity was detected in spleen or lymph node extracts, suggesting

that the active compound is confined to, or at least present at an active concentration in, the thymus only. Since all the experiments described were carried out with culture medium supplemented with 15% fetal calf serum, the possibility that THF acts as a mere xenogeneic stimulus seems rather improbable. A THF-like activity has been detected in the serum of some mammals (20) and in particular in calf serum (21). It is therefore possible that the differences in activity between serum batches vis-à-vis in vitro stimulation of lymphocytes depends upon the amount of thymic factor present in the batches.

Cell-mediated responses have been found to depend mainly on T-lymphocyte function. However, a recent report indicates that B cells may also participate in such reactions, involving in particular the rejection of syngeneic tumor cells (22). It was therefore of interest to investigate which is the type of lymphocyte engaged in sensitization and cytotoxicity in the present system in which tumor cells were challenged with syngeneic spleen cells. When adult thymectomized, lethally irradiated mice were restored with either bone marrow cells or thymocytes, we observed that the spleen cells from T-restored animals were active against the tumor cells, while spleen cells from B animals did not mediate significant cytotoxicity. These findings are in accordance with those obtained by other investigators using similar or slightly different systems of in vitro sensitization against fibroblasts (9) or lymphocytes of allogeneic origin (23).

Although the role of a thymic humoral component in the chain of events leading to immunologically competent T lymphocytes has been well established, the precise stage at which this humoral factor is active is still unclear. Several reports suggest that thymus hormone would act upon prethymic elements still devoid of thymus antigenic markers (24, 25). On the other hand, previous observations from this laboratory indicate that THF activates a postthymic cell population (7, 26). The present effect of THF upon in vitro sensitization could be explained either by activation of non-T precursor cells present in the normal spleen and their transformation into active T lymphocytes, or by direct interaction with T cells themselves, which leads to an increase in their immunological competence. Our present results indicate that the effect of THF is exerted on T cells rather than on B cells, since T-restored spleen cells were found to be more cytotoxic when sensitized in the presence of THF. This was further supported by the finding that thymocytes, which are poorly reactive in themselves, became as cytotoxic as mature T cells when tested in the presence of THF. Still, the possibility of an increase in newly differentiated T cells out of the B spleen cell population, too small to be detected under the present experimental conditions, cannot be totally discarded.

All the results presently reported have been obtained by the use of in vitro procedures. More information using in vivo models is required in order to understand the general significance of the role of THF in cell-mediated immunity.

SUMMARY

Unprimed mouse spleen cells cultured in vitro on syngeneic tumor cell monolayers have been previously shown to become specifically sensitized and to mediate cytotoxicity against the same type of tumor cells. This complete in vitro system of cell-mediated response has been presently used to test the effect of a thymic humoral factor (THF) upon the differentiation process leading to the generation of specifically committed lymphocytes. Culture media were supplemented with 2% THF during either the sensitization or effector phase, or both phases of the reaction. Whereas the addition of THF during both phases or during sensitization only resulted in a significant increase in the cytotoxicity index, THF added during the effector phase was ineffective. The behavior of unsensitized spleen cells and of spleen cells sensitized against nonrelated transplantation antigens remained unmodified by THF. After showing that the entire reaction is mediated by lymphocytes of thymic origin, THF was directly tested on T or B spleen cells. It was found that only T cells reacted to THF by an increased cytotoxic capacity, while B cells remained inactive after addition of THF. It was therefore concluded that THF activates a postthymic population of lymphoid cells, transforming them into fully competent lymphocytes.

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