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Abstract

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**ALLOGENEIC INHIBITORY ACTIVITY OF REGIONAL
LYMPH NODE CELLS IN THE MOUSE ISOGRAFTED
WITH METHYLCHOLANTHRENE-INDUCED TUMOR**

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Abstract: In mouse bearing progressive cancer a decrease was present in the allogeneic inhibitory activity of T-lymphocytes, which constitutes the core of immunological surveillance system in mammals. For tests, methylcholanthrene-induced tumor (MC-tumor) was isografted subcutaneously on the back between scapulae of C3H mice, and the lymphocytes were prepared from the regional axillary lymph nodes removed from these mice at 1, 2, 3, or 4 weeks after grafting. These lymph nodes cells were cultured together with 40-fold numbers of allogeneic JTC-11 cells derived from Ehrlich cancer cells in a culture medium containing 2.0% (v/v) PHA for 24 or 48 hours. The proliferation rate of JTC-11 cells (increased numbers) at weekly interval was considered the allogeneic inhibitory activity of lymph node cells. As a result it was demonstrated that in the early stage after tumor transplantation, i. e., in the first or second week, regional lymph node cells showed a strong allogeneic inhibitory activity, as in the case with lymph-node cells from normal mice, but at progressive stage of cancer, i. e., the third or fourth week when tumors were larger, such activity was completely lost. It seems that mice with progressive cancer showed a decrease of allogeneic inhibitory activity, i. e., a disruption of homeostasis was present.

In our previous study of cell-mediated immunity of the cancer bearing body from non-specific and specific aspects we reported that the cell-mediated immunity is decreased in progressive cancer (1, 2).

This time we studied the correlation between the change in allogeneic inhibitory activity of lymphocytes, which belongs to the immunological surveillance system (3), and the degree of cancer progress, and report that such an allogeneic inhibitory activity decreased just as the lymphocytotoxic activity of lymphocytes to target tumor cells decreases in progressive cancer.

MATERIALS AND METHODS

Animals: The animals used were C3H male mice 6-10 weeks old procured from The Mouse Colony of Okayama University.

Methods of successive maintenance of tumors: Tumors are maintained by successive subcutaneous transplantations of methylcholanthrene-induced tumor (MC-tumor) obtained after injecting the arabian gum suspension containing 1 mg of 20-methylcholanthrene on the back of mice. MC-induced tumors obtained at the sixth-eighth generations of successive transplantation were used for the experiment.

Allogeneic target cells: JTC-11 cells derived from Ehrlich cancer cells and being maintained at Department of Pathology, Okayama University, were used as allogeneic target cells.

The preparation of lymphocyte suspension: After taking out lymph nodes aseptically and slicing them into small pieces, they are passed through the 150-mesh filters, the filtrate is centrifuged at 1,000 rpm for 10 min. and suspended in the YLE solution (containing 30 μ /ml Cephalothin) supplemented with 20% bovine serum to make the lymph node cell suspension.

Preparation of phytohemagglutinin (PHA): Immediately before use, one vial (50mg) of phytohemagglutinin-M (Difco) was dissolved in 5ml YLE solution and this serves as 100% (v/v) PHA solution.

Isografting of MC-tumor: Forty C3H mice are divided into four groups, and 1 mm³-piece of MC-tumor is transplanted subcutaneously on the back between the scapulae to each group of 10 mice at an interval of one week. After lapse of one week from the last transplantation, i. e. at the time when all the four groups of the first, second, third and fourth groups after MC-tumor transplantation are ready for the test, 5 mice having tumors of similar size are selected from each group, and under the ether anesthesia regional axillary lymph nodes are extracted aseptically from each group of mice, and finally lymph node cell suspensions are prepared from each group.

Estimation of allogeneic inhibitory activity: This is done according to the method of KONAGA (4). Lymph node cells and JTC-11 cells are mixed in the ratio of 40:1, i. e. the mixture is 80 \times 10⁴ cells: 2 \times 10⁴ cells/ml and to the mixture is added 2.0% (v/v) of PHA to make the final volume 10ml. The mixed solution is put into 6 short test tubes each to contain 1.5ml of it and the stationary culture is carried out at 37°C by the method of EVANS *et al.* (5).

At 24 and 48 hours after the start of culture, three short test tubes are picked out at random from each group, by decanting the culture medium, 1.5ml crystal violet solution (composed of 2.1g citric acid and 50mg of crystal violet dissolved in 100ml of distilled water) is added to each tube, warmed to 37°C for 30min. Cells on the test tube wall are scraped off gently with a cleaner, and after shaking well the nuclear counts of tumor cells are taken of each test tube several times with Bürker-Türk hemocytometer. Those cells with a thin nucleus showing distinct nucleoles are taken as tumor cells.

RESULTS

Effects of the proliferation of MC-tumor after its isografting on the allogeneic inhibitory of the host are as shown in Table. After the sub-

TABLE. ALLOGENEIC INHIBITORY ACTIVITY OF REGIONAL LYMPH NODE CELLS IN THE MOUSE ISOGRAFTED WITH MC-TUMOR

lymph node cells	PHA	No. of JTC-11 cells after culture* (Mean \pm S.E.**)		t-test***
		24 hr	48 hr	
1 week after isografting	+	3.17 \pm 0.40	8.63 \pm 0.66	0.7 < P < 0.8
2 week after isografting	+	2.97 \pm 0.32	7.96 \pm 0.83	0.6 < P < 0.7
3 week after isografting	+	3.93 \pm 0.51	12.69 \pm 1.19	P < 0.001
4 week after isografting	+	3.90 \pm 0.29	11.12 \pm 1.03	P < 0.001
normal lymph node cells (control)	+	3.13 \pm 0.28	8.25 \pm 0.59	
JTC-11 cells alone	-	4.31 \pm 0.28	11.94 \pm 0.64	

* $\times 10^4$

** Standard error

*** Significant difference between control group by Student's t-test.

cutaneous transplantation of 1 mm³ MC-tumor, the tumor grows to the size of a rice grain one week later, by two weeks it is as big as small finger tip, by 3 weeks it is the size of thumb head, and by the fifth to sixth week the animals die of tumor. The tumors at the third to fourth post-transplantation week correspond to advanced stage. The allogeneic inhibitory activity of regional axillary lymph node cells of cancer-bearing mice at relatively early stage of one to two weeks after the isografting against JTC-11 cells is as strong as that of normal lymphocytes, showing a strong suppression of the proliferation of JTC-11 cells. However, the allogeneic inhibitory activity of regional lymph node cells 3-4 weeks after transplantation is markedly disturbed, showing not any inhibitory effect on the proliferation of JTC-11 cells, and there can be observed an increase in the number of JTC-11 cells just as in the case of the culture of JTC-11 cells alone (control group) or mixed culture of normal lymph node cells and JTC-11 cells in the absence of PHA. In other words, the allogeneic inhibitory activity of the lymph node cells at the third or fourth week is completely lost.

DISCUSSION

As for the non-specific functions of T-lymphocytes in the cancer bearing body the studies in the past were concerned with the skin reactions *in vivo*

such as tuberculin reaction and DNCB reaction (1), and *in vitro* the blastformation rate of lymphocytes against allogeneic cells or the blastformation rate to PHA, and in either instance it is reported that the blastformation rate is decreased in progressive cancer (6). However, there is as yet no report about allogeneic inhibitory activity that is one of the important functions of T-lymphocytes. Allogeneic inhibition has been noticed relatively recently by MÖLLER (7), and under certain culture conditions it destroys allogeneic cells by inducing non-immune lymphocytes to adhere and aggregate non-specifically onto allogeneic cells. It is considered that this allogeneic inhibition is the activity possessed by all somatic cells including lymphocytes in order to eliminate any undesirable cells that have deviated from their normal activity and it is believed to play an important role in the immunological surveillance system of the body.

On the other hand, lymphocytotoxicity or immune lymphocytes, one of the most representative of the specific cell-mediated immunity, is also initiated by the adherence and aggregation of lymphocytes onto target cells. The similarity between the mechanism of the destruction of allogeneic cells by non-immune lymphocytes and the mechanism of destruction of specific target cells by immune lymphocytes has already been reported in detail by KONAGA (8), and there appears to be a quite similarity between the two mechanisms.

In the present experiment likewise observations were made on the lymphocytotoxicity in the mixed cultures of regional axillary lymph node cells prepared from C3H mice to which the MC-tumor of the same strain of animals was transplanted subcutaneously on the back and with lapse of one, two, three, and four weeks after transplantation. As a result a strong cytotoxic activity being generated in the regional lymph node cells was observed at a relatively early stage of the first or the second week, whereas by the third or fourth week when the tumor had grown larger, i. e. in the advanced stage, such an activity was completely lost as mentioned by our previous study (9).

The change in the specific cytotoxic activity of immune lymphocyte and that in the allogeneic inhibitory activity are well correlated.

In the background of the decreasing tendency of specific cell-mediated immunity in the progressive cancer there can be assumed a decrease in allogeneic inhibitory activity. The mechanisms of such an activity decline are quite complex, but the loss of such an allogeneic inhibition means an irreparable deviation of homeostasis, hence indicating the worsening of progressive cancer.

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