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Natural cytotoxicity of lymphocytes and monocytes and its augmentation by OK432 in melanoma patients

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Summary. *Lymphocytes and monocytes from the peripheral blood of 30 patients with malignant melanoma were tested for natural cytotoxicity against K562 cells in a 3-h ^{51}Cr-release assay, and the effects of OK432 (a streptococcal preparation) on the cytotoxicity were examined. The lymphocyte cytotoxicity of melanoma patients was similar to that of normal donors and control patients with benign skin disease. Furthermore, the lymphocyte cytotoxicity of melanoma patients was not correlated to the stage of the disease. Similarly, lysis of K562 cells by monocytes isolated by adherence to autologous serum-coated plastic dishes in melanoma patients was comparable to that of controls and not associated with the stage of the disease. Positive monocyte reactions were recorded in 10 of 30 (33%) melanoma patients, seven of 21 (33%) normal donors and three of 10 (30%) control patients. There was no correlation between lymphocyte cytotoxicity and monocyte cytotoxicity. Overnight treatment of monocytes and lymphocytes with OK432 resulted in an increase in cytotoxicity. Significant augmentation of cytotoxicity by OK432 was observed in 28% of the monocyte samples and 86% of the lymphocyte samples, while partially purified human interferon augmented cytotoxicity in 63% of the monocyte samples and all the lymphocyte samples. These results suggest that neither lymphocyte nor monocyte cytotoxicities are depressed in melanoma patients as compared with normal donors and patients with benign disease and that OK432 has a stronger stimulatory effect on lymphocytes than on monocytes.*

Introduction

Lymphocytes [8, 19] and monocytes [15, 20] from the peripheral blood of normal individuals have been shown to express natural cytotoxicity against a variety of tumor cells and some normal cells. Cumulative evidence suggests that these natural effector cells play an important role in host defense mechanisms against tumors [1, 8]. Both lymphocyte and monocyte cytotoxicities appear to be highly regulated in both positive and negative ways [8]. Interferon (IFN) has been shown to play a central role in the augmentation of natural cytotoxicities [8, 12, 13, 24, 25]. Natural killer (NK) cell activity of blood lymphocytes has been reported to be comparable to that in normal donors in patients with localized tumors but to be impaired in patients with advanced stages of cancer [18, 21]. The incidence of tumor recurrence and survival in melanoma patients seem to be related to the level of their lymphocyte cytotoxicity against melanoma target cells [9]. Although there is evidence of monocyte cytotoxicity in normal donors, little is known about the cytotoxicity of monocyte/macrophages in cancer patients [3, 6, 14]. In these studies monocyte-mediated lytic activity was measured in a long-term (48–72 h) assay. Recently, highly purified monocytes isolated from the peripheral blood of normal individuals by EDTA-reversible adherence to plastic surfaces precoated with autologous serum, but not with fetal calf serum (FCS), have been shown to lyse a variety of tumor cells, including highly NK-sensitive K562 cells in a short-term (3–4 h) assay [5]. Furthermore, the cytotoxicity of these monocytes has been augmented by lymphokines and IFN [4]. No attempts have been made to examine whether K562 cells can be lysed by blood monocytes from cancer patients.

OK432, a heat- and penicillin-treated lyophilized powder of the Su strain of *Streptococcus pyogenes* A3, has been shown to have antitumor activity in cancer patients through stimulation of host immunity [22, 23]. Recently, we reported that OK432 augments NK-cell activity both in vivo and in vitro, independently of IFN induction [24–26]. In mice and rats OK432 has been shown to stimulate the cytotoxic or cytostatic activity of peritoneal macrophages [10, 16]. Our previous report demonstrated that blood monocytes isolated by adherence to FCS-coated plastic dishes cannot kill K562 cells even after treatment with OK432 [24]. It seems of interest to examine whether the cytotoxicity of blood monocytes purified by adherence to autologous serum-coated plastic dishes can be augmented by OK432. The present study was designed to investigate the natural cytotoxicity of peripheral blood monocytes obtained from plastic dishes precoated with autologous serum in melanoma patients in comparison with NK-cell activity of lymphocytes. Furthermore, the in vitro effects of OK432 on monocyte cytotoxicity were compared with those on NK-cell activity of lymphocytes.

Materials and methods

Patients. Of the 30 patients with malignant melanoma selected for this study, 16 had stage I melanoma, eight had stage II and six had stage III. At the time of the study the 18 male and 12 female patients, ranging in age from 29 to 61 years, had never received any anticancer agents. Twenty-one healthy donors were used as roughly age- and sex-matched normal controls. Ten patients suffering from acne served as benign skin disease controls.

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Preparation of lymphocytes and monocytes. Peripheral blood lymphocytes and monocytes were separated according to the method of Fischer et al. [5]. Blood mononuclear cells were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. The mononuclear cells were suspended at a concentration of $3-4 \times 10^6$/ml in RPMI-1640 supplemented with 2 mM L-glutamine, 25 mM HEPES, 100 U penicillin/ml, 100 µg streptomycin/ml, and 10% heat-inactivated FCS (Gibco, Glasgow, Scotland; complete medium). The cells were incubated for 1 h at 37° C in a humidified 5% CO_2 atmosphere in plastic tissue culture plates (no. 3002, Falcon Plastics, Oxnard, Calif., USA) which had been precoated with autologous serum for 15 min at 37° C. After incubation the nonadherent cells were collected from the plates by gentle washing, washed and suspended in complete medium. The preparation contained more than 96% lymphocytes as judged by Giemsa staining and morphology, and was used as lymphocytes. After the plates had been thoroughly washed with RPMI-1640 to remove all nonadherent cells, the adherent cells were collected by incubating the plates with Versene (1 : 5,000, Gibco) for 15 min at room temperature and by gentle scraping with a rubber policeman, washed and suspended in complete medium. The recovered cells contained more than 96% monocytes as judged by peroxidase staining and these were used as monocytes. Every fraction was more than 96% viable according to the trypan blue dye exclusion test.

Treatment with OK432 and IFN. OK432 was supplied by Chugai Pharmaceutical Co., Tokyo, Japan. The unit "KE" is used to express the strength of the preparation, 1 KE corresponding to 0.1 mg dried streptococci [17]. Human leukocyte IFN, partially purified and frozen, was obtained from Immunoloski Zabod, Zagreb, Yugoslavia. The specific activity was 2×10^6 U/mg protein. Lymphocytes or monocytes at a concentration of 1×10^6/ml in complete medium were incubated overnight alone or with OK432 (0.5 KE/ml) or IFN (10^3 U/ml) in plastic tubes (no. 2051, Falcon Plastics) in a humidified 5% CO_2 atmosphere, unless otherwise stated. After incubation the cells were washed and resuspended in complete medium. No significant difference was observed in the recovery of viable cells incubated alone and with OK432 or IFN.

Cytotoxic assay. A 3-h ^{51}Cr-release assay was performed using K562 cells as target, as described in detail elsewhere [24–26]. Briefly, 100 µl of ^{51}Cr-labelled target cells (1×10^4) and 100 µl of effector cells of varying number were added to wells of round-bottom microtiter plates (Nunc, Roskilde, Denmark). The plates were then centrifuged at 150 *g* for 3 min and incubated for 3 h at 37° C in a humidified 5% CO_2 atmosphere. After incubation 100 µl of supernatant was collected and the activity was counted in an auto-gamma scintillation counter. Spontaneous release was determined in wells containing target cells and medium, and maximum release by the addition of Triton X-100. The percentage specific lysis for each individual assay was calculated using the following formula for triplicate samples: % specific lysis = (test cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm) × 100.

Statistical analysis. Results were evaluated for statistical significance by Student's *t*-test and X^2-test. A percentage specific lysis greater than 10% was always statistically significant at $P < 0.05$ by Student's *t*-test and therefore considered to be positive.

Results

Natural cytotoxicity of lymphocytes and monocytes

Lymphocytes and monocytes from the peripheral blood of melanoma patients were tested for cytotoxicity against K562, and compared to normal donors and patients with benign skin disease. The NK-cell activity of blood lymphocytes of melanoma patients ranged from 10.4% to 79.6%, with the mean value of 45.9 ± 2.8% at an effector to target cell ratio (E : T) of 20 : 1 (Fig. 1). This value was comparable to that of normal donors (range, 14.5%–68.2%; mean, 38.2 ± 3.6%) and patients with benign disease (range, 21.7%–73.9%; mean, 43.3 ± 5.4%). Similarly, the natural cytotoxicity of blood monocytes of melanoma patients ranged from 1.0% to 33.6%, and the mean value (11.2 ± 1.7%) was comparable to that of normal donors (range, 1.9%–22.3%; mean, 9.4% ± 1.4%) and patients with benign disease (range, 1.7%–21.7%; mean, 8.8 ± 2.0%). Significant levels of monocyte cytotoxicity over 10% were detected in 10 of 30 (33%) melanoma patients, seven of 21 (33%) normal donors and three of 10 (30%) control patients. There were no differences in the frequencies of positive monocyte cytotoxicity among these groups.

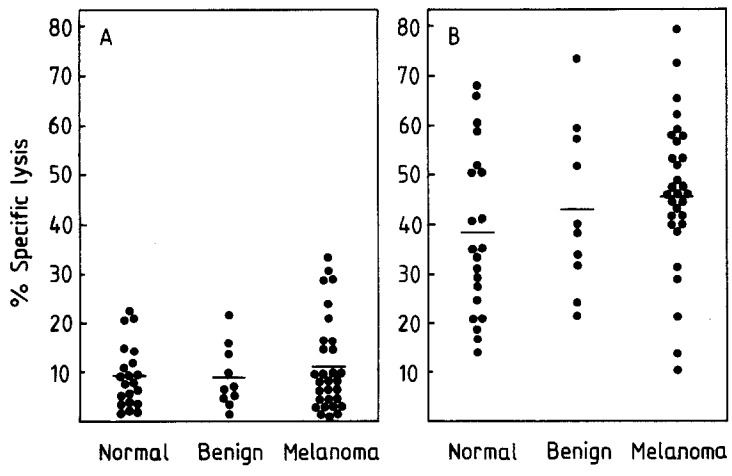

Fig. 1. Cytotoxic activity of blood monocytes **(A)** and lymphocytes **(B)** of melanoma patients and controls. Cytotoxic activity was measured in a 3-h assay at an effector to target cell ratio of 20 : 1. Cytotoxic activity over 10% was considered to be significant. *Horizontal bar* indicates the mean value

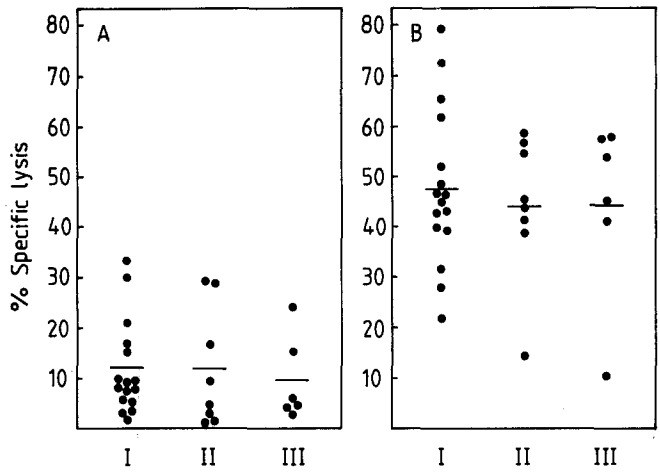

Fig. 2. Cytotoxic activity of blood monocytes **(A)** and lymphocytes **(B)** of melanoma patients at various stages. Cytotoxic activity was measured in a 3-h assay at an effector to target cell ratio of 20 : 1. Cytotoxic activity over 10% was considered to be significant. *Horizontal bar* indicates the mean value

Table 1. Augmentation of monocyte cytotoxicity by different doses of OK432

Experiment	OK432 (KE/ml)	% Specific lysis[a] at an E : T[d] of	
		20 : 1	10 : 1
1	0	20.2	–[c]
	1×10^{-4}	15.9	–
	1×10^{-3}	22.7	–
	1×10^{-2}	29.1[b]	–
	1×10^{-1}	34.3[b]	–
	5×10^{-1}	33.1[b]	–
	1	18.3	–
	5	15.3	–
2	0	10.1	6.2
	1×10^{-4}	13.2	9.4
	1×10^{-3}	13.6	7.7
	1×10^{-2}	12.0	8.4
	1×10^{-1}	13.4	9.9
	5×10^{-1}	10.6	9.0
	1	7.5	5.0

[a] Blood monocytes from normal donors were incubated overnight with various concentrations of OK432, then washed and tested for cytotoxicity in a 3-h assay

[b] The value is significantly higher than that of control cells ($P < 0.05$)

[c] Not tested

[d] Effector to target cell ratio

Natural cytotoxicity in melanoma patients at various stages of the disease

Patients with malignant melanoma were divided into three groups according to the stage of disease. As shown in Fig. 2, the mean value of NK-cell activity of blood lymphocytes was 47.4% ± 4.0% (range, 21.7%–79.6%) at stage I, 43.9% ± 5.4% (range, 14.1%–58.1%) at stage II and 44.1% ± 7.3% (range, 10.4%–57.6%) at stage III at an E : T of 20 : 1. No significant differences were observed in the mean values of NK-cell activity among these groups. Similarly, there were no differences in the monocyte cytotoxicities in stage I (range, 2.0%–33.6%; mean, 12.0% ± 2.3%), stage II (range, 1.0%–29.3%; mean, 11.8% ± 4.2%) and stage III (range, 2.6%–24.3%; mean, 9.5% ± 3.4%) at an E : T of 20 : 1. Significant monocyte cytotoxicity was detected in five of 16 (31%) patients, three of eight (38%) patients, and two of six (33%) patients with malignant melanoma at stage I, II, and III, respectively.

Correlation between lymphocyte cytotoxicity and monocyte cytotoxicity

To ascertain whether an individual showing significant cytotoxic activity of blood monocytes has higher activity of lymphocytes, the cytotoxic activity of monocytes was compared with that of lymphocytes in 21 normal donors and 30 patients with malignant melanoma. As shown in Fig. 3, no correlation between lymphocyte cytotoxicity and monocyte cytotoxicity was observed in melanoma patients ($r = 0.175$) and in normal donors ($r = 0.240$) at an E : T of 20 : 1.

Optimal conditions for augmentation of monocyte cytotoxicity by OK432

Blood monocytes from normal donors were incubated overnight with various concentrations of OK432, then washed and tested for cytotoxicity against K562 cells. Results of repre-

Table 2. Kinetics of augmentation of monocyte cytotoxicity by OK432

Preincubation period	% Specific lysis[a]	
	Medium	OK432
2 h	11.9	11.2
4 h	12.7	16.8
16 h	13.6	25.4[b]
20 h	14.6	26.9[b]
24 h	15.2	25.6[b]

[a] Blood monocytes from a normal donor were incubated with or without OK432 (0.5 KE/ml) for 2–24 h, then washed and tested for cytotoxicity in a 3-h assay at an effector to target cell ratio of 20 : 1

[b] The value is significantly higher than that of corresponding control cells ($P < 0.05$)

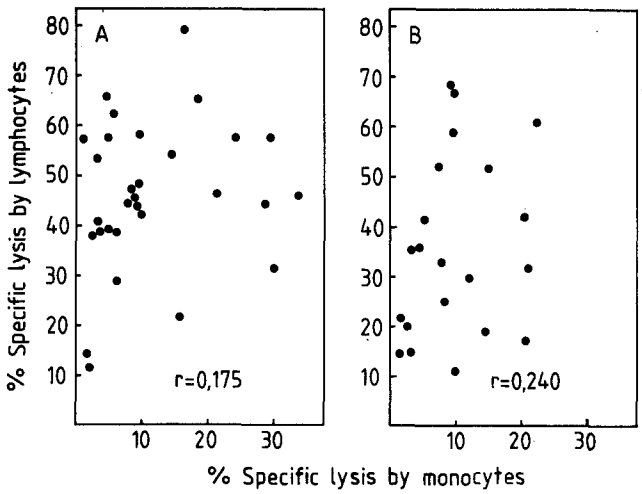

Fig. 3. Correlation between monocyte cytotoxicity and lymphocyte cytotoxicity in melanoma patients **(A)** and normal donors **(B)**. Cytotoxic activity was measured in a 3-h assay at an effector to target cell ratio of 20 : 1

sentative experiments are shown in Table 1. Significant augmentation of cytotoxicity was observed when monocytes were treated with doses of 1×10^{-2}, 1×10^{-1}, and 0.5 KE/ml of OK432 (experiment 1). On the other hand, it was also observed that treatment of monocytes with any doses of OK432 failed to augment cytotoxicity (experiment 2). A kinetics study in which monocytes were preincubated with OK432 (0.5 KE/ml) for time intervals varying from 2 to 24 h demonstrated that significant augmentation of cytotoxicity was detected after 16, 20, and 24 h preincubation with OK432, while monocytes preincubated in medium in the absence of OK432 showed no significant change in lytic function during 24 h (Table 2).

Table 3. Augmentation of cytotoxic activity of blood monocytes and lymphocytes by OK432 in normal donors

Experiment	% Specific lysis[a] by monocytes		% Specific lysis by lymphocytes	
	Medium	OK432	Medium	OK432
1	14.9	28.9[b]	51.6	72.7[b]
2	5.3	3.7	41.4	65.6[b]
3	10.1	10.6	31.7	64.2[b]
4	8.3	10.4	21.1	38.9[b]
5	22.3	31.1[b]	60.8	72.5[b]
6	7.6	10.5	51.9	69.7[b]
7	8.1	11.3	32.9	45.7[b]
8	10.9	7.1	27.8	47.4[b]
9	1.9	5.9	–[c]	–
10	6.9	13.8[b]	–	–
11	2.7	5.6	–	–

[a] Blood monocytes and lymphocytes were incubated overnight with OK432 (0.5 KE/ml) or medium alone, then washed and tested for cytotoxicity in a 3-h assay at an effector to target cell ratio of 20:1

[b] The value is significantly higher than that of control cells ($P < 0.05$)

[c] Not tested

Table 4. Augmentation of cytotoxic activity of blood monocytes and lymphocytes by OK432 in patients with benign disease

Donor	% Specific lysis[a] by monocytes		% Specific lysis by lymphocytes	
	Medium	OK432	Medium	OK432
1	13.9	24.6[b]	52.3	63.6[b]
2	6.2	8.9	39.1	46.9[b]
3	9.8	17.1[b]	21.7	37.8[b]
4	4.7	4.5	31.5	39.6[b]
5	1.7	3.3	23.9	21.4
6	15.9	22.4[b]	40.0	58.6[b]
7	6.5	5.1	57.4	65.7[b]
8	3.4	4.9	73.9	73.9

[a] Blood monocytes and lymphocytes were incubated overnight with OK432 (0.5 KE/ml) or medium alone, then washed and tested for cytotoxicity in a 3-h assay at an effector to target cell ratio of 20:1

[b] The value is significantly higher than that of control cells ($P < 0.05$)

Augmentation of cytotoxic activity of lymphocytes and monocytes by OK432

Blood lymphocytes and monocytes were incubated overnight alone or with OK432 (0.5 KE/ml), then washed and tested for cytotoxicity against K562 cells. There was no difference in cytotoxicity between freshly prepared effectors and effectors cultured overnight with medium alone (data not shown). An

Table 5. Augmentation of cytotoxic activity of blood monocytes and lymphocytes by OK432 in melanoma patients

Donor	% Specific lysis[a] by monocytes		% Specific lysis by lymphocytes	
	Medium	OK432	Medium	OK432
1	8.3	15.9[b]	44.5	61.6[b]
2	15.6	17.1	21.7	29.2[b]
3	4.7	6.4	65.6	62.5
4	8.9	12.5	45.4	55.7[b]
5	9.3	10.8	47.9	65.7[b]
6	21.1	36.7[b]	46.5	76.9[b]
7	3.7	9.0	39.0	48.9[b]
8	24.3	21.6	57.6	80.7[b]
9	29.3	30.4	54.5	66.2[b]
10	28.8	38.7[b]	44.3	77.9[b]
11	9.5	10.4	42.7	70.5[b]
12	4.7	5.4	47.5	47.1
13	30.2	39.5[b]	–[c]	–
14	9.0	13.7	–	–
15	8.4	9.5	–	–
16	1.9	5.7	–	–
17	5.7	6.7	–	–

[a] Blood monocytes and lymphocytes were incubated overnight with OK432 (0.5 KE/ml) or medium alone, then washed and tested for cytotoxicity in a 3-h assay at an effector to target cell ratio of 20:1

[b] The value is significantly higher than that of control cells ($P < 0.05$)

[c] Not tested

Table 6. Effects of OK432 and IFN on cytotoxic activity of blood monocytes and lymphocytes

Experiment	% Specific lysis[a] by monocytes			% Specific lysis by lymphocytes		
	Medium	OK432	IFN	Medium	OK432	IFN
1	14.9	28.9[b]	17.8	51.6	72.7[b]	66.5[b]
2	8.3	10.4	12.6	21.1	38.9[b]	36.7[b]
3	7.6	10.5	16.3[b]	51.9	69.7[b]	60.8[b]
4	8.1	11.3	17.7[b]	32.9	45.7[b]	42.8[b]
5	1.9	5.9	11.2[b]	–[c]	–	–
6	15.9	22.4[b]	26.3[b]	40.0	58.6[b]	52.2[b]
7	8.3	15.9[b]	10.0	44.5	61.6[b]	56.2[b]
8	3.7	9.0	12.3[b]	39.0	49.8[b]	48.0[b]

[a] Blood monocytes and lymphocytes from normal donors (expts. 1–5), a control patient (expt. 6) and melanoma patients (expts. 7, 8) were incubated overnight with medium, OK432 (0.5 KE/ml) or IFN (10^3 U/ml), then washed and tested for cytotoxicity in a 3-h assay at an effector to target cell ratio of 20:1

[b] The value is significantly higher than that of cells cultured with medium alone ($P < 0.05$)

[c] Not tested

induction of monocyte cytotoxicity was observed in one of seven normal donors who had no spontaneous cytotoxicity, and an augmentation was observed in two of four individuals who showed significant monocyte cytotoxicity at an E : T of 20 : 1 (Table 3). In total, monocyte cytotoxicity was induced or augmented by OK432 in three of 11 (27%) individuals, while lymphocyte cytotoxicity was enhanced in all eight individuals studied. The frequency of positive augmentation by OK432 was significantly lower in monocyte cytotoxicity than in lymphocyte cytotoxicity ($P < 0.01$). In patients with benign disease, monocyte cytotoxicity was induced by OK432 in one of six previously nonreactive cases and augmented in the two previously reactive samples, whereas lymphocyte cytotoxicity was enhanced in six of eight patients (Table 4). In melanoma patients, as shown in Table 5, OK432-induced monocyte cytotoxicity was observed in only one of 11 patients and cytotoxicity was augmented in three of six patients. In total, monocyte cytotoxicity was able to be augmented by OK432 in four of 17 (24%) patients, while lymphocyte cytotoxicity was augmented in 10 of 12 (83%) melanoma patients. The frequency of OK432-augmented monocyte cytotoxicity was significantly lower than that for lymphocytes ($P < 0.01$).

Effects of OK432 and IFN on cytotoxic activity of lymphocytes and monocytes

To find out whether the effect of OK432 on monocyte cytotoxicity is comparable to that of IFN, blood lymphocytes and monocytes were incubated overnight with medium, OK432 (0.5 KE/ml) or IFN (10^3 U/ml), then washed and tested for cytotoxicity. Although OK432 and IFN augmented lymphocyte cytotoxicity in more than 85% of the samples tested, significant augmentation of monocyte cytotoxicity was observed in five of eight (63%) samples by IFN (Table 6) and in 10 of 36 (28%) samples by OK432 (Tables 3–5). However, OK432 was able to augment monocyte cytotoxicity in cases where IFN failed to increase monocyte cytotoxicity (experiments 1 and 7 in Table 6).

Discussion

The present study confirmed that the NK-cell activity of peripheral blood lymphocytes of melanoma patients is comparable to that of normal donors and patients with benign skin disease. Furthermore, the NK-cell activity of melanoma patients was found not to be correlated to the stage of the disease. In contrast, it has been demonstrated that NK-cell activity is not impaired in various cancer patients with localized tumor, but depressed in patients with advanced disease [18, 21]. Although the reason why melanoma patients have considerable NK-cell activity even in stage III is not clarified in the present study, it seems likely that most of our patients with stage III had no metastasis to visceral organs and therefore showed normal levels of NK-cell activity.

It has been shown that blood monocytes recovered from plastic dishes precoated with FCS do not lyse highly NK-susceptible K562 cells [5, 24]. Recently, monocytes obtained from plastic dishes precoated with autologous serum have been reported to kill K562 cells in normal donors [5]. In the present study lysis of K562 by blood monocytes isolated by adherence to autologous serum-coated plastic dishes was observed in 33% of normal donors. Since K562 cells are highly sensitive to lysis by NK cells, the possibility that the contaminating lymphocytes are responsible for the observed monocyte cytotoxicity cannot be completely ruled out in this study. However, there was no correlation between monocyte cytotoxicity and lymphocyte cytotoxicity. In addition, our monocyte preparation with less than 4% contaminated lymphocytes sometimes expressed higher reactivity to K562 than the value calculated from the parallel determination of lymphocyte cytotoxicity. Furthermore, we have observed that OK432 or IFN fails to augment monocyte cytotoxicity in cases where NK-cell activity of lymphocytes is markedly enhanced by these agents.

In the present study we first demonstrated that blood monocytes of cancer patients isolated by adherence to autologous serum-coated plastic dishes express considerable levels of cytotoxicity against K562 in a 3-h assay. The mean value of monocyte cytotoxicity and the frequency of positive reactions in melanoma patients was comparable to that of normal donors and patients with benign disease. Furthermore, monocyte cytotoxicity of melanoma patients was found not to be correlated to the stage of the disease. Our findings are in agreement with other observations that the cytotoxic activity of blood monocytes is not depressed in patients with various types of tumors [3, 6], but differ from another report that circulating monocytes of ascites ovarian cancer patients show lower cytolytic activity than monocytes of normal donors in a 48-h assay, although by 72 h there is little difference between normal donors and cancer patients [14]. On the other hand, we have previously observed that the cytostatic activity of monocytes of cancer patients is neither impaired nor correlated to the stage of the disease [27, 28]. The cytostatic activity of monocytes has also been shown to be higher in breast and lung cancer patients (especially in patients with disseminated disease) than in normal donors [11]. These conflicting results may be due to differences in the disease and in the assay system used to evaluate the antitumor activity of monocytes.

We have recently shown that OK432 enhances in vitro the NK-cell activity of lymphocytes and the maximum enhancement is observed when lymphocytes are cultured overnight with a dose of 0.5 KE/ml of OK432 [24, 25]. Since similar results were obtained in monocyte cytotoxicity, lymphocytes and monocytes were incubated overnight with a dose of 0.5 KE/ml of OK432, and then tested for cytotoxicity. It has been confirmed that monocyte cytotoxicity is augmented by OK432 in 28% of specimens, although OK432 augments lymphocyte cytotoxicity in most (86%) cases. The frequency of OK432-augmented monocyte cytotoxicity was significantly lower that of OK432-enhanced lymphocyte cytotoxicity in normal donors and melanoma patients. These results suggest that OK432 has a stronger stimulatory effect on nonadherent lymphocytes than on monocytes. Similar results have been observed by other investigators (Mantovani A, personal communication).

The mechanisms by which OK432 enhances monocyte cytotoxicity are not clarified in this study. IFN has been shown to play a major role in the activation of monocytes [4, 12, 13]. It has also been reported that lymphokines other than IFN can augment the tumoricidal activity of human monocytes [2, 7, 13]. It seems likely that OK432-induced augmentation of monocyte cytotoxicity is independent of IFN for the following reasons. The supernatant produced by cultures of monocytes and OK432 contained no detectable amount of IFN and did not augment monocyte cytotoxicity (data not shown). OK432 augmented monocyte cytotoxicity in cases where IFN failed to do so. In addition, OK432 has been demonstrated to enhance NK-cell activity independently of IFN induction [24–26].

In conclusion, the present study demonstrated that the natural cytotoxicities of lymphocytes and monocytes of melanoma patients are comparable to those of normal donors and patients with benign disease. In addition, there were no differences in the reactivities to OK432 in these groups. However, macrophages in regional lymph nodes and in the site of tumor growth will also have to be examined to understand better the role of monocyte/macrophages in host resistance against tumors. Such studies are currently in progress.

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