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Natural species-restricted attachment of human and murine T lymphocytes to various cells

(natural attachment/self-recognition)

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ABSTRACT **Murine and human T lymphocytes bear on their surface a receptor that confers on them the ability to attach to a variety of target cells from the same species, derived *in vivo* and *in vitro*. Thymocytes and activated T cells attached readily to target cells, while blood T lymphocytes were able to do so only after the removal of sialic acid from either their cell membrane or that of the target cell. The natural attachment (NA) receptor and the corresponding site on the target cells are trypsin sensitive and the conjugation between them is temperature dependent.**

The phenomenon may be a manifestation of self recognition in a broader sense—recognizing the species—which is also reflected in the reactivity of mitogen-activated T cells and specific immune responses against allo- or other antigens expressed on target cell surfaces.

The attachment of T lymphocytes to their target cells is probably the first step in cell-mediated immune processes. Immunization with allogeneic tumors cells leads to generation of T lymphocytes that can attach specifically to the relevant target cells (1, 2). The consequence of this attachment is damage of the target cells (3).

The present study describes receptors on T cells at their various stages of differentiation, which confer the ability to attach to any cell of the same species. Attachment occurred to both lymphoblastoid and monolayer cell lines as well as to cells derived *in vivo*. Because T cells from immunologically unmanipulated donors exhibited the phenomena, we defined it as *natural attachment* (NA), an operational term.

MATERIALS AND METHODS

Lymphoid Cells. (*a*) Human thymocytes. Thymus tissues obtained from patients undergoing open heart surgery were teased gently in minimal essential medium (MEM). (*b*) Human peripheral blood lymphocytes (PBL). PBL isolated from heparinized blood were collected from the top of a Ficoll/Isopaque barrier after centrifugation (4). (*c*) Mouse thymocytes. Cell suspensions were prepared from thymuses obtained from young A/Sn and C57BL mice. The suspensions were washed twice and resuspended at a concentration of 1×10^6 cells per ml in MEM.

Cell Lines. The cell lines used as target cells for the attachment experiments were of two kinds, those carried as monolayers and those carried in suspensions. The monolayers included the human CAMA-1 and SW982 lines (received from J. Fogh, Sloan-Kettering Institute, NY) and the mouse A9HT, IT-22, and SB lines (Table 1). These were grown in MEM supplemented with 10% fetal calf serum (GIBCO), streptomycin (100 μg/ml), and penicillin (100 international units/ml). The remaining lines (Table 1) were grown as suspension cultures in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin.

Human Lung Cells. Malignant and normal lung tissues were obtained during operations on patients bearing lung carcinoma, at the Thorax Clinic in the Karolinska Hospital. The tissues were minced, passed through an iron mesh, and centrifuged on a Ficoll/Isopaque barrier in order to eliminate dead cells. The cells that remained in the barrier were collected, washed, and suspended in MEM. Such suspensions contained over 60% viable cells as indicated by trypan blue staining.

Neuraminidase Treatment. To 10^6 cells suspended in 1 ml of phosphate-buffered saline containing 3 mM $CaCl_2$, 5 units (as defined by the supplier) of *Vibrio cholerae* (BDH, Poole, England) was added. After incubation for 30 min at 37°, the cells were washed twice.

Mixed Lymphocyte Cultures (MLC). For generation of activated T cells, MLC were prepared as described elsewhere (5).

Isolation of Non-T PBL by Removal of Sheep Erythrocyte Rosette-Forming Cells. PBL were allowed to form (E) rosettes with sheep erythrocytes (SRBC) treated with neuraminidase (10 units/ml). The mixture of cells and rosettes was layered on top of a Ficoll/Isopaque barrier, and the test tubes were spun at $800 \times g$ for 30 min. The subset collected from the interphase was washed. On rerosetting, it contained less than 2% lymphocytes with attached SRBC.

Attachment of Lymphocytes to Target Cells. (*a*) Attachment of lymphocytes to target cells in suspension. All attachment experiments, unless otherwise stated, were performed in MEM supplemented with 10% fetal calf serum. Lymphocytes and target cells were adjusted to a concentration of 1×10^6 cells per ml. Lymphocyte aliquots of 0.5 ml were added to 0.1 ml of target cell suspensions in Falcon 2058 tubes. The tubes were centrifuged for 4 min at $200 \times g$, and the pellets were incubated at 37° for 20 min. At the end of incubation the pellets were gently resuspended. For identification of T lymphocytes, 0.1 ml of 1% washed SRBC was added to each mixture. The tubes were spun for 3 min at $200 \times g$ and incubated at 22–24° for 30 min. Following this incubation, the pellets were gently resuspended. The porportion of target cells with attached lymphocyte conjugates (mouse thymocytes and non-T cells) or with attached E-rosetting cells (Fig. 1) was determined by scoring 200 target cells in a counting chamber.

(*b*) Attachment of T cells to target cells in monolayers. The method has been described in detail elsewhere (6, 7). Three hundred target cells were seeded to the wells of Falcon 3034 microplates. Following the removal of the medium, 20-μl aliquots of lymphocyte suspensions (10^6/ml in MEM with 10%

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Abbreviations: E rosettes, rosettes formed with sheep erythrocytes; MEM, minimal essential medium; MLC, mixed lymphocyte cultures; NA, natural attachment; PBL, peripheral blood lymphocytes; SRBC, sheep erythrocytes; T lymphocytes, thymus-derived lymphocytes.

Table 1. Attachment of lymphocytes from T origin to human and mouse cell lines

Species	Cell line	Classification* (ref.)	Attachment, %† Human thymocytes	Human PBL Untreated	Human PBL Neuraminidase	MLC-activated T cells	Mouse thymocytes
Human	K-562	CML (8)	50 ± 24.7	1	20 ± 9.0	20 ± 10.1	0
	Molt-4	ALL (9)	27 ± 17.1	1	15 ± 8.2	16 ± 7.5	1
	Daudi	BL (10)	48 ± 21.0	1	14 ± 7.2	20 ± 6.5	0
	P3HR	BL (11)	22 ± 8.7	1	12 ± 4.5	21 ± 5.8	0
	Namalwa	BL (12)	53 ± 26.1	2	19 ± 6.9	19 ± 9.1	0
	Raji	BL (13)	47 ± 14.6	5	18 ± 8.5	16 ± 6.7	1
	Ramos	BL (14)	75 ± 22.2	3	22 ± 5.3		0
	BJAB	BL (15)	64 ± 19.8	0	20 ± 5.5	22 ± 5.0	0
	1301	ALL (16)	1	0	1	3	1
	CAMA-1	Ma. Car.	84 ± 18.2	4	69 ± 18.9	74 ± 22.1	
	2T‡	OS	88 ± 16.9	4	76 ± 10.3	84 ± 19.7	
	SW-982	FS	75 ± 15.0	0	60 ± 12.3	60 ± 15.5	
Mouse	YAC-1	MVL (17)	0	0	0	2	53 ± 22.9
	YAC	MVL (17)	0	0	0		27 ± 13.1
	RBL-5	RVL (18)	1	0	0	1	21 ± 9.3
	EL-4	BPL (19)	3	0	0	1	34 ± 12.7
	P-815	Mast. (20)	1	0	0	1	17 ± 7.7
	YCAB	MVL (17)	1	0	0	0	40 ± 13.5
	MCP-11	Plast. (21)	1	0	0	0	22 ± 6.0
	136-6‡	Rad. L	1	0	0	1	22 ± 18.5
	RPC-5	Plast. (22)	1	0	0	1	42 ± 20.0
	A9HT	L cells (23)	2	0	1	0	
	IT-22	L cells (24)	5	0	0	2	
	SB‡	FS	0	0	0	4	
Monkey	26CB-1‡	HVL	49 ± 17.3	0			1
	22CM-61‡	HVL	52 ± 20.1	0			2

* CML, chronic myeloid leukemia; ALL, acute lymphocytic leukemia; BL, Burkitt lymphoma, Ma. Car., mammary carcinoma; OS, osteosarcoma; FS, fibrosarcoma; MVL, Moloney virus lymphoma; RVL, Rauscher virus lymphoma; BPL, benzopyrene-induced lymphoma; Mast., mastocytoma; Plast., plasmocytoma; Rad. L., radiation leukemia; HVL, herpesvirus lymphoma.

† The numbers indicate the mean percentage of target cells with attached T lymphocytes (three to five experiments), ± SD.

‡ Line 2T was received from J. Pontén (Department of Pathology, Uppsala, Sweden). Line 136-6 was received from N. Haran-Ghera (Weizmann Institute, Rehovot, Israel). The line SB was received from N. Kuzumaki (Department of Tumor Biology). Lines 26CB- and 22CM-61 were received from D. Johnson (Department of Tumor Biology).

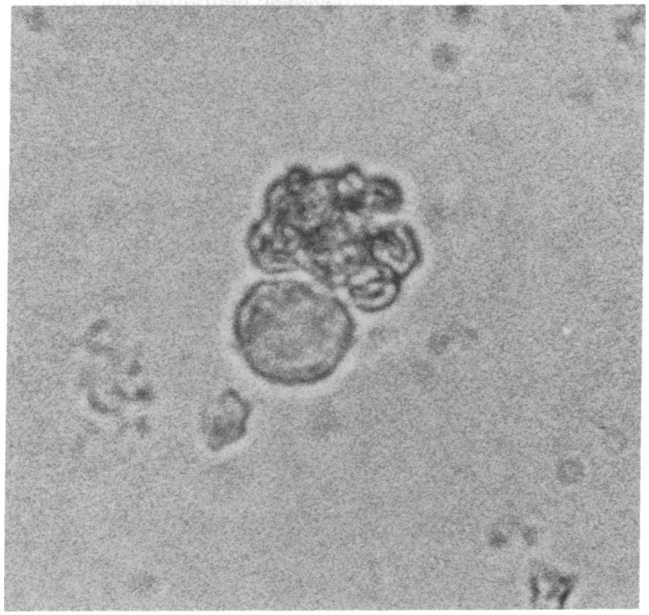

FIG. 1. Conjugate between a human thymocyte and K562. Proof for the T nature of the lymphocyte is the attachment of SRBC. (×400.)

fetal calf serum) were added. The plates were incubated for 20 hr—in order to allow time for sedimentation of lymphocytes—at 37° in a humidified atmosphere containing 5% CO_2. Thereafter the plates were washed in saline to remove the nonadherent lymphocytes. Subsequently, 20 μl of 1% SRBC suspension (in saline) was added to the wells, and the plates were centrifuged for 3 min at 200 × *g* and incubated for 30 min at room temperature (22–24°). At the end of incubation, the plates were carefully washed three times with saline to remove excess SRBC. The T cells attached to the monolayer cells were identified by their E rosette formation. No T cells attached to the bottom of the wells in the absence of target cells. The proportion of target cells to which T lymphocytes attached was determined in the microscope. This test can be performed only with human T cells because their attachment can be identified by the formation of E rosettes.

RESULTS

All except one human cell line formed conjugates with human thymocytes (Table 1). There were practically no target–target or thymocyte–thymocyte conjugates. The attachment was found to be species specific and did not occur between human thymocytes and mouse cells. Conversely, mouse thymocytes attached to mouse and not to human cells. There was no influence of the histocompatibility relationship of lymphocyte and target, because the results with the two mouse strains (A

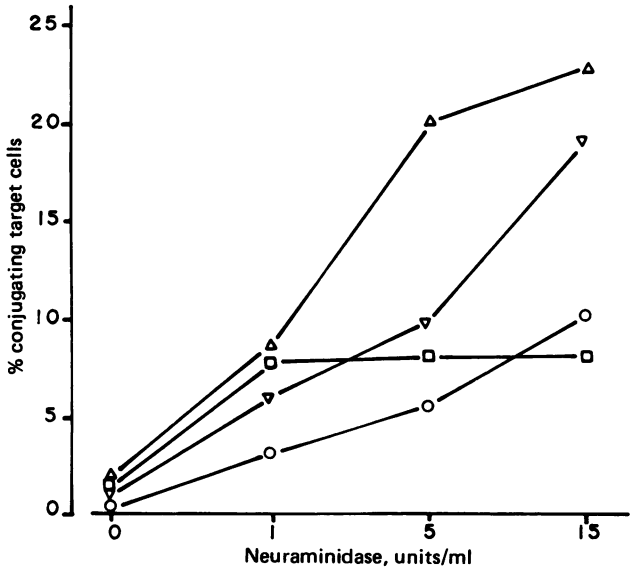

FIG. 2. Conjugate formation between neuraminidase-treated target cells and blood T lymphocytes. ○, Molt-4; △, K-562; ▽, Daudi; □, Namalwa.

and C57BL) were similar. The tumor carried *in vivo* (YAC) conjugated less efficiently with mouse thymocytes than the subline carried in culture (YAC-1). Monkey cell lines formed conjugates with human but not with mouse thymocytes. Blood T cells did not attach unless they were activated in MLC or the sialic acid was removed from their surface by treatment with neuraminidase. In these cases as well, the attachment was species specific. The untreated blood T cells could attach to target cells if these were preincubated with neuraminidase (Fig. 2).

Out of the 12 human lines tested only 1, the acute lymphocytic leukemia line 1301, lacked the ability to conjugate with T cells. Only viable target cells showed the NA phenomenon. Variation in the results probably reflects the condition of the cultures, because these were not standardized.

The non-T subset of PBL was NA negative even after neuraminidase treatment (Table 2).

When thymocytes and target cells were mixed in a counting chamber, the accidental collisions between the two cell types were sufficient for attachment. In order to see the stability of the conjugates, we also vortex-mixed such cell mixtures; the conjugates were not dissociated.

Table 2. Attachment of human non-T PBL to various target cells

Species	Cell line	Attachment, %* Untreated lymphocytes	Attachment, %* Neuraminidase-treated lymphocytes
Human	K-562	2	2
	Molt-4	1	1
	Daudi	1	2
	Namalwa	1	1
Mouse	P-815	1	0
	YAC-1	0	1
	YCAB	1	2
	EL-4	1	0

* The numbers indicate the percentage of target cells with attached lymphocytes and are the means of four experiments.

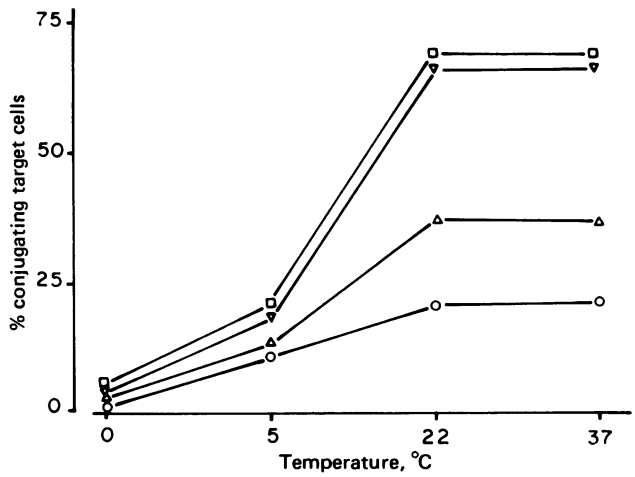

FIG. 3. Conjugate formation between human thymocytes and target cells when confronted at various temperatures. ○, Molt-4; △, K-562; ▽, Daudi; □, Namalwa.

The conjugate formation was temperature dependent; it did not occur at 0°, and at 5° only a few conjugates were formed. No difference was detected between the attachment of thymocytes at 22° and 37° (Fig. 3).

Phosphate-buffered saline contains Ca^{2+} and Mg^{2+} ions. The phenomenon was dependent on their presence. When 2 mM EDTA was added the attachment was almost completely eliminated (Table 3).

Treatment with 0.25% trypsin of the target cells or thymocytes also abolished NA (Table 4). There was a difference between the effect on the target and the thymocyte. Trypsin treatment at room temperature affected the target cells, but the thymocytes had to be treated at 37° to abolish the attaching ability. When the trypsin-treated cells were incubated in medium at 37° for 18 hr, NA recurred. After 7–8 hr conjugates could already be seen.

The phenomenon was not confined to cell lines grown *in vitro*. Both autologous and allogeneic neuraminidase-treated blood T lymphocytes attached to normal and malignant lung cells (Table 5).

In these cases substitution of fetal calf serum with autologous serum in the medium did not influence the results.

Table 3. Attachment of thymocytes to target cells in absence of Ca^{2+} and Mg^{2+}

Species	Cell line	Attachment, %* Phosphate-buffered saline	Attachment, %* Phosphate-buffered saline + 2 mM EDTA
Human†	Molt-4	17	0
	K-562	30	4
	Daudi	63	1
	Ramos	57	0
	Namalwa	48	7
	BJAB	33	1
Mouse†	YAC-1	48	6
	RBL-5	34	7
	EL-4	36	3

* Mean percentage of target cells with attached T lymphocytes (three to five experiments).

† Each cell line was tested with thymocytes from the same species.

Table 4. Effect of trypsin treatment on the ability of thymocytes or target cells to attach and form conjugates

Species	Cell line	Attachment, %* Untreated cells	Trypsinized thymocytes 22°, 20 min	Trypsinized thymocytes 37°, 30 min	Trypsinized target cells 22°, 20 min
Human†	Molt-4	23	21	0	0
	K-562	38	37	0	6
	Raji	40	43	4	3
	Daudi	46	47	0	5
	Namalwa	42	39	3	5
Mouse†	RBL-5	29	—	0	1
	YAC-1	65	—	3	11
	EL-4	47	—	0	1

* Mean percentage of cells with attached T lymphocytes (three to five experiments).
† Each cell line was tested with thymocytes from the same species.

DISCUSSION

We found that human and murine T lymphocytes attach to a variety of cells derived from the same species. This characteristic, which we have termed natural attachment, appears on T cells at early stages of differentiation and could be readily demonstrated by the small lymphocytes that compose the majority of the thymus lymphocytes and populate its cortex (25). The expression of the NA activity seems to be dependent on the amount of negative charge on the cell surface, which is mainly due to the sialic acid portion of the membrane glycoproteins. Cortical thymocytes have been shown to carry low amounts of negative charge (26, 27). There are indications that activated T cells have the same property (5). Blood T cells possess relatively high amounts of negative charge. Once their negative charge or that of the target cells is reduced, attachment can take place, because the repulsion due to electrostatical force is eliminated. We have found that activated T lymphocytes obtained from the synovial fluid of rheumatoid patients also attach in a similar manner (unpublished data).

There was only one exception among the target lines, the acute lymphocytic leukemia line 1301, which did not form conjugates. This line is exceptional in that no lymphocyte surface markers are expressed on it (28). In view of the strict species specificity of the conjugate formation one would question the identity of this line. Karyotype analysis, however, confirmed its human origin (Francis Wiener, personal communication).

The NA phenomenon reflected species relationships, because human thymocytes bound to marmoset monkey lymphoblastoid lines, but mouse thymocytes did not conjugate with these cells.

The responsible sites on both the T and target cells were sensitive to trypsin treatment.

The interaction between the cells is an active process: it did not take place at 0°. The fact that only a portion of target cells bound thymocytes may be due to variation in the expression of the receptor during the cell cycle.

It can be questioned whether the E receptors on T cells are involved in the phenomenon. On the basis of our present findings this is unlikely for the following reasons: (*a*) Divalent cations are needed, in contrast to the E rosette formation (29). (*b*) Treatment of human thymocytes with trypsin (0.25%) at room temperature eliminates the E receptor (28), but this did not affect NA. The relationship between the NA and the T antigen in mouse and in man remains to be defined.

T cells of immunized mice were found to conjugate specifically with allogeneic cells (1, 2). Previously one of us reported specific attachment of peripheral T cells from blood of bladder cancer patients to a bladder cancer line (6). Following an initial antigen-determined specific recognition, the NA surface receptor may contribute to the affinity between the T lymphocyte and the target cell. In fact the NA receptor was found to be exposed on the surface of activated T lymphocytes.

Also, the NA receptor may play a role in mitogen-induced T cell cytotoxicity. Primarily this effect was designated as nonspecific. It has been shown, however, that a species specificity prevails in that effects with reactants from the same species are considerably stronger than those with reactants derived from different species (30). This is also the case for the mixed lymphocyte (31) and graft-versus-host responses (32).

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Table 5. Attachment of T lymphocytes to normal and malignant lung cells

Donor	Target cells	Attachment, % Autologous PBL Untreated	Autologous PBL Neuraminidase	Allogeneic PBL Untreated	Allogeneic PBL Neuraminidase
1	Malignant	1	14	1	48
	Normal	0	13	0	18
2	Malignant	3	12	3	10
	Normal	2	15	1	16
3	Malignant	0	10	0	13
	Normal	0	12	2	15
4	Malignant	1	18	0	14
	Normal	0	13	0	10

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