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Adoptive immunotherapy of a BALB/c lymphoma by syngeneic anti-DBA/2 immune lymphoid cells: Characterization of the effector population and evidence for the role of the host's non-T cells

Mario P. Colombo, Mariella Parenza, and Giorgio Parmiani

Division of Experimental Oncology D, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1 I-20133 Milan, Italy

Summary. It has been previously shown that the BALB/c lymphoma YC8 is susceptible to lysis by syngeneic anti-DBA/2 lymphocytes and that YC8-bearing BALB/c mice can be cured by adoptive transfer of such immune effectors. In this study in vivo and in vitro functions of the curative immune lymphocytes have been evaluated together with the role of the host immune system in the mechanism of tumor eradication. It was found that the curative anti-DBA/2 lymphocytes were not directly cytotoxic to YC8 cells although they developed into YC8-lytic cells after in vitro restimulation with YC8. In vivo, the immune lymphocytes were able to mediate a tumor-specific delayed type hypersensitivity reaction against YC8 but had a low tumor-neutralizing activity in the Winn assay. Proliferation of infused BALB/c anti-DBA/2 lymphocytes was necessary for the in vivo therapeutic effect, since irradiation of effector cells or treatment of the donor immune lymphocytes with vinblastine abolished their curative capacity. Immunodepression of the T cell compartment of the prospective tumor-bearing animals by thymectomy plus irradiation or its abrogation in B mice (thymectomized, lethally irradiated, and reconstituted with fetal liver cells) did not interfere with the therapeutic effect of the transferred anti-DBA/2 lymphocytes. Blocking the macrophage functions of the host by carrageenan, however, abolished the therapeutic effect of immune lymphocytes. These data indicate that a radiation-resistant, non-T cell is involved in the tumor eradication induced by anti-DBA/2 lymphocytes. It was also shown that cured mice, tested 90 days after therapy, become resistant to 5×10^3 LD_{80} YC8 cells and that this resistance was due to the presence of memory cells derived from the transferred and not from the host lymphocyte population.

Introduction

Immune response against tumors has been studied mostly with chemically or viral-induced antigenic tumors. However, since spontaneous human and rodent cancers are often not detectably immunogenic [15], one may argue whether findings obtained with immunogenic experimental neoplasms are biologically and clinically relevant. Thus, immunologists tried to bypass this problem by several procedures. One is that of mutagenizing nonantigenic, spontaneous tumors that, by this treatment, become immunogenic and able to induce a cytotoxic T lymphocyte response which also destroys the parental, apparently nonantigenic cell line [3]. Another possibility for obtaining tumor cytotoxic lymphocytes, though less specific, is that offered by alloimmunization or lectin activation of syngeneic or autologous lymphocytes. In fact, allostimulated lymphocytes can kill syngeneic murine [17, 18, 21, 24] and autologous human tumors [1, 10, 14, 28], independently from their antigenic features. Thus, availability of efficient antitumor effectors allowed a successful adoptive immunotherapy of weakly or nonimmunogenic tumors by alloimmunized lymphocytes [5], lectin-activated lymphocytes [19, 20] and lymphocytes activated against highly immunogenic mutant sublines [6].

We have recently described an experimental model of passive adoptive immunotherapy in which BALB/c mice bearing a syngeneic lymphoma (designated YC8) growing either in the peritoneal cavity (ascitic form) or in the liver, can be cured by transferring BALB/c anti-DBA/2 lymphocytes [5]. In fact, although this tumor has a low immunogenicity, it expresses antigens cross-reacting with those of the DBA/2 strain [22, 24]. The therapeutic effect was obtained when immune lymphocytes were injected both at the site of tumor growth (i.e., i.p.) and i.v., indicating that such lymphocytes can either recirculate and reach tumor cells seeded at distant organs or induce a host-mediated reaction which would ultimately kill the neoplastic cells. It was also shown that, as in several other experimental systems [9, 11, 13], the main lymphocyte subset involved in the curative effect was the Thy 1.2+, Lyt 1+, as detected by negative selection with monoclonal antibodies and complement [5]. At variance with other reports [4], however, it was not necessary to eliminate suppressor cells by cyclophosphamide or irradiation [2, 9, 13] in order to obtain the curative effect.

To gain more information about the mechanisms of YC8 eradication by anti-DBA/2 lymphocytes, we have further evaluated (a) the effector cell population by studying its in vitro and in vivo functional activities, and (b) the possible role of the host by manipulating its immune system during immunotherapy of the growing lymphoma.

Material and methods

Mice. Adult BALB/c/LacDp (*H-2^d*), DBA/2J/Dp (*H-2^d*) mice were obtained from our animal colony and used

Offprint requests to: M. P. Colombo

when 6–8 weeks old. All mice were kept under standard conditions and fed with standardized pellets (Charles River, Calco, CO, Italy).

Tumors. The BALB/c lymphomas YC8 and LSTRA were originally induced by Moloney virus and passed weekly by i.p. injections of 10^6 cells in our BALB/c mice; tumor cells were frozen at different passages and thawed when necessary. YC8 has been characterized from the genetic and antigenic point of view [22], as a BALB/c lymphoma expressing class I and minor histocompatibility but not class II antigens of the *H-2*^{d} haplotype, Thy 1.2, Lyt 2.2, Lyt 5.1, and gp70 antigens. The RL♂1/SK is a cloned line of X-ray-induced BALB/c lymphoma obtained from Dr. E. Fleissner (Sloan-Kettering Cancer Center, New York, NY, USA) and transplanted as ascites in our BALB/c mice. The P815 is a DBA/2 mastocytoma serially transplanted i.p. in our DBA/2 mice.

Preparation of immune lymphocytes. BALB/c female mice of 6–8 weeks of age were injected i.p. three times at weekly intervals with $10-30 \times 10^6$ DBA/2 lymphoid cells obtained from the spleen and s.c. lymph nodes. Then 7 days later the mice were sacrified and axillary, inguinal, mesenteric lymph nodes, and spleen were removed, perfused with Hank's balanced salt solution (HBSS) without heterologous serum, minced and teased to provide lymphoid cell suspensions. Lymphoid cells were similarly obtained from nonimmunized, age-matched, control animals.

Cytotoxic assay. Cytotoxicity was determined by 4 h ^{51}Cr release assay. Labeled target cells (10^4) were admixed with different numbers of effector cells in triplicate (peritoneal exudate cells and/or splenocytes plus lymph node cells) in a volume of 0.2 ml of RPMI-1640 plus 9% fetal bovine serum and incubated for 4 h. Supernatants were collected and the isotope release determined in a gamma counter. The percentage specific ^{51}Cr release was calculated as follows:

$$\% \text{ specific } ^{51}\text{Cr release} = \frac{\text{Experimental release} - \text{control release}}{\text{Maximum release} - \text{control release}} \times 100.$$

The spontaneous release of the various targets was in the range of 10% to 20% of the maximum release obtained by the incubation of labeled cells in distilled water with 1% NP-40.

Winn assay. Alloimmmune, control nonimmune spleen and lymph node cells and lymphoma cells were suspended in HBSS without heterologous serum. Tumor cells (10^3/mouse) were admixed at various ratios with lymphocytes as indicated in the text. A total volume of 0.4 ml and 0.2 ml/mouse was injected i.p. and s.c. respectively. Mice were then checked twice weekly until death or for at least 3 months.

Delayed type hypersensitivity (DTH) assay. BALB/c anti-DBA/2 lymphocytes (8×10^6) were admixed with mitomycin C (Kyowa Hakko Kogyo, Tokyo, Japan) – treated YC8 or LSTRA lymphoma cells (4×10^7) and injected in a volume of 30 µl of HBSS in the right hind footpad. Footpad swelling was measured 24 h later using a spring loaded micrometer (Schnelltaster, Jena, DDR) and specific swelling was evaluated by comparing the mean difference between foot pads before and after injection. Six mice per group were used.

Thymectomy and B mice. Thymectomy was carried out by suction after midsternal incision. B mice were obtained by lethal irradiation (850 R) of thymectomized animals and reconstituted with i.v. injection of 2×10^7 syngeneic fetal (14-day-old) liver cells.

Pharmacological treatment. Each BALB/c anti-DBA/2 immune mouse received 200 µg of vinblastine sulfate (Velban, Eli Lilly Labs., Indianapolis, Ind., USA) i.v. 15 h before harvesting lymph node and spleen lymphoid cells to be used in adoptive transfer.

Carrageenan (Type V, Iota Carrageenan, Sigma Co., St. Louis, Mo. USA) was dissolved saline by sonication and then given i.p. at the dose of 50 mg/kg per mouse for three times, starting 8 h before each of the three adoptive transfers of lymphoid cells.

Results

In vitro and in vivo functional activities of therapeutic lymphocytes

To see whether BALB/c anti-DBA/2 lymphoid cells can proliferate and develop into tumor cytotoxic cells when they encounter the YC8 tumor, aliquots of immune effectors used in the immunotherapy experiments were tested in vitro. As shown in Table 1, BALB/c anti-DBA/2 lympho-

Table 1. In vitro restimulation of BALB/c anti-DBA/2 lymphocytes by YC8 cells [a]

Effector cells	E:T ratio [b]	% ^{51}Cr specific release on the following targets		
		YC8	P815	RL♂ 1/SK
BALB/c anti-DBA/2 restimulated in vitro	50:1	55.4	74.0	6.8
	25:1	49.9	72.8	3.5
	12:1	43.5	61.0	1.2
BALB/c anti-DBA/2 not restimulated	50:1	3.4	1.7	4.5
	25:1	2.8	0.8	1.9
	12:1	2.5	0	1.4

[a] Restimulation was carried out at the effector : stimulator ratio of 5:1 for 5 days in vitro with mitomycin C-treated lymphoma cells

[b] Effector : target ratio

Table 2. Specific DTH reaction of BALB/c anti-DBA/2 lymphocytes [a]

Sensitizing cells [b]	Footpad swelling
	ΔL, mm ± SE [c]
YC8	1.01 ± 0.02 [d]
LSTRA	0.31 ± 0.05

[a] 8 × 10^6 lymphocytes
[b] 4 × 10^7 mitomycin C-treated tumor cells
[c] Swelling was evaluated 24 h after injection. ΔL is the difference between the thickness of the footpad (in mm) before and after injection with the mixture of lymphocytes and tumor cells
[d] $P < 0.01$

cytes were not directly cytotoxic against YC8 cells at the time of their infusion into tumor-bearing mice. After in vitro restimulation with YC8 cells, however, immune lymphocytes developed a significant lytic activity against both YC8 and the DBA/2 target P815 but not against the BALB/c lymphoma RL♂1 (Table 1). Aliquots of the same effector cells did not proliferate or produce detectable amounts of interleukin-2 (IL-2) or interferon gamma (IFN-γ) when kept in culture with YC8 cells, whereas they proliferated after re-exposure to DBA/2 normal and neoplastic cells (data not shown, and [5]).

The immune function of the BALB/c anti-DBA/2 effectors was then evaluated by two different in vivo assays, namely foot pad swelling and Winn neutralization. The first assay is known to detect a reaction which is mediated by Lyt 1+ helper subset [16] whereas the Winn assay is used to evaluate the direct, tumor-neutralizing activity of the immune cells and is considered to reflect the activity of Lyt 2+ cytotoxic cells [25]. Table 2 indicates that BALB/c anti-DBA/2 lymphoid cells specifically induced a DTH effect in syngeneic animals when mixed with YC8 cells but not when mixed with a different BALB/c lymphoma LSTRA. No DTH reaction was induced by non immune BALB/c lymphocytes admixed with YC8 cells (data not shown). When used in the Winn assay, the immune anti-DBA/2 lymphocytes had a relatively low tumor-neutralizing activity as shown in Table 3. In fact, although mean survival time (MST) was significantly longer in mice injected with tumor plus immune lymphocytes, only a few mice survived either s.c. or i.p. injections even at the high effector: tumor cell ratio of 500:1. These data suggest that the lymphoid population harvested from in vivo primed mice contains at least two subpopulations of lymphocytes with different functional activity. The first one includes inactive or undetectable cytotoxic T cells (CTL) or CTL precursors that can differentiate, after in vitro restimulation with tumor cells, into active tumor cytotoxic effectors without the need to proliferate. The other subpopulation is that of activated helper or inducer cells revealed by the positive DTH activity against YC8. The low tumor neutralizing activity of anti-DBA/2 immune lymphocytes can be explained by the low content of cytotoxic cells. In fact the same effector cell population that became cytotoxic against YC8 after restimulation gave complete protection in the Winn assay against YC8 cell growth (Colombo, unpublished data).

Table 3. Neutralization activity of BALB/c anti-DBA/2 lymphocytes in the Winn assay

Effector lymphocytes	E:T ratio [a]	No. of mice with tumor/No. of mice injected and MST ± SD Route of injection			
		s.c.		i.p.	
BALB/c anti-DBA/2	100:1	17/20	36 ± 15	10/20	24 ± 6
	500:1	12/20	42 ± 10 [b]	15/20	43 ± 11 [c]
BALB/c	500:1	13/15	24 ± 7	15/15	18 ± 5

[a] Effector:target ratio
[b] $P < 0.05$ calculated by the Gehan test
[c] $P < 0.01$ calculated by the Gehan test

Table 4. In vivo proliferation of immune lymphocytes is necessary for eradicating tumor cells

Lymphocytes injected [a]	Treatment of curative lymphocytes before injection	No. of mice with tumor [b]/ No. of mice treated	MST ± SD
BALB/c anti-DBA/2	None	0/12	–
	800 R [c]	12/12	16.6 ± 3
	2000 R	12/12	16.4 ± 4
	Vinblastine [d]	5/8	33.0 ± 3.5
BALB/c anti-BALB/c	None	12/12	17.3 ± 5

[a] Mice were injected i.v. with 10^3 YC8 cells and 3, 5, and 7 days later given 30 × 10^6 immune or control lymphocytes
[b] Mice invariably died with liver and kidney metastases with the indicated MST ± SD.
[c] Irradiation of lymphocytes was 2 h before injection
[d] Vinblastine (200 μg/mouse) was injected 15 h before killing immune lymphocyte donors

In vivo proliferation of effector cells is required for therapeutic activity

Although in vitro studies indicate that anti-DBA/2 lymphocytes may develop into tumor cytotoxic cells without or with a weak proliferation, we do not know whether the same phenomenon occurs in the host. Thus, we tested whether the proliferative capacity is necessary for the curative effect to take place in mice infused with alloimmune lymphoid cells. Therapy was carried out as previously described [5] by i.v. injection of 30×10^6 BALB/c anti-DBA/2 lymphocytes at days 3, 5, and 7 after injection of 10^3 YC8 cells i.v. Table 4 shows that when the therapeutic lymphocytes were blocked by irradiation 2 h before infusion into tumor-bearing mice or by treating the donors with vinblastine, a drug which inhibits cell division, the therapeutic effect was largely inhibited.

This finding indicates that infused lymphocytes need to proliferate in order to cure the tumor-bearing animals.

Role of the host's immune system in the eradication of YC8 lymphoma

To investigate whether the immune T cell compartment of the host is involved in the elimination of tumor cells triggered by the transferred lymphocytes, the recipient mice were either thymectomized and sublethally irradiated with 500 R, thymectomized and lethally irradiated with 850 R and reconstituted with 14-day-old fetal liver cells (B mice) or irradiated only. After i.v. injection of YC8 tumor cells, the mice were treated with BALB/c anti-DBA/2 lymphoid cells as usual. Table 5 shows that none of the immunodepressive manipulations interfered with the therapeutic effect.

In the same experiment, groups of mice were treated with a macrophage-blocking compound and then used as recipients in the immunotherapy experiment, to see whether macrophage-like cells of the host may have a role in the mechanism of tumor eradication by immune cells. Table 5 shows that macrophage-poisoned mice could not be cured by the adoptive transfer of immune lymphocytes indicating that host macrophages play a key role in the eradication of YC8 via their possible antigen-presenting cell function to the donor lymphocytes or as final cytotoxic effector cells stimulated by donor lymphocytes or their products.

Acquisition of systemic tumor immunity in cured mice

Animals cured by immunotherapy were challenged 3 months later with increasing doses of YC8 cells to evaluate their immunological status against the cured tumor. As shown in Table 6, mice first cured by immunotherapy develop an efficient reaction against high challenging doses

Table 5. Host involvement in the eradication of YC8 lymphoma by adoptive transfer of alloimmune lymphocytes

Treatment of [a] recipient mice	Lymphocyte injected	No. of mice with tumor [b]/ No. of mice treated	MST ± SD
500 R	BALB/c anti-DBA/2	0/5	–
Thymectomy + 500 R	BALB/c anti-DBA/2	0/5	–
B-mice [c]	BALB/c anti-DBA/2	0/10	–
Carrageenan	BALB/c anti-DBA/2	7/9	42.5 ± 12.0
Untreated	BALB/c anti-DBA/2	0/6	–
Thymectomy + 500 R	BALB/c	6/6	18.3 ± 3.4
Untreated	BALB/c	9/9	24.5 ± 6.2

[a] After treatment mice were injected i.v. with 10^3 YC8 cells and 3, 5, and 7 days later given 30×10^6 BALB/c anti-DBA/2 or BALB/c lymphocytes

[b] Mice developed liver and kidney metastases and died with the indicated MST ± SD

[c] B mice were prepared by lethal irradiation (850 R) of thymectomized animals and i.v. injection of 2×10^7 liver cells from 14-day-old fetuses

Table 6. Systemic tumor immunity in mice cured by adoptive transfer of BALB/c anti-DBA/2 lymphocytes [a]

Mice	No. of mice with tumor/No. of mice i.v. challenged with					
	YC8 cells				RL ♂ 1 cells	
	10^3	10^6	5×10^6	10^7	10^3	10^4
Cured [b]	–	1/6	2/15	6/6	10/10	5/5
Normal	10/10	ND	ND	ND	10/10	ND

[a] BALB/c mice were given 30×10^6 immune lymphocytes 3, 5, and 7 days after the i.v. administration of 10^3 YC8 cells

[b] Cured tumor-free mice were challenged 90 days after the last therapeutic injection of immune lymphocytes

Table 7. Detection of memory cells in YC8-cured BALB/c mice

Previous lymphocyte treatment[a]	Host[b]	Booster[c]	% ^{51}Cr release on target			
			YC8		P815	
			50:1	12:1	50:1	12:1
BALB/c anti-DBA/2	Tumor-cured mice	YC8	37	19	50	27
		DBA/2	43	22	64	42
BALB/c anti-DBA/2	Tumor-cured B mice	YC8	32	23	57	25
		DBA/2	38	21	58	40
BALB/c anti-DBA/2	Normal mice	YC8	5	1	8	4
		DBA/2	19	9	24	16
BALB/c	Normal mice	YC8	2	0	2	1
		DBA/2	5	2	9	4

[a] 30 × 10^6 lymphocytes were injected three times at days 3, 5, and 7 after tumor injection or in normal mice
[b] Mice with or without tumor that received lymphocyte transfer
[c] Booster was carried out 90 days after the last treatment by i.p. injection of 10^7 DBA/2 lymphocytes or 5 × 10^5, 8000 R treated YC8 cells; cytotoxic assay was done 5 days later

($> 5 \times 10^3$ LD_{80}) of lymphoma cells. Acquired immunity is tumor-specific since cured mice were unable to resist a low challenging dose (10^3 cells) of another syngeneic lymphoma RL♂1.

Systemic YC8 immunity in cured mice is due to the presence of memory cells. In fact, Table 7 shows that normal and B mice cured by BALB/c anti-DBA/2 lymphocytes and i.p. boosted with irradiated YC8 cells or DBA/2 lymphocytes, both developed cytotoxic T cells that were able to lyse YC8 and P815 targets. Negative controls were BALB/c mice that received only an i.p. injection equivalent to the booster in cured mice. These findings suggest that memory cells derive from BALB/c anti-DBA/2 transferred lymphocytes, but require restimulation which occurs during the eradication of the tumor without the need to recruit the host's T cells. In fact, BALB/c mice without tumor which received the curative dose of immune lymphocytes did not develop cytotoxicity against YC8 and P815 when boosted with YC8; as expected from previous data, mice boosted with DBA/2 cells developed effectors with a low lytic activity against YC8 targets [5].

Discussion

Although several papers have described adoptive immunotherapy of immunogenic tumors by transfer of immune T lymphocytes [4], analysis of the main cell population involved in mediating the curative effect(s) is often limited to the phenotype determination obtained by negative selection, and to monitoring the cytotoxicity of effector cells.

In a previous communication we examined the phenotype and activity of alloimmune cells used in the adoptive therapy of YC8 lymphoma. It was found that Lyt 1+2− cells were necessary for the therapeutic effect and that the whole population of immune effector cells lacked significant in vitro cytotoxic or proliferating activity against YC8 [5]. Nevertheless, the therapeutic activity of such lymphocytes given to tumor-bearing mice implies that BALB/c anti-DBA/2 effectors can recognize tumor cells and can be activated against them. In the present study we show that noncytotoxic BALB/c anti-DBA/2 immune cells may differentiate into YC8 lytic effectors after 5 days of in vitro restimulation with mitomycin C-treated YC8 cells, albeit without detectable proliferation, production of IL-2, or IFN-γ. Lack of or weak proliferating activity of BALB/c anti-DBA/2 lymphocytes against YC8 but not against DBA/2 cells [5] might be due to the absence of lymphocyte activating molecules on YC8 cells like class II MHC products [22] or DBA/2-like *Mls* locus products. By contrast, proliferation is required in vivo for the therapeutic effect to take place since irradiation or vinblastine treatment of lymphocytes before their transfer abolished the curative effect. Similar findings have been reported by other investigators [8, 26]. The discrepancy between in vivo and in vitro data may be ascribed to the physiological environment that lymphocytes find in vivo and which may not be reproducible in vitro. On the other hand, the differentiation of a fraction of T lymphocytes into tumor cytotoxic cells may occur also in vivo but be undetectable as this cytotoxicity is insufficient to affect the course of tumor development.

Using a different assay, we also investigated the presence and function of helper or inducer cells in the population of the immune anti-DBA/2 lymphocytes. Such cells are known to be Lyt 1+2− and to represent the main subpopulation involved in the therapy of YC8 although they at best proliferate weakly in the presence of YC8 cells [5]. DTH reaction is thought to be mediated by Lyt 1+ cells [16] and be involved in antitumor immunity in vivo [23]. We found that BALB/c anti-DBA/2 lymphocytes, when admixed with YC8 cells, developed a specific DTH reaction whereas the same cells displayed a low neutralizing effect in the Winn assay. This last finding is not unexpected because it has been shown that the neutralization of YC8 cells requires cytotoxic T cells [25] which represent a small, inactive fraction of the whole population of BALB/c anti-DBA/2 cells.

Although the study of different lymphocyte subpopulations allows the collection of important information on the mechanism of immunotherapy, the possiblity of successfully using the whole lymphocyte population has some distinct practical advantages. In fact it permits the use of a high number of cells, avoids homing problems encountered by injecting T lymphocyte clones [7] and excessive

cell manipulation. In addition, it is possible that by transferring an homogeneous lymphocyte population containing the physiological array of all subpopulations, the immunotherapeutic effect may be largely independent from the immunological status of the host. An important finding of the present investigation was the demonstration that whole BALB/c anti-DBA/2 lymphoid cells are able to mediate the curative effect in T-depleted animals including B mice. These data suggest that: (a) the whole lymphocyte population contains all the necessary cell subsets some of which may be lacking or suppressed in the tumor-bearing host; (b) the T cell system of the host is not necessary in the mechanisms of eradication of tumor cells by infused immune lymphocytes. In agreement with our result, it has been reported that B guinea pigs were fully capable of rejecting syngeneic hepatoma after transfer of immune cells [27]. However, this finding does not rule out the possibility that other nonlymphoid cells of the host may participate in the eradication of tumor cells since it is known that macrophages cannot be removed by the techniques used in these and other experiments.

To test the possibility that the host's macrophages may be involved in the phenomenon, mice were treated with an inhibitor of monocyte-macrophage function before onset and/or during immunotherapy. This type of treatment abolished the therapeutic effect. According to the different functions of such cells in immunity, however, it is not clear whether the antigen-presenting cell or the cytotoxic activity of macrophages or both are important in the mechanism of immunotherapy. In fact, macrophages may be needed either for presenting the DBA/2-like antigens of YC8 cells (which lack Ia expression) to donor lymphocytes or may be the final antitumor effectors stimulated by lymphokines released by donor lymphocytes after in vivo recognition of YC8 cells. In fact YC8 cells are not susceptible to NK lysis [23] but are destroyed by syngeneic or allogeneic macrophages activated with lipopolysaccharide (Colombo, unpublished data).

Since we have shown that the lymphocyte compartment of the host is not necessary for tumor eradication, the resistance of our tumor-cured mice to challenge with high doses of YC8 cells and the presence of memory tumor cytotoxic cells support the conclusion that donor lymphocytes proliferate and expand into cytotoxic cells after encountering tumor cells in vivo, leaving the identification of the ultimate tumor killing cells unsettled, since both host macrophages and donor lymphocytes may be present in the host.

In agreement with our data, Greenberg and Cheever have recently shown, using Thy 1 congenic mice, that host T cells did not contribute substantially to tumor eradication or to the long-term maintainance of tumor immunity [12].

In conclusion, BALB/c anti-DBA/2 lymphocytes mediate the cure of YC8 lymphoma in BALB/c mice independently from the host's T cell participation but not from the macrophage-monocyte, radiation-resistant host involvement. This is likely to be possible because the injected lymphocytes contain a main Lyt 1+ subset endowed with a DTH function against YC8, together with precursor cytotoxic T cells which may differentiate into YC8 cytotoxic effectors after restimulation with YC8 cells. One can speculate that the fraction of precursor cytotoxic cells may attack tumor targets early after their infusion, being activated by neoplastic cells themselves, whereas the helper subpopulation will need more time to interact with the tumor-antigen possibly presented by host macrophages, to release interleukins and, finally, to mobilize the ultimate tumor killers.

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