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DR antigen expression on ovarian carcinoma cells does not correlate with their capacity to elicit an autologous proliferative response

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Summary. Expression of HLA-DR antigens by purified preparations of human ovarian carcinoma cells freshly isolated from surgical specimens was examined in parallel with the capacity of tumor cells to elicit a blastogenic response from autologous lymphocytes in mixed lymphocyte-tumor culture (MLTC) assay. Of 21 tumor preparations, 11 (52%) reacted with monoclonal antibodies 279 and/or 949 specific for a monomorphic determinant of HLA-DR antigens, with heterogeneous positivity, ranging between 30% and 95%. In this series of patients positive MLTC occurred in 8/21 individual experiments. The HLA-DR expression was proportionally similar in tumors giving positive MLTC (4/8 = 50%) and negative MLTC (7/13 = 53%). The lack of correlation between DR expression on tumor cells and stimulatory activity in autologous MLTC and the fact that DR-negative tumors could induce lymphocyte stimulation, support the hypothesis that blastogenesis occurs upon recognition of tumor-associated antigens, different from DR molecules, possibly tumor-specific antigens.

Introduction

The proliferative response of lymphocytes stimulated in vitro with autologous tumor cells (mixed lymphocyte-tumor culture, MLTC) was investigated in a homogenous caseload of 43 patients with ovarian carcinoma. The results demonstrated a proliferative response in about 48.8% of the patients. Blastogenesis in MLTC assay is supposed to reflect the recognition of putative tumor antigens [5, 16, 18, 19]. Since T lymphocytes recognize antigens in conjunction with Class II molecules (HLA-DR) of the Major Histocompatibility Complex [7], which also play a key role in lymphocyte stimulation, we have investigated whether ovarian carcinoma cells expressed HLA-DR antigens on their membrane and whether this expression correlated with their ability to elicit an immune proliferative response.

The HLA-DR (Ia) antigens, were first described on hematopoietic cells, but it is now clear that they are expressed on cells from various tissues, including endothelial, Langherans cells, and some tumors [3, 4, 6, 8, 11, 13, 14, 17]. A proportion of ovarian carcinomas have been reported to express DR antigens defined by monoclonal antibodies (MoAb) [8, 17]. The significance of the presence of Ia antigens on neoplasms is, at present, unclear. Previous studies on the role of DR antigens in MLTC gave conflicting results. It has been reported that a positive proliferative response in MLTC was observed in most DR-positive primary (but not metastatic) melanomas [4], while other authors have demonstrated that autologous MLTC is independent of the expression of DR antigens on tumor cells [17], although they contribute to the allogeneic MLTC response.

To clarify this issue we have studied the lymphocyte blastogenesis of 21 patients with ovarian carcinoma in the autologous MLTC assay and the correlation with DR antigen expression on the stimulator cell. Our results demonstrate that the presence of Ia antigens on ovarian tumor cells does not correlate with the ability, and it is not a prerequisite to elicit an autologous MLTC.

Materials and methods

Patients. A total of 21 patients with histologically confirmed epithelial ovarian carcinoma admitted to the Department of Oncology, Obstetrics and Gynecology Clinic of the University of Milan, S. Gerardo Hospital, Monza, Milano, formed the caseload of this study. All patients had cancer classified as stages III and IV. Histology, grading, and cytology are presented in Table 1. All the patients but 2 (nos. 36 and 44) had not received any prior treatment.

Peripheral blood lymphocytes. The peripheral blood lymphocytes (PBL) were separated as described elsewhere [1, 2] on Ficoll-Hypaque gradients (Eurobio, Paris, France) and depleted of monocytes by plastic adherence and of B lymphocytes by passage over a nylon wool column. Nonadherent lymphocytes eluted from the column were used as responder lymphocytes in the MLTC assay.

Tumor cells. A single cell suspension of tumor cells from solid tumors was prepared as described elsewhere [12], by mechanical disaggregation and exposure to 0.3% collagenase (40130, Sigma Chemical Co. St. Louis, Mo.) in BME (Eurobio). Disaggregated cells were centrifuged on a Ficoll-Hypaque gradient and depleted of adherent macrophages by plastic adherence. In some experiments nonadherent tumor cells were passed over a nylon wool column as described for PBL.

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Purified preparations of tumor cells from peritoneal effusions were obtained by centrifugation on discontinuous Ficoll-Hypaque gradients (75%–100%), as previously described [1]. Tumor cells were harvested at the 75%-medium interface, lymphocytes and macrophages were collected at the 75%–100% interface. The tumor cell population was then processed as described for tumor cells from solid specimens.

Histopathological examination was performed on each tumor cell preparation by an independent pathologist who confirmed the presence of tumor cells with 70%–98% purity.

Tumor-associated lymphocytes (TAL). Lymphocytes from peritoneal effusions or infiltrating solid tumors were isolated on a discontinuous Ficoll-Hypaque gradient 75%–100% as described for ascitic tumor cells [1, 2]. The various phases of the separation procedure were monitored by morphological examination of Wright-stained smears; when the separation was not satisfactory, the discontinuous gradient was repeated or, alternatively, lymphocyte-enriched cells were layered on a discontinuous gradient of Percoll (Pharmacia Chemicals AB, Uppsala, Sweden) [2]. Viability of enriched fractions always exceeded 90%.

Mixed lymphocyte-tumor culture. The MLTC was performed by the technique described by Vanky et al. [16, 17]. The PBL and TAL were mixed with irradiated (2000 rads) stimulator autologous tumor cell to give responder/stimulator ratios ranging from 2/1 to 32/1 [2, 4, 8, 16, 32]. Control cultures consited of lymphocytes cultured alone, lymphocytes stimulated with 1 μg/ml phytohemagglutinin A (Wellcome, England), and tumor cells cultured alone at the highest concentration used in the assay. Proliferation was assessed by uptake of 1 μCi/well of ^{3}H-thymidine, specific activity 2.5 μCi/mmol, (Radiochemical Centre, Amersham, Bucks, England) after 6 days incubation. The MLTC was arbitrarily considered positive when the stimulation index (SI) was greater than 3, calculated as follows:

$$SI = \frac{\text{mean cpm in test wells} - \text{mean cpm of stimulator cells}}{\text{mean cpm of lymphocytes alone}}$$

In some experiments, lymphocytes alone had a high counts per minute value (i.e., >6000), and in these cases, an SI as low as 1.8 was considered positive.

Avidin-biotin immunoperoxidase staining. Cytocentrifuged preparations of highly purifed ovarian carcinoma cells, prepared as a single cell suspension from solid masses or peritoneal effusions were air-dried, fixed in acetone for 10 min and stained by the avidin-biotin complex method as described elsewhere [9]. The MoAbs against a common determinant of HLA-DR were 279 [10] and 949 [15], diluted in phosphate-buffered saline pH 7.4+2% human pooled serum and incubated at room temperature for 30 min. Positive controls consisted of normal lymph nodes and ovarian carcinoma sections with predetermined reac-

Table 1. Histopathological characteristics and DR expression of ovarian carcinoma cells and lymphocyte reactivity in autologous MLTC

Patient no.	Age	Histology	Grade	Cytology	Stimulation index[a]			Reactivity with:[b]			
					PHA	MLTC		279	949	279	949
						Primary	Ascitic	Primary		Ascitic	
10	60	Serous	G3	IV	9.3	0.3		10	0		
11	46	Serous	G3	IV	11.6	9.5*	46*	80*	80*	70*	80*
13	61	Serous	G3	IV	1.9	1.3		0	0		
14	50	Mucinous	G2	III	4.1	−0.3		80*	80*		
18	66	Serous	G3	IV	nt[c]	1.1		50*	70*		
19	49	Anaplastic	G3	III	15.6	3.7*		80*	50*		
20	60	Serous	G3	III	8.7	1.3	−0.2	nt	40*	50*	40*
30	61	Serous	G3	IV	nt	−17.1	−33.5	10	0	0	0
31	52	Serous	G3	IV	10.5	16.1*	91.4*	0	0	0	0
33	57	Serous	G2	III	nt	3.3*		0	0		
35	62	Serous	G3	IV	nt	4.8*		10	0		
36	48	Serous	G3	IV	2.8	1.8		0	10		
38	82	Serous	G3	IV	9.5	0.2		0	20		
39	57	Serous	G3	IV	1.5	1.4		0	40*		
40	63	Endometroid	G3	IV	1.1	0.8	−3.9	0	0	20	50*
41	41	Serous	G3	IV	1.8	1.8*	1.1	0	0	50*	50*
42	60	Endometroid	G3	IV	4.4	9.5*		50*	50*		
43	54	Mixed[d]	G3	IV	2.8	0.8		30*	50*		
44	47	Serous	G3	IV	15.0	0.8		90*	95*		
45	58	Serous	G3	IV	13.9	9.1*	1.1	40*	50*	60*	40*
46	68	Serous	Borderline		2.5	0.4		90*	90*		

[a] PBL from cancer patients were incubated for 6 days with different numbers of ovarian carcinoma cells or phytohemagglutinin (PHA), proliferation was assessed as ^{3}HTdr uptake and calculated as stimulation index. Positive MLTC are designated with an asterisk

[b] MoAbs 279 and 949 recognize a common determinant of HLA-DR antigens. Reactivity was assessed with peroxidase staining. Only tumors with at least 30% of DR-positive cells were considered positive. These preparations are marked with an asterisk

[c] nt, not tested

[d] Mixed Mullerian adenocarcinoma

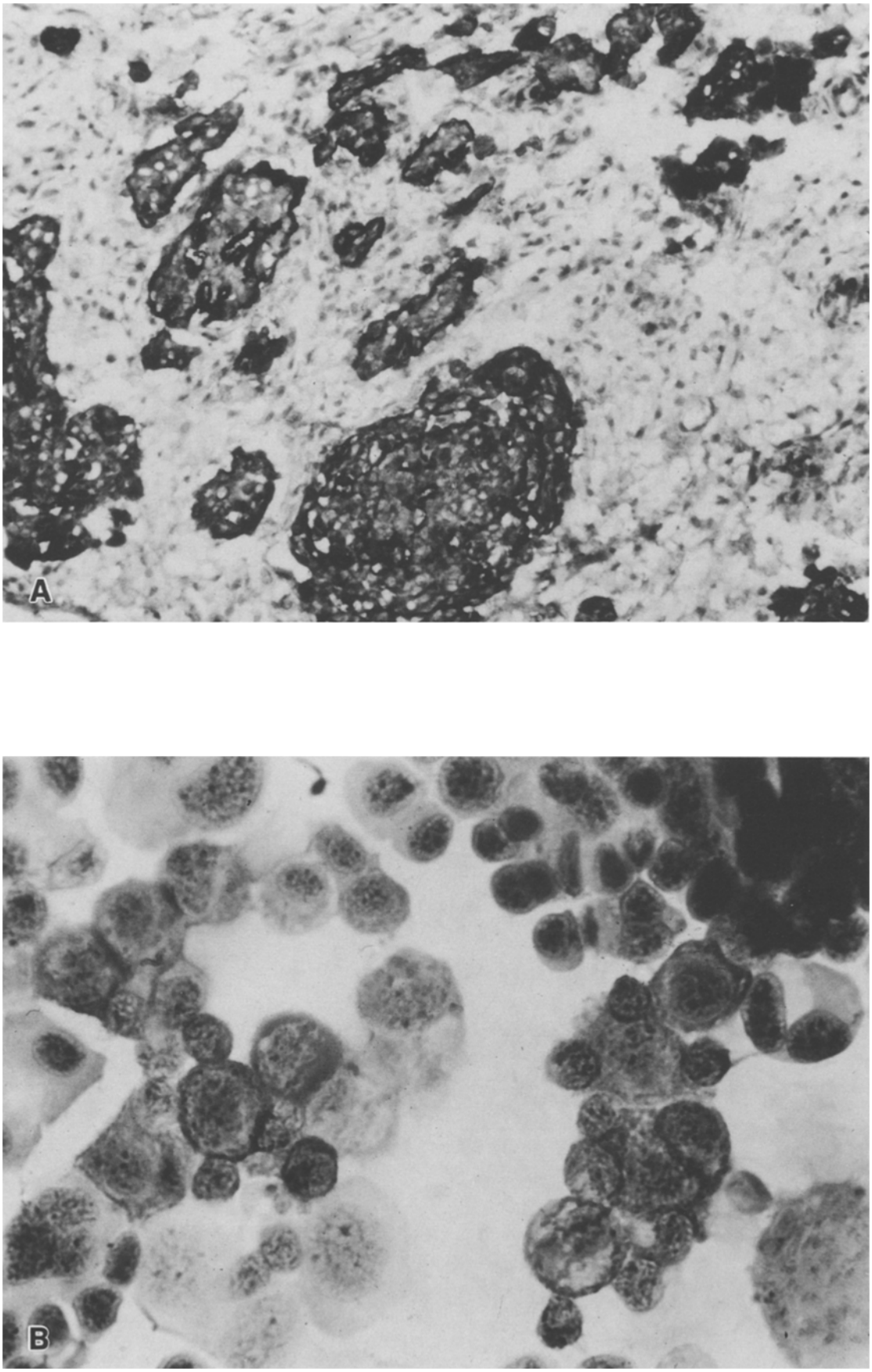

Fig. 1. Reactivity with monoclonal antibody (MoAb) 279 (monomorphic HLA-DR determinant) on cryostat section (*panel A* 100×) and cytocentrifuge preparation (*panel B* 400×) of two different ovarian carcinomas

tivity. Negative controls were tissue sections preincubated with an irrelevant antibody. Cell preparations were scored positive when at least 30% of the cells were peroxidase stained.

Results

We evaluated the expression of DR antigens on purified preparations of ovarian carcinoma cells. Immunohistochemical staining of two representative tumor populations is shown in Fig. 1. Most frequently the expression of DR antigens was heterogeneous and restricted to only a proportion of tumor cells. As shown in Table 1, 11/21 solid tumors and 5/7 tumor preparations from carcinomatous ascites expressed DR antigens, reacting with one or both MoAbs 279 and 949, specific for a monomorphic epitope of DR antigens. One patient (no. 39) had cells reacting only with MoAb 949, a quite surprising finding also because 949 positivity was quite high: 40% positive cells. Although the vast majority of the ovarian carcinoma analyzed were of serous histology, a few other cases (2 endometroid, 1 mucinous, 1 anaplastic, and 1 mixed Mullerian) were studied and all of them were DR-positive. As a matter of fact 9 of 10 DR-negative cases were serous adenocarcinomas, 1 being an endometroid tumor.

Table 2. DR expression and stimulatory activity in autologous MLTC of ovarian carcinoma cells

	MLTC positive = 8		MLTC negative = 13	
	DR-positive	DR-negative	DR-positive	DR-negative
Solid tumors (total = 21)	4	4	7	6
	MLTC positive = 2		MLTC negative = 5	
	DR-positive	DR-negative	DR-positive	DR-negative
Ascitic tumors (total = 7)	1	1	4	1

As also shown in Table 1 it was occasionally possible to analyze tumor cells from the peritoneal effusions in parallel with the primary tumor. While in 3 cases we found similar results, in patient nos. 40 and 41, ascitic tumor cells reacted with MoAbs 279 and 949 while the primary tumor did not. It is unlikely that this discrepancy could be attributed to normal Ia-positive macrophages from the perito-

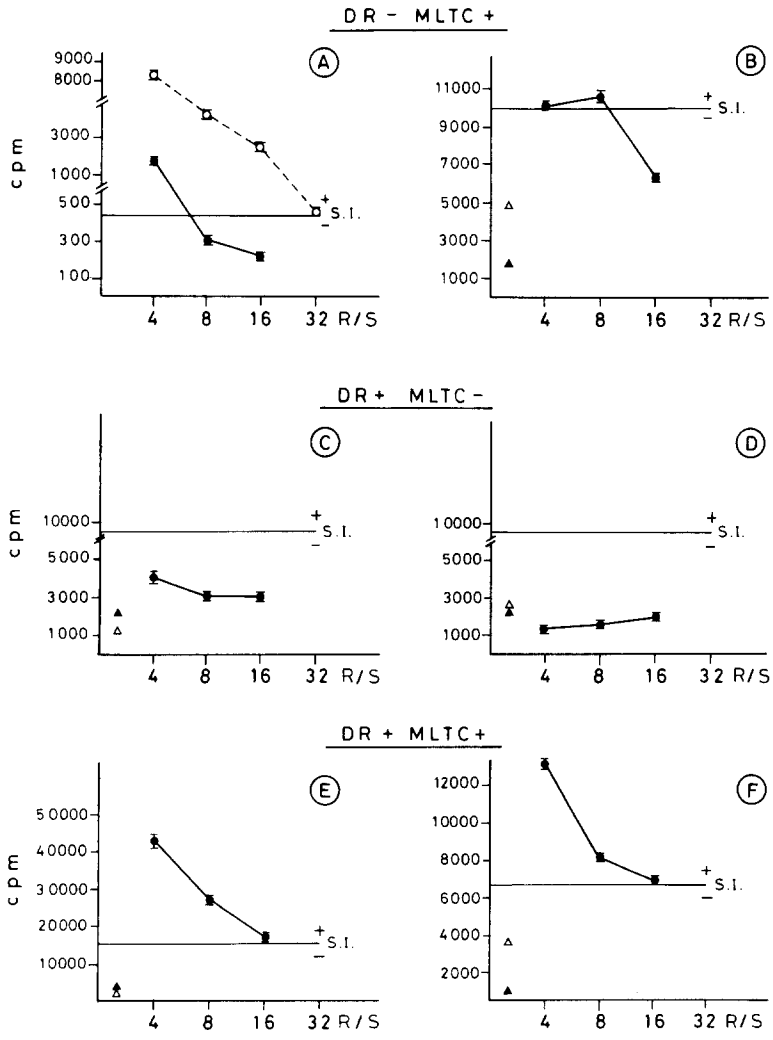

Fig. 2. Curves of mixed lymphocyte-tumor cell (MLTC) with autologous tumor cells. DR-positive (panels C, D, E, F) and negative (*panels A, B*) from ascitic (○——○) and solid tumors (●——●). Peripheral blood lymphocytes (PBL) were cocultured with different responder/stimulator (R/S) ratios ranging from 4/1 to 32/1 for 6 days. Proliferation was assessed by ^{3}H-thymidine (^{3}H Tdr) uptake. Stimulation index >3 was considered positive. △ irradiated tumor cells alone; ▲ PBL alone

neal cavity contaminating the tumor preparation, as 50% of the cells from these patients reacted with MoAbs 279 and 949 (patient no. 40 had 20% 279-positive cells but 50% positive for 949) and the isolation procedure with discontinuous density gradients and plastic adherence usually results in highly purified tumor cell preparations (70%–98% purity).

The proliferative activity of patients' lymphocytes stimulated with autologous tumor cells in vitro was evaluated and compared to the surface expression of DR antigens. As summarized in Table 2 positive MLTC occurred in 8/21 primary ovarian carcinomas. DR expression was almost identical in both MLTC-positive and MLTC-negative cases. Similar results were obtained with cell preparations isolated from peritoneal effusions: of these, only 2 gave positive MLTC, 1 being positive and 1 negative. Thus, the expression of DR antigens did not seem to positively correlate with the ability to elicit an autologous lymphocyte response.

Figure 2 shows some representative experiments. DR-negative tumors (panel A and B) did not differ in their stimulatory ability in MLTC from DR-positive tumors (panels E and F): panel A also shows that solid as well as ascitic DR-negative tumor cells elicted autologous MLTC. Moreover, DR expression was not sufficient to induce lymphocyte blastogenesis (e.g., panel C and D) where tumors were DR-positive.

If DR antigens are not necessary for autologous MLTC, other experiments demonstrated that they are a prerequisite in allogeneic conditions. In fact, as shown in Fig. 3, 2 DR-positive tumor preparations gave negative autologous MLTC (case nos. 43 and 44) but did stimulate allogeneic lymphocytes (panel C and D), while case no. 41, a DR-negative solid tumor was not stimulatory towards two different allogeneic donors but did induce positive autologous MLTC (panel B).

Discussion

The presence of DR antigens on tissues other than hematopoietic cells and in particular on human tumors is now well-established [3, 4, 6, 8, 11, 13, 14, 17]. As these surface molecules play a key role in antigen presentation to lymphocytes and in their stimulation [7], we have investigated whether the ability of tumor cells to elicit blastogenesis of autologous T lymphocytes was related to the expression of HLA-DR antigens. In our study 11/21 of primary ovarian carcinoma and 5/7 ascitic tumor cells reacted with 949 and/or 279 MoAb specific for a monomorphic DR determinant. This high reactivity of ovarian tumor cells has also been reported by other authors [8, 17]. Positive MLTC was found in 8/21 primary tumors and 2/7 ascitic tumor preparations. Lymphocyte stimulation occurred with both DR-negative and DR-positive tumors with equal frequencies:

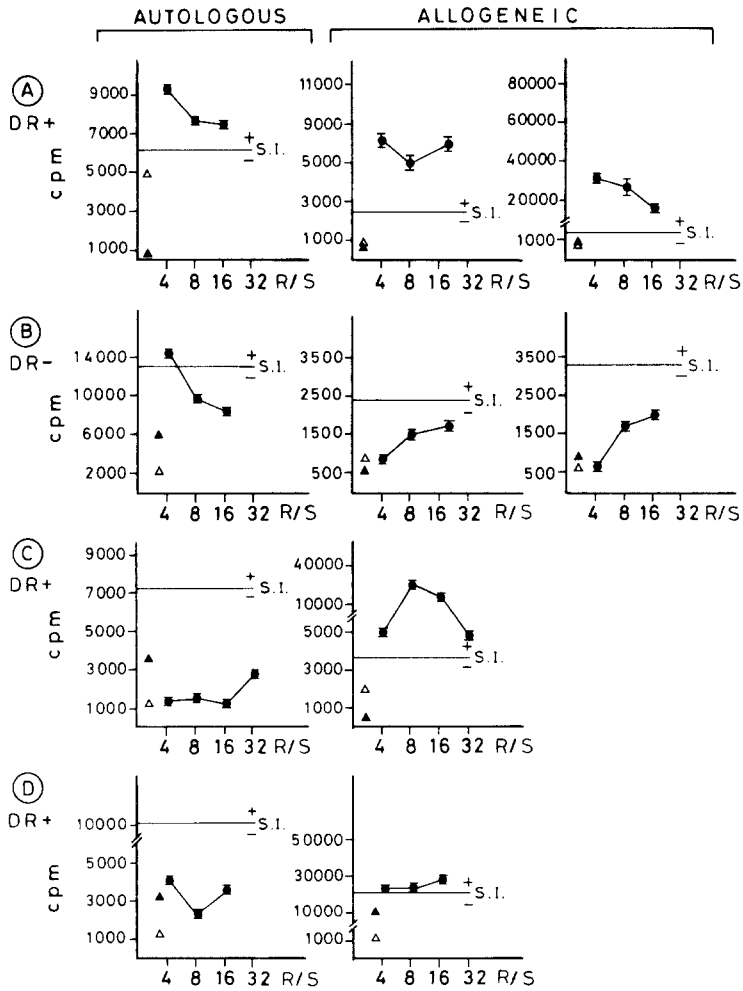

Fig. 3. MLTC with autologous and allogenic PBL with DR-positive (*panels A, C, D*) and DR-negative (*panel B*) tumors cells. △ irradiated tumor cells alone; ▲ PBL alone

therefore indicating that DR antigens are not a prerequisite and may not be sufficient for the autologous sensitization.

These data are in agreement with those of Vanky et al. [17] who also found no positive correlation between DR expression and MLTC in lung tumors. However, the data are at variance with those of Fossati et al. [4] who demonstrated that DR-positive (but not DR-negative) primary melanomas can stimulate (6/6 cases) lymphocytes in MLTC. These different results may be explained by the different biology of the tumors. Vanky et al. [17] reported that among a large range of tumors of different histologies (lung, ovarian and thyroid adenocarcinomas, hypernephromas, osteosarcomas, and others), lung tumors were, by far, the most antigenic. Therefore intrinsic properties of the tumor may well account for their different stimulatory capacity and the different role played in this by DR antigens.

A consistent finding was that DR antigens are important in the stimulation of allogeneic lymphocytes [4, 17]. This is not surprising if we consider the physiological role of DR antigens in lymphocyte stimulation [7]. In this context, these data also provide evidence that DR antigens associated with tumor cells appear to be functionally similar to those of normal cells. On the other hand, DR-negative tumors are functionally negative because they do not induce allogeneic MLTC.

In conclusion, these results indicate that DR antigen expression on ovarian carcinoma cells does not correlate with the capacity of tumor cells to elicit an autologous MLTC. As in some positive cases tumor preparations did not react with MoAb specific for the monomorphic determinant of HLA-DR antigens and therefore lacked the most potent stimulatory molecules in autologous mixed lymphocyte reaction, these experiments support the hypothesis that blastogenesis is triggered by recognition of tumor-associated antigens. The identification of antigenic molecules associated with human tumors and of surface structures with high stimulatory potential on the immune system, may have important therapeutic interest.

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