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Lack of suppressive activity of human primary melanoma cells on the activation of autologous lymphocytes*

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Summary. Previous studies have indicated that primary but not metastatic melanomas were able to stimulate the proliferation of autologous (Auto) peripheral blood lymphocytes (PBL) in 73% of cases. On the other hand, 57% of the metastatic melanomas were shown to be suppressive when melanoma cells (Me) were admixed with Auto-PBL stimulated with allogeneic (Allo) PBL or interleukin 2 (IL-2) at the beginning of a 6-day incubation period. Here, we report that the suppressive activity of Me is a functional characteristic associated with a particular stage of the disease. In fact, we found that none of the 11 primary tumors tested were able to inhibit the proliferative response of Auto-PBL to Allo-PBL or IL-2 at all the doses of tumor cells used. The generation of lymphocytes cytotoxic against Auto-Me or K562 was also not inhibited. Of the 11 primary tumors checked for suppression, 8 were able to stimulate Auto-PBL in a primary mixed lymphocyte tumor culture. We conclude that opposite functions, stimulation and inhibition of autologous lymphocyte responses are characteristics of primary and metastatic Me, respectively.

Introduction

Previous studies on cell-mediated immunity of peripheral blood lymphocytes (PBL) from cancer patients against autologous (Auto) melanoma cells (Me) have shown that primary and metastatic tumors may differ in their ability to interact with the immune system of the host [11, 17]. In particular, only in a low percentage of cases (< 10%) were metastatic Me able to induce the proliferation of Auto-PBL and the generation of tumor cytotoxic lymphocytes [21]. On the contrary, in more than 70% of the cases a positive mixed lymphocyte tumor culture (MLTC) was obtained when Auto-Me isolated from primary tumors were used as stimulators [10]. It has not been possible however to ascribe the stimulatory activity of primary melanomas to the expression of particular melanoma-associated antigens on the surface of the cells. We have only found that the majority of the primary tumors, which were able to stimulate Auto-PBL, were HLA-DR$^+$, whereas HLA-DR$^-$ tumors were not stimulatory. In contrast, metastatic melanomas either DR$^+$ or DR$^-$ were not stimulatory [10].

In addition, we have shown that 57% of metastatic melanomas were able to inhibit the response of Auto-PBL when added at the beginning of the stimulation with alloantigens or interleukin 2 (IL-2) [21]. The inhibition was dose-dependent, tumor-restricted, and associated with high levels of HLA-DR antigens expressed on the surface of the metastatic cells. In fact, HLA-DR$^-$ tumors were not suppressive and the treatment in culture of metastatic cells with recombinant interferon γ, which augments the expression of HLA-DR, also induced a strong suppressive activity [21].

Although suppressor cancer cells and suppressor T cells, either tumor specific or not, have been described in several murine and human systems [2, 3, 12, 14–16, 18, 25], no clear association between the stage of the tumor (primary vs metastases) and suppressive activity or generation of suppressor T cells has ever been made. Therefore, the availability of Me isolated from primary lesions and their Auto-PBL prompted us to investigate whether the suppressive activity described for the metastatic cells [21] was also present in the primary melanomas.

Here we report that none of the 11 primary melanomas tested was inhibitory when added at different doses at the beginning of the stimulation of Auto-PBL with alloantigens or IL-2. On the contrary, 8 out of these 11 tumors were capable of stimulating Auto-PBL.

Materials and methods

Tumor cells. Me were isolated from cutaneous primary lesions of 12 patients and from lymph node metastases of 28 patients as previously described [9]. Briefly, the tumor mass was cleaned and minced in RPMI 1640. Dead cells were eliminated by treatment of the tumor cell suspension with 270 units/ml of DNase and 0.25% trypsin for 1 min at room temperature, followed by two washings. Infiltrating lymphocytes were removed by Ficoll gradient centrifugation. Tumor cell suspensions were then frozen and stored at −80° C or in liquid nitrogen. When possible, tumor cells were also cultured in tissue culture flasks (Corning 25110, Corning Glass Works, New York, NY, USA) with RPMI 1640 plus 10% heat-inactivated fetal calf serum (FCS) and antibiotics (10% FCS-RPMI 1640). Before testing, fresh or mechanically harvested tumor cells were washed twice and used in the assays. They showed at least 80% viability as assessed by trypan blue exclusion and less than 5% nonmalignant cells. By electron microscopy analysis tumor

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cell preparations appeared to have premelanosomes and to be mycoplasma free.

Lymphocytes. PBL were obtained by Ficoll gradient centrifugation from heparinized blood of melanoma patients (Pt-PBL) 7–10 days after surgery or from normal donors. Interface cells were washed three times and resuspended in RPMI 1640 supplemented with 10% heat-inactivated human serum and antibiotics (10% HS-RPMI 1640). PBL were depleted of adherent cells by incubation in Petri dishes (Corning 25020) at 37° C for 2 h. Nonadherent PBL (NA-PBL) were then recovered, washed, and used in the assays.

The E-rosette positive subpopulation of lymphocytes was obtained by incubating NA-PBL with neuroaminidase-treated sheep red blood cells followed by Ficoll gradient centrifugation.

Lymphocyte activation systems. Pt-PBL (10^6/ml) were stimulated in vitro as follows:

1. with irradiated (8,000–10,000 R) Auto-Me at different responder:stimulator cell ratios ranging from 5:1 to 20:1 (MLTC);
2. with IL-2 from different sources, either nonpurified supernatants of phytohemagglutinin-stimulated normal PBL used at 25% final concentration (crude IL-2) or recombinant IL-2 (rIL-2, Hoffmann-La Roche, Nutley, NJ, USA) used at different concentrations from 25 units/ml to 100 units/ml;
3. with an irradiated pool of normal PBL (Allo-PBL) at a responder:stimulator ratio of 1:1. The cultures were set up in 24-well plates (Costar 3524, Costar, Cambridge, Mass., USA) in 2 ml of 10% HS-RPMI 1640 for the tumor cytotoxicity assay and in round-bottomed 96-well plates (Costar 3596) in 0.2 ml of 10% HS-RPMI 1640 for the proliferation assay; they were incubated at 37° C for 6–7 days.

^{3}H-Thymidine incorporation. The cultures tested for proliferation were pulsed during the last 18 h of a 6-day incubation with 1 μCi/well of ^{3}H-thymidine (^{3}H-TdR) (New England Nuclear, Boston, Mass., USA, sp. act. 6.7 Ci/mmol). The cells were harvested on fiberglass filters using a Skatron Cell Harvester, dissolved in liquid scintillation fluid (Filter-Count, Packard, La Grange, Ill., USA), and counted in a β counter.

The stimulation index (SI) was calculated in the following way:

$$SI = \frac{\text{cpm test}}{\text{cpm lymphocytes alone}}$$

All the tests were performed in triplicate and the SD of the three replicate samples was usually less than 10% of the mean. The proliferation assay was considered positive only when the SI was >3.

Cell-mediated cytotoxicity. Stimulated NA-PBL were recovered after 6–7 days of culture, washed twice, and used as effectors against tumor target cells in an 18-h Cr release assay as previously described [9]. Briefly, targets were labeled for 90 min at 37° C with 200 μCi of $Na_2\,{}^{51}CrO_4$ (Radiochemical Centre, Amersham, UK), washed three times, resuspended at a final concentration of 5×10^4/ml and admixed with effectors to obtain effector:target ratios of 40–20:1 in round-bottomed 96-well plates in 0.2 ml final volume of 10% HS-RPMI 1640. After 18 h of incubation, the plates were centrifuged and 0.1 ml of supernatant was collected and counted in a γ-scintillation counter (Packard). The percentage specific cytotoxicity was calculated from the average of triplicate samples as follows:

$$\%\ \text{cytotoxicity} = \frac{\text{cpm test} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \times 100$$

Spontaneous release was determined by incubating the targets without effectors and maximum release by incubating the targets with 1% Nonidet P40 detergent (BDH Biochemicals 56009, Poole, UK). Cell-mediated cytotoxicity was considered positive when the percentage cytotoxicity was >15.

Assay for suppressive activity. The suppressive activity of Me was determined by adding different numbers of these irradiated cells to the autologous NA-PBL stimulated with IL-2 or Allo-PBL at the beginning of the stimulation.

The percentage inhibition of proliferation was calculated in the following way:

$$\%\ \text{inhibition of proliferation} = \frac{\text{cpm test} - \text{cpm test with Auto-Me}}{\text{cpm test}} \times 100$$

where cpm test was the ^{3}H-TdR incorporation of Pt-PBL in the presence of stimuli (Allo-PBL or IL-2). Only inhibition values >20 were considered positive.

Indirect immunofluorescence. Indirect immunofluorescence was performed by incubating $3-6 \times 10^5$ cell with the optimal dilution of each monoclonal antibody (MAb) in 0.1 ml of 5% FCS-RPMI 1640 at 4° C for 30 min. After three washings, the cells were resuspended in 0.1 ml of medium with a 1:30 dilution of fluorescein-conjugated $F(ab')_2$ fragment of sheep anti-mouse antibody (NEN 504, New England Nuclear, Boston, Mass., USA). After 30 min of incubation on ice, the samples were washed three times, resuspended in 0.2 ml of 1% paraformaldehyde medium for storage and finally analyzed by flow cytometry (FACS IV, Becton Dickinson) or UV microscopy. Results were expressed as the percentage of positive cells above the background fluorescence determined using the same procedure without the first antibody. A percentage fluorescent cells >10 was considered positive. The mean channel fluorescence intensity ranging from 0 to 255 was also calculated for each MAb. The following MAbs were used:

- the anti-HLA-A, B, C monomorphic, w6/32 (Sera Laboratories, Sussex, UK);
- the anti-HLA-DR monomorphic, D1-12 [1];
- the anti-HLA-DQ, SPV-L3 [20];
- the anti-high molecular weight MAA (HMW-MAA) 225.28S [26].

Results

Pathological and antigenic features of primary melanomas

Table 1 shows the pathological characteristics and the antigenic profile of Me isolated from 12 different primary lesions. All tumors, except 2, were classified at the IV level of invasion and 10 of them were >4 mm in thickness. Of the 12 cases, 5 were defined as nodular melanomas, whereas the remaining 7 cases were superficial spreading melanomas. Only 1 tumor (Me 5737) appeared to be completely not pigmented.

From the antigenic point of view, all the 6 tumors tested were positive for the expression of HLA-class I antigens and HMW-MAA with a high percentage of positive cells. Of the 10 tumors examined, 7 were positive for HLA-DR antigens and 2 out of 7 for HLA-DQ antigens. As shown elsewhere [22], the distribution of these antigens on primary melanomas, although heterogenous, was not dissimilar from that observed for the metastatic lesions.

Failure of primary melanomas to suppress Auto-PBL stimulation

Table 2 shows a representative case in which NA-PBL from patient 872 proliferated and became cytotoxic when stimulated with Auto-Me, alloantigens, or IL-2. The cytotoxicity was dose-dependent and directed against both Auto-Me and K562. Autologous normal lymphocytes used as targets were not lysed (data not shown). This particular melanoma belongs to the series of primary tumors which are DR^+ and able to stimulate Auto-PBL [10]. However,

Table 1. Histopathological features and antigenic phenotype of human primary melanomas used throughout this study

Melanoma	Level of invasion	Thickness (mm)	Histologic type[a]	Phenotype (% positive cells)[b]			
				HLA-A, B, C	HLA-DR	HLA-DQ	HMW-MAA
11581	IV	>4	NM (P)	ND[c]	ND	ND	ND
5737	IV	>4	SSM (NP)	83	56	29	53
872	III	>4	NM (±P)	ND	67	ND	ND
1722	IV	>4	NM (P)	ND	30	ND	ND
5301	IV	3.3	NM (±P)	95	61	2	32
163	IV	2.8	SSM (±P)	ND	ND	ND	ND
3122	IV	>4	SSM (±P)	ND	2	0	ND
3772	IV	>4	SSM (±P)	ND	37	ND	ND
1007	IV	4	SSM (P)	97	5	8	100
1402	IV	>4	NM (P)	92	14	6	96
5469	IV	>4	SSM (P)	60	3	2	41
10538	V	>4	SSM (±P)	76	80	44	98

[a] NM = nodular melanoma; SSM = superficial spreading melanoma; P = pigmented; NP = not pigmented
[b] All tumors were examined by indirect immunofluorescence using the following monoclonal antibodies: w6/32, anti-HLA-A, B, C; D1.12, anti-HLA-DR; SPV-L3, anti-HLA-DQ; 225.28S, anti-HMW-MAA and fluorescein-conjugated $F(ab')_2$ fragment of sheep anti-mouse antibody
[c] ND = not done

Table 2. Failure of primary Auto-Me 872 to inhibit the cytotoxic and proliferative response of NA-PBL to Allo-PBL and IL-2

Stimulus	Irradiated tumor cells added	^{3}H-TdR uptake[a] cpm ± SD	SI[b]	% Specific cytotoxicity[c]			
				Auto-Me		K562	
				40 : 1	20 : 1	40 : 1	20 : 1
Medium	–	997 ± 453		8.6	3.1	21.9	11.9
Auto-Me[d]	–	4857 ± 173	4.5	26.9	21.4	40.9	26.9
Allo-PBL	–	39055 ± 9407	38.9	33.1	25.1	60.6	53.7
Allo-PBL	Auto-Me[d]	44097 ± 6809	43.6	27.6	24.5	56.6	41.9
IL-2 crude[e]	–	31900 ± 656	31.9	ND[f]	ND	ND	ND
IL-2 crude	Auto-Me	33312 ± 5382	33.4	ND	ND	ND	ND

[a] 18 h pulse with ^{3}H-TdR (1 μCi/well) on day 6
[b] SI = stimulation index
[c] 18 h ^{51}Cr release assay after 6–7 days of in vitro sensitization; the spontaneous release was 31% and 11% of the total cpm incorporated by 5×10^3 Me 872 and K562 cells, respectively
[d] Irradiated (10,000 R) autologous Me were used as stimulator or suppressor cells at 10% final concentration
[e] Crude supernatant of phytohemagglutinin (PHA)-activated human PBL (25% v/v)
[f] ND = not done

when checked for suppression, we found that the addition of 10% cells from Me 872 to Auto-PBL during stimulation did not modify the responses, thus suggesting that, differently from a DR^+ metastatic tumor, a DR^+ primary melanoma was not suppressive.

To check whether the failure of primary melanomas to inhibit the response of autologous lymphocytes was a general property of these cells, a series of primary tumors was analyzed in different experimental situations (Table 3). The overall results seemed to indicate that freshly explanted primary tumors cannot interfere with the stimulation of Auto-PBL. This was verified at all the doses of tumor used, from 20% to 5% of the total number of responder cells and with different subpopulations of effector cells (PBL vs NA-PBL vs E-rosette positive T lymphocytes). Lack of suppressive activity was seen with melanomas which directly stimulated Auto-PBL (Me 11581, Me 5737, Me 5301, Me 3772) and with those which were not stimulatory (Me 163, Me 3122). Moreover, from the phenotypic analysis of the cells both DR^+ (Me 5737 and Me 5301) and DR^- (Me 3122) primary tumors were not suppressive.

In a parallel experiment, shown in Fig. 1, the suppressive activity of a primary (Me 1007) was compared with that of a metastatic melanoma (Me 5222) using different doses of rIL-2 and Allo-PBL as stimuli. In the absence of tumor cells, PBL from both patients proliferated in response to the stimuli. However, when graded numbers of Auto-Me were added at the beginning of the culture period, a dose-dependent inhibition of the proliferation was seen with the metastatic Me only; the primary Me were not suppressive at any dose tested.

Stimulatory and suppressive activity of primary and metastatic melanomas

Table 4 shows a summary of the data collected so far on the immunogenicity and suppressive capacity of human primary and metastatic melanomas. Overall, we have examined 11 primary tumors and 28 metastases for suppressive activity. Of the primary tumors, 73% were able to sti-

Table 3. Failure of primary Me to inhibit the proliferative response of autologous lymphocytes to Allo-PBL or IL-2

Responder cells	Stimulus	R/S ratio	Irradiated tumor cells added (%)	^{3}H-TdR uptake[a] cpm ± SD	SI[b]	% Inhibition
NA-PBL	Medium		–	1018 ± 87		
11581	Auto-Me	10 : 1	–	9440 ± 2081	9.4	
	Allo-PBL	1 : 1	–	16616 ± 1299	16.3	
	Allo-PBL	1 : 1	Auto-Me (10)	17062 ± 3642	16.7	− 2.5
	Allo-PBL	1 : 1	Auto-Me (5)	21609 ± 3380	21.2	−30
	Allo-PBL	1 : 1	Auto-Me (2.5)	20412 ± 7555	20.0	−23
NA-PBL	Medium		–	400 ± 155		
163	Auto-Me	5 : 1	–	441 ± 137	1.1	
	Auto-Me	10 : 1	–	625 ± 249	1.5	
	Auto-Me	20 : 1	–	742 ± 239	1.8	
	Allo-PBL	1 : 1	–	30048 ± 4811	75.1	
	Allo-PBL	1 : 1	Auto-Me (10)	30527 ± 5350	76.3	− 1.6
	Allo-PBL	1 : 1	Auto-Me (5)	28791 ± 4358	71.9	+ 4.2
	Allo-PBL	1 : 1	Auto-Me (2.5)	28670 ± 1568	59.1	+ 4.6
E-rosette						
positive 5737	Medium		–	251 ± 23		
	Auto-Me	5 : 1	–	1999 ± 214	7.9	
	Allo-PBL	1 : 1	–	81244 ± 11473	323.6	
	Allo-PBL	1 : 1	Auto-Me (10)	91380 ± 3721	364.0	−12
NA-PBL	Medium		–	1708 ± 56		
5301	Auto-Me	10 : 1	–	7361 ± 333	3.4	
	Allo-PBL	1 : 1	–	123023 ± 3698	71.4	
	Allo-PBL	1 : 1	Auto-Me (10)	112646 ± 6858	64.5	+ 8.4
	Allo-PBL	1 : 1	Auto-Me (5)	102983 ± 4051	58.7	+16.2
	Allo-PBL	1 : 1	Auto-Me (2.5)	127171 ± 2882	72.3	− 3.3
NA-PBL	Medium		–	2003 ± 899		
3122	Auto-Me	10 : 1	–	1399 ± 174	0.7	
	Allo-PBL	1 : 1	–	50766 ± 5000	25.3	
	Allo-PBL	1 : 1	Auto-Me (10)	45739 ± 3545	22.8	+10
	IL-2 crude[c]		–	41491 ± 6476	20.7	
	IL-2 crude		Auto-Me (10)	36191 ± 2517	18.0	+13
PBL 3772	Medium		–	3321 ± 960		
	Auto-Me	10 : 1	–	21430 ± 1405	6.4	
	Allo-PBL	1 : 1	–	64449 ± 7465	19.4	
	Allo-PBL	1 : 1	Auto-Me (10)	77579 ± 4574	23.3	−20
	Allo-PBL	1 : 1	Auto-Me (5)	69660 ± 12655	20.1	− 8
	Allo-PBL	1 : 1	Auto-Me (2.5)	57199 ± 10370	17.2	+11
	IL-2 crude[c]		–	62963 ± 2528	18.9	
	IL-2 crude		Auto-Me (10)	55177 ± 5904	16.6	+12
	IL-2 crude		Auto-Me (5)	61088 ± 711	18.3	+ 3
	IL-2 crude		Auto-Me (2.5)	74142 ± 4504	22.3	−18

[a] 18 h pulse with ^{3}H-TdR (1 μCi/well) on day 6
[b] SI = Stimulation index
[c] Supernatant of PHA-activated human PBL (25% v/v)

mulate Auto-PBL and the stimulation was preferentially associated with the expression of DR antigens on the surface of the cells. Moreover, we have shown here that none of the 11 tumors examined was suppressive for the response of Auto-PBL to alloantigens or IL-2. On the contrary, only 1 out of 28 (4%) metastatic tumors stimulated Auto-PBL independently from the expression of DR antigens, whereas more than 50% of the metastases were suppressive [21]. A high percentage (>80%) of tumors with suppressive activity expressed DR antigens. Thus it seems that opposite functions, stimulation and inhibition of autologous lymphocyte responses are features of primary and metastatic Me, respectively.

Discussion

In previous reports we have shown that human metastatic Me were unable to stimulate Auto-PBL, but inhibited the stimulation of Auto-PBL induced by alloantigens or IL-2 [10, 17, 21].

The ability of metastatic Me to suppress the lymphocyte stimulation provides a suggestive explanation for the escape mechanism of invasive tumor cells from host immune attack. Particularly significant is the finding that metastatic Me could block the activation of Auto-PBL by IL-2 thus preventing the triggering and development of a complete immune response, a phenomenon which can actually occur in an in vivo microenvironment.

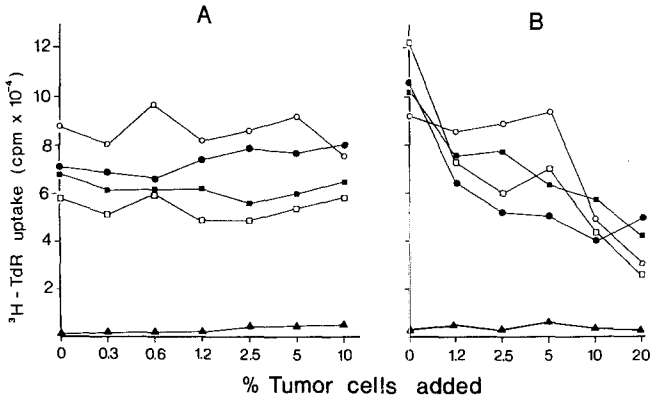

Fig. 1. Comparison of suppressive activity between melanoma cells (Me) obtained from primary (Me 1007, panel A) and metastatic (Me 5222, panel B) lesions on the proliferative response of autologous peripheral blood lymphocytes (Auto-PBL) to allogeneic (ALLO)-PBL or recombinant interleukin 2 (rIL-2). Autologous non-adherent (NA)-PBL (5×10^4/w) were cultured with medium alone (▲——▲), or with rIL-2, 100 units/ml (●——●), 50 units/ml (■——■), 25 units/ml (□——□), or with irradiated Allo-PBL (○——○) in the presence of graded numbers of irradiated (8000 R) Auto-Me. Cultures were pulsed on day 6 with ^{3}H-TdR for 18 h

The results of the present report clearly indicate that tumor cells, derived from primary melanoma lesions, failed to show any suppressive activity on the stimulation of Auto-PBL.

The lack of inhibitory activity is independent from the presence of HLA-DR antigen expression on primary Me. This finding seems to be at variance with that reported by Brocker et al. [5] indicating that the expression of HLA-DR in histological sections of primary Me was associated with high risk of metastasis.

Suppressive activity of human tumor cells by themselves or through the release of soluble factors has been reported [2, 4, 7, 8, 12, 13, 18, 19]. Moreover, evidence for cell-mediated suppression of in vitro cytotoxic lymphocytes by Auto-Me specific suppressor $CD8^+$ cells induced by the helper ($CD4^+$) population derived from a metastatic lymph node has been reported [14, 15].

Another interesting finding is the presence of significant immune suppressor cells in the tumor proximal lymph nodes of most melanoma patients [6] which supports our results on the inhibitory activity of metastatic Me obtained from invasive lymph nodes [21].

Previously we have shown that PBL from patients with primary melanoma were stimulated by Auto-Me thus suggesting that these patients could mount an immune response to Auto-Me [10, 11, 17]. The immune response of lymphocytes from cancer patients to autologous tumors, mainly to localized neoplasms, has been evidenced by the extensive studies of Vose et al. [24] and Vanky et al. [23] for a variety of histologically different tumors. Therefore, from this collective data it can tentatively be concluded that primary but not metastatic tumors may express tumor-associated antigens against which the patient can mount an immune response; in addition metastatic invasion and progression may be implemented by the ability acquired by these tumor cells to suppress the immune attack of the host.

In conclusion it appears that primary melanomas exhibit stimulatory, but not inhibitory, activity on the stimulation of Auto-PBL, whereas, metastatic melanomas are far less stimulatory and can display pronounced inhibitory activity.

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References

1. Accolla RS, Gross N, Carrell S, Corte G (1981) Distinct forms of both α and β subunits are present in human Ia molecular pool. Proc Natl Acad Sci USA 78: 4549
2. Akiyama A, Bean MA, Sadamoto K, Takamashi Y, Branko-van V (1983) Suppression of the responsiveness of lymphocytes from cancer patients triggered by co-culture with autologous tumor-derived cells. J Immunol 131: 3085
3. Berendt MJ, North RJ (1980) T-cell mediated suppression of antitumor immunity. An explanation for progressive growth of an immunogenic tumor. J Exp Med 151: 69
4. Bettetini D, Garrouste F, Remacle-Bonnet M, Culouscou J-M, Marvaldi J, Pommier G (1987) Enhancement of production of superoxide anion by human monocytes exposed to products of HT29 human colonic adenocarcinoma cell line. Int J Cancer 39: 203
5. Brocker EB, Suter L, Bruggen J, Ruiter DJ, Macher E, Sorg C (1985) Phenotypic dynamics of tumor progression in human malignant melanoma. Int J Cancer 36: 29
6. Cochran AJ, Hoon DB (1987) Immunological aspects of malignant melanoma. In: Veronesi U, Cascinelli N, Santinami M (eds) Cutaneous melanoma. Academic Press Inc London (LTD), p 47

Table 4. Summary of the results on the stimulatory and suppressive activity of primary and metastatic human melanomas

Stimulation of PBL with	Primary melanomas			Metastatic melanomas		
	Stimulatory/ total cases (%)	Suppressive[a]/ positive cases (%)	% DR+ Auto-Me	Stimulatory/ total cases (%)	Suppressive[a]/ positive cases (%)	% DR+ Auto-Me
Auto-Me	8/11 (73)		5/7[b] (71)	1/28 (4)		18/28[b] (64)
IL-2	6/ 6 (100)	0/ 6 (0)	3/4[b] (75)	28/28 (100)	16/28 (57)	13/16[c] (81)
Allo-PBL	11/11 (100)	0/11 (0)	5/7[b] (71)	25/25 (100)	13/25 (52)	12/13[c] (92)

[a] Number of cases in which Auto-Me could suppress the activation of PBL induced by IL-2 or Allo-PBL
[b] Positive cases/examined cases
[c] Positive cases/suppressive cases

7. Ebert EC, Roberts AI, O'Connell SM, Robertson FM, Nagase H (1987) Characterization of an immunosuppressive factor derived from colon cancer cells. J Immunol 138: 2161
8. Fontana A, Hengartner H, de Tribolet N, Weber E (1984) Glioblastoma cells release interleukin 1 and factors inhibiting interleukin 2-mediated effects. J Immunol 132: 1837
9. Fossati G, Balsari A, Taramelli D, Sensi ML, Pellegris G, Nava M, Parmiani G (1982) Lysis of autologous human melanoma cells by in vitro allosensitized peripheral blood lymphocytes. Cancer Immunol Immunother 14: 99
10. Fossati G, Taramelli D, Balsari A, Bogdanovich G, Andreola S, Parmiani G (1984) Primary but not metastatic human melanomas expressing DR antigens stimulate autologous lymphocytes. Int J Cancer 33: 591
11. Fossati G, Anichini A, Taramelli D, Balsari A, Gambacorti-Passerini C, Kirkwood JM, Parmiani G (1986) Immune response to autologous human melanoma: implication of class I and II MHC products. Biochim Biophys Acta 865: 235
12. Gately MK, Glaser M, McCarron RM, Dick SJ, Dick MD, Mettetal RW jr, Kornblith PL (1982) Mechanisms by which human gliomas may escape cellular immune attack. Acta Neurochir 64: 175
13. Hersey P, Bindon C, Czerniecki M, Spurling A, Wass J, McCarthy WH (1983) Inhibition of interleukin 2 production by factors released from tumor cells. J Immunol 131: 2837
14. Mukerji B, Wilhelm SA, Guha A, Ergin MT (1986) Regulation of cellular immune response against autologous human melanoma. I. Evidence for cell-mediated suppression of in vitro cytotoxic immune response. J Immunol 136: 1888
15. Mukerji B, Nashed AL, Guha A, Ergin Mt (1986) Regulation of cellular immune response against autologous human melanoma. II. Mechanism of induction and specificity of suppression. J Immunol 136: 1893
16. Naor D (1983) Coexistence of immunogenic and suppressogenic epitopes in tumor cells and various types of macromolecules. Cancer Immunol Immunother 16: 1
17. Parmiani G, Fossati G, Taramelli D, Anichini A, Balsari A, Gambacorti-Passerini C, Sciorelli G, Cascinelli N (1985) Autologous cellular immune response to primary and metastatic human melanomas and its regulation by DR antigens expressed on tumor cells. Cancer Met Rev 4: 7
18. Roth Ja, Osborne BA, Ames RA (1983) Immunoregulatory factors derived from human tumors. II. Partial purification and further immunobiochemical characterization of a human sarcoma-derived immunosuppressive factor expressing HLA-DR and immunoglobulin related determinants. J Immunol 130: 303
19. Santoli D, Tweardy DJ, Ferrero D, Kreider BL, Rovera G (1986) A suppressor lymphokine produced by human T leukemia cell lines. J Exp Med 163: 18
20. Spits H, Borst J, Giphart M, Cougan J, Terhorst C, De Vries JE (1984) HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. Eur J Immunol 14: 299
21. Taramelli D, Fossati G, Balsari A, Marolda R, Parmiani G (1984) The inhibition of lymphocyte stimulation by autologous human metastatic melanoma cells correlates with the expression of HLA-DR antigens on the tumor cells. Int J Cancer 34: 797
22. Taramelli D, Fossati G, Mazzocchi A, Delia D, Ferrone S, Parmiani G (1986) Classes I and II HLA and melanoma-associated antigen expression and modulation on melanoma cells isolated from primary and metastatic lesions. Cancer Res 46: 433
23. Vanky F, Klein E (1982) Specificity of autotumor cytotoxicity exerted by fresh, activated and propagated human T lymphocytes. Int J Cancer 29: 547
24. Vose BM (1987) Activation of lymphocyte antitumor responses in man: effector heterogeneity and the search for immunomodulators. Cancer Met Rev 5: 299
25. Vose BM, Moore M (1979) Suppressor cell activity of lymphocytes infiltrating human lung and breast tumors. Int J Cancer 24: 579
26. Wilson BS, Imai K, Natali PC, Ferrone S (1981) Distribution and molecular characterization of a cell-surface and a cytoplasmic antigen detectable in human melanoma cells with monoclonal antibodies. Int J Cancer 28: 293

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