Treatment of spontaneous murine lymphomas with syngeneic lymphoid cells[1]

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Summary. Syngeneic thymus grafts and spleen cells were administered to thymectomized and intact (C57BL/1XA)F_1 mice with spontaneous lymphomas. Their life span was prolonged significantly compared to untreated tumor-bearing controls. Dramatic clinical and histologic evidence of tumor regression was observed.

The (C57BL/1XA)F_1 ((BA)F_1) strain of mice has a very low incidence of spontaneous lymphomas until old age (table 1). However neonatally thymectomized (Tx) mice of this strain manifested a much higher incidence of lymphoma (table 1)[2], had impaired cellular immunity[3], and when tumors arising in such Tx mice were transplanted into intact syngeneic 3-week-old hosts, the acceptance rate was significantly lower than for tumors obtained from intact (BA)F_1 mice[3]. These results suggested that spontaneous lymphomas of Tx mice were more strongly antigenic than those of intact mice. It therefore seemed possible that transfer of T cells into Tx-tumor-bearing mice might cause regression of the tumor.

Materials and methods. (BA)F_1 mice were thymectomized within 24 h of birth. A panel of intact (BA)F_1 mice was set up for comparison. The panels of Tx and intact mice were each divided into 2 subgroups. Each of the 4 subgroups was a closed population. All mice within each subgroup which developed lymphomas, up to 24 months of age, constituted 1 of 4 experimental groups. Mice were examined weekly for evidence of tumor. A peripheral lymph node 5 mm or more in diameter, a definitely palpable (under ether anesthesia) mesenteric node or a spleen over 300 mg in mass[4] was considered evidence of lymphoma. This diagnosis was confirmed where possible by biopsy or laparotomy. Grafting was usually carried out within 2 days of tumor diagnosis. 2 thymuses from 1-month-old syngeneic mice of the same sex were grafted s.c. in the axilla, and simultaneously, a suspension of spleen cells from the same donors was injected i.p. Grafted mice were observed up to 4 times daily and examined twice weekly. The grafting was repeated if tumor enlargement or lack of regression was observed, but no more frequently than monthly. Control mice were subjected to incision only, without grafting. Autopsies were performed on terminal and dead mice.

Results and discussion. The survival times of the Tx and intact group, nongrafted and grafted, respectively, were similar so the data was pooled (table 1). The overall mean survival time for 56 nongrafted mice was 65 days, and for 30 grafted mice, 201 days ($p < 0.01$). The pooled data for the peripheral node category was also highly significant ($p < 0.01$); the median survival time was increased from 95 to 510 days. Clinical and/or pathological evidence of antitumor effect was evident in 29 of 30 of the grafted mice. Indeed, this was the most striking evidence of the effect of grafting. Clinical evidence consisted of definite regression or disappearance of lymphoma-involved lymph nodes or a definite decrease in size of an enlarged spleen. Pathologic evidence involved decrease in size or disappearance of tumor and the presence of focal or massive necrosis of tumor. In the presence of multifocal tumor, both the clinical and pathologic changes were usually selective for particular nodes, other nodes showing no changes whatsoever. These changes did not occur in the tumors of untreated control mice.

Some examples will illustrate dramatically the response to this mode of therapy. At age 11.5 months, a Tx-(BA)F_1-mouse presented with 3 enlarged lymph nodes: a neck node of 10, an axillary node of 7, and an inguinal node of 5 mm in diameter. Excisional biopsy of the axillary node revealed reticulum cell sarcoma. The neck node showed slight transitory regression after the 1st graft, but a 2nd graft 2 months after the 1st resulted in marked regression to about 3 mm in diameter. A3rd graft 5 months after the 2nd

Table 1. Cumulative lymphoma incidence in 77 neonatally thymectomized (Tx) and 149 intact (BA)F_1 mice*

Age (months)	Tx(BA)F_1 No. (%)	Intact (BA)F_1 No. (%)	p** χ^2
4.0	0 (0.0)	0 (0.0)	n.s.
6.0	4 (5.2)	1 (0.7)	< 0.05
8.0	6 (7.8)	1 (0.7)	< 0.01
8.5	6 (7.8)	1 (0.7)	< 0.01
10.0	7 (9.0)	3 (2.0)	< 0.02
11.0	7 (9.0)	3 (2.0)	< 0.02
12.0	9 (11.7)	3 (2.0)	< 0.01
14.0	9 (11.7)	3 (2.0)	< 0.01
15.0	12 (15.6)	3 (2.0)	< 0.01
16.0	14 (18.0)	3 (2.0)	< 0.001
16.5	14 (18.0)	3 (2.0)	< 0.001
18.0	16 (20.8)	5 (3.4)	< 0.001
20.0	25 (32.5)	8 (5.4)	< 0.001
22.0	31 (40.3)	12 (8.1)	< 0.001
24.0	36 (47.0)	25 (16.8)	< 0.001

* Incidence based on clinical diagnosis with autopsy proof. Clinically undetectable tumors found in necropsied moribund mice are not included. Incidence also based on number of mice alive at 4 months i.e. in the Tx-group, mice dying from post-thymectomy wasting syndrome are excluded. The original groups consisted of equal numbers of males and females; also there was no sexual predilection in tumor incidence. ** χ^2 test; n.s., not significant.

Table 2. Effect of lymphoid tissue grafts from syngeneic donors on survival times of (BA)F_1 mice with spontaneously occurring lymphomas

Group Initial tumor site	No grafts[a] No. mice	$\bar{\chi}$ survival time (days)	Grafted[b] No. mice	$\bar{\chi}$ survival time (days)	p[c]
Peripheral nodes	11	136.5	7	500.6	< 0.01
Mesenteric node	12	41.0	10	154.6	n.s.
Spleen	24	54.6	2	67.2	n.s.
Generalized[d]	9	35.0	11	76.3	n.s.
Total	56	64.7 ± 8.7[e]	30	200.8 ± 42.9[e]	0.01

[a] 42 neonatally thymectomized and 14 intact mice. [b] 18 neonatally thymectomized and 12 intact mice. [c] t-test; n.s., not significant. [d] More than 1 of above 3 sites. [e] Mean survival time ± SE.

was followed by disappearance of the neck node. 2 months later, tumor recurred in the left thigh muscles. A 4th graft was followed by temporary regression, then enlargement of the thigh mass. The mouse was autopsied when found dying. Large blood containing spaces were present in some areas of tumor, the characteristic appearance of tumors undergoing postgraft regression. Death appeared due to a combination of the effects of a moderate tumor load, tumor necrosis (see below), and renal disease. This mouse survived in good clinical condition for 11 months after the diagnosis of multinodal tumor, more than one third of the life span of the longest survivors of this hybrid (personal observation). 1 Tx and 2 intact mice which presented originally with a single or 2 enlarged peripheral lymph nodes showed complete node regression after a single graft, and remained tumor free for 26, 21, and 25 months respectively. 1 died from a nonlymphomatous tumor and 2 from lymphoma.

2 serious life shortening complications of grafting were observed, 'tumor necrosis syndrome', and hemorrhage. Usually within 1-4 weeks of grafting, a previously healthy mouse rapidly weakened and died within a few h. At autopsy dark red or black foci of varying size were present in the tumor. Microscopically tumor necrosis resulted in large blood-filled spaces (up to 6 mm in diameter) within the tumor. Pathologic evidence for this syndrome was present in 14 of 18 mice in the Tx and in 7 of 12 mice in the control group. The 2nd complication was that of hemorrhage, usually into necrotic tumor. In 1 such case ending in sudden death, a spleen observed to be lymphomatous at laparotomy was converted by the 12th day after a 2nd graft into a 2.0-g sac of blood with only a few islands of cells.

In these experiments, prolongation of survival and regression of tumor was greater following treatment of small tumors than following treatment of large tumors. The longest survivals were obtained with peripheral nodal tumors, probably for this reason. Nevertheless, with serial grafts, some animals survived and appeared healthy for a large proportion of the normal life span of this strain, as a result of only partial regression or absence of progression of a large or multifocal tumor.

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Chemotaxis is not a special case of haptotaxis[1]

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Summary: Serum peptides containing classical anaphylatoxin (CAT) produce marked chemotactic orientation of human neutrophil granulocytes without modifying cell attachment to the substratum. Furthermore gradients of adhesion produced with gammaglobulins fail to induce morphological orientation of neutrophils. The results suggest that chemotaxis is not a special case of haptotaxis.

Various theories have been developed to explain orientation of cells responding to chemotactic stimuli. One such hypothesis implies that the direction of moving metazoan cells is determined by a gradient of adhesion[2] (haptotaxis[3]). Carter[3] produced gradients of adhesion by depositing palladium on cellulose acetate and reported movement along a gradient of increasing substrate adhesion. He suggested that chemotactic mediators might in one way or another form such gradients of adhesion, which direct moving cells by the relative strengths of their peripheral contacts, and that chemotaxis is therefore just a special case of haptotaxis. Such an inference is necessarily based on the reasonable but unproved supposition that chemotactic mediators specifically increase the strength of adhesion between moving cells and the substratum. The following experiments were performed in order to clarify this point.

The results of such experiments are only conclusive if the following 2 requirements are fullfilled: a) the cells studied must be capable of responding to chemotactic mediators and b) the chemotactic agents used must lack chemokinetic

Relation between neutrophil adhesion and chemotaxis

Test material:* Gey's solution containing	% Neutrophils sticking (± SD)	% Neutrophils migrated (± SD) incubation time 1 h	3 h
No addition	54.6 ± 3.7	0.13 ± 0.08	1.9 ± 0.9
HSA (20 mg/ml)	2.8 ± 0.4	0.18 ± 0.04	2.3 ± 0.8
HSA (20 mg/ml) + S-CAT 1.6.1 (4.6 µg/ml)	3.2 ± 1.3	25.2 ± 4.6	50.6 ± 9
HSA (20 mg/ml) + S-CAT 1.6.1 (1.53 µg/ml)	4.9 ± 1.8	29.7 ± 2.0	40.4 ± 4.9
HSA (20 mg/ml) + S-CAT 1.6.1 (0.92 µg/ml)	2.8 ± 1.2	27.8 ± 5.8	48 ± 11.9
HSA (20 mg/ml) + S-CAT 1.6.1 (0.46 µg/ml)	2.3 ± 1.0	21.8 ± 7.1	40.9 ± 10.4
HSA (20 mg/ml) + S-CAT 1.6.1 (0.05 µg/ml)	4.8 ± 0.2	0.58 ± 0.42	5.9 ± 0.6
Standard γ-globulin (20 mg/ml)	65.3 ± 3.6	0.13 ± 0.08	8.4 ± 2

* Neutrophil adhesion was tested using the respective test material as medium for the cells. In contrast, locomotion was tested with cells suspended in 2% HSA in Gey's solution, the respective test material being only present in the lower compartment of the chamber.