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Anchorage and lymphocyte function. Antibodies as adhesion and spreading factors for human T lymphocytes

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Summary. When attached to a solid surface coated with protein A various antibodies reacting with lymphocyte membrane antigens (anti-β_2m, OKT3, OKT8, Leu2, 3, 4 and certain patient sera) catalyse the formation of peripheral lamellar activity, i.e. an active spreading process in human T lymphocytes. In contrast, binding only of the same antibodies to the cells or allowing antibody-coated cells to settle and bind to a protein A-coated surface did not induce spreading although the number of cells attached to the solid surface was virtually the same as in the former case. The peripheral lamellar activity markedly facilitated short-range lymphocyte interactions and appeared to constitute the region of the lymphocyte that actively contacts other cells. These results show that antibodies can act as spreading factors, and indicate that this function is critically dependent on the presentation of the inducing ligand. The asymmetry in the induction of active cell edges may influence functional lymphocyte interactions with environmental surfaces.

INTRODUCTION

Lymphocytes, as circulating cells, must interact selectively with the substrata encountered during circulation. Interactions between lymphocytes and accessory cells are required for immune responses. It is well established that non-lymphoid cells interact by means of filopodia (broad-based projections, length 1 to 5 μm) and lamellipodia (flattened lamellar projections). These structures are active cell edges (Vasiliev & Gelfand, 1978) which characterize the spreading process induced by substratum contract in non-lymphoid cells, and which in these cell systems play a crucial role in substrate adhesion (Rajaraman *et al.*, 1974), cell–cell interaction (Vasiliev & Gelfand, 1978), cell migration (Harris & Dunn, 1972) and morphogenesis (Wessels, 1964). It is, however, also likely that filopodia and lamellar activity are important for lymphocyte interactions with cellular and non-cellular surfaces. Thus, when brought into contact with concanavalin A (Con A)- and phytohaemagglutinin (PHA)-coated surfaces under appropriate conditions, peripheral T cells and mature thymocytes show pronounced formation of filopodia and lamellae, whereas immature thymocytes do not (Sundqvist *et al.*, 1980; Otteskog, Friskopp & Sundqvist, 1982; Otteskog & Sundqvist, 1983). The present study is an analysis of the possibility that antibodies may induce spreading of human T lymphocytes. The results obtained demonstrate that antibodies to lymphocyte surface components can function as spreading factors. The most remarkable observation, however, was that the way of presentation of the ligand to the cell determined the degree of spreading.

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MATERIAL AND METHODS

Lymphocytes

Lymphocytes were obtained from defibrinated venous blood of healthy human adults or from normal spleens removed in the preparation for renal transplantation. The mononuclear cells were isolated by gelatin sedimentation followed by treatment with carbonyl iron and a magnet. Adherent cells were removed by incubation in tissue culture flasks in RPMI medium with 10% foetal calf serum (FCS) for 1 hr. Of the leucocytes 98% or more were lymphocytes as judged by May-Grünewald/Giemsa stain. T lymphocyte-enriched and -depleted cells respectively were obtained using sheep erythrocyte rosette sedimentation (E-rosetting) with normal non-treated erythrocytes. The T-enriched cells comprising 83–96% E-rosetting cells were used in the experiments performed to study spreading. Erythrocytes were lysed by addition of 0·83% NH_4Cl. The cells were cultivated ($1{\cdot}5 \times 10^6$/culture) in 1 ml RPMI-1640 medium with 10% FCS in Falcon petri-dishes.

Ligands

OKT3, OKT4 and OKT8 were obtained from Ortho Pharmaceuticals, Raritan, NJ, U.S.A. Leu2, Leu3 and Leu4 was obtained from Becton & Dickinson, Sunnywhale, CA, U.S.A. A rabbit anti-β_2m antiserum was obtained from Dakopatt. Patient sera containing anti-DNA antibodies were selected from the diagnostic determination of anti-tissue autoantibodies at the Department of Clinical Immunology, Huddinge University Hospital. The presence in these sera of antibodies reacting with surface antigens on T lymphocytes was determined by indirect immunofluorescence (IF). Sera containing both anti-DNA antibodies and anti-lymphocyte antibodies or anti-DNA antibodies only were used as inducers of spreading. Sera from healthy individuals at the laboratory which lacked demonstrable anti-lymphocyte as well as other anti-tissue antibodies were used as controls.

Spreading

The antibodies used as adhesion and spreading factors were either attached to the cells by a 30 min incubation period at 4° or room temperature or were bound to a protein A-coated glass cover slip (protein A 100 μg/ml for 30 min at room temperature). The working concentration of each antibody and serum used for induction of spreading was determined by counting the number of attached cells at different antibody dilutions. The antibody dilution corresponding to the plateau end point in number of attached cells was chosen for the experiments. Con A was used at a concentration of 100 μg/ml both for coating of substratum (30 min at room temperature) and when present in the medium during adhesion.

Scanning electron microscopy (SEM)

Cells incubated on glass in the absence and presence of ligand were fixed *in situ* after removal of the medium by addition of an excess of 2·5% glutaraldehyde. The fixed lymphocytes were rinsed in distilled water and dehydrated in a series of acetone dilutions followed by incubation in absolute acetone and critical point drying. The preparations were covered with gold (7 nm) using a Polaron gold sputter and then examined in a Philips S501 scanning electron microscope (grid voltage 15 kV).

Live-cell microscopy

The behaviour of lymphocytes on the substratum was observed continuously in a microscope with phase contrast equipment or interference contrast optics according to Normarski. The cells were allowed to settle in petri-dishes placed in a room with adjustable temperature which was kept at 37°. Photographic exposures were made every 2nd min. The films were analysed by projection on a screen.

RESULTS

Antibodies induce spreading of human T lymphocytes

The capacity of different antibodies reacting with defined antigenic specificities on the lymphocyte surface to induce peripheral lamellar activity in human T lymphocyte enriched mononuclear cells was studied (Table 1). The antibodies were either attached to a solid surface coated with protein A, after which the cells were allowed to settle on this surface, or were bound to the cells which subsequently were allowed to settle on a protein A-coated surface. Con A, which has been applied extensively as an inducer of spreading in previous experiments (Sundqvist *et al.*, 1980; Otteskog *et al.*, 1982; Otteskog & Sundqvist, 1983), was used as a positive control. When bound to a solid surface, the antibodies OKT3, Leu4 and anti-β_2m induced peripheral lamellar activity in 50–90% of the T-enriched

Table 1. Adhesion and spreading of human T cells induced by antibodies to defined cell surface antigens

Coating of substratum	Coating of the lymphocytes	% Attached cells*	% Spread cells†
—	—	<1	6
—	OKT3	<1	9
—	OKT4	<1	5
—	OKT8	<1	7
—	Leu2	<1	8
—	Leu3	<1	5
—	Leu4	<1	5
—	anti-β_2m	<1	8
Prot. A-OKT3	—	70	87
Prot. A-OKT4	—	56	11
Prot. A-OKT8	—	39	21
Prot. A-Leu2	—	36	50
Prot. A-Leu3	—	50	81
Prot. A-Leu4	—	79	92
Prot. A-anti-β_2m	—	77	62
Prot. A	—	6	<1
Prot. A	OKT3	72	8
Prot. A	OKT4	51	4
Prot. A	OKT8	36	5
Prot. A	Leu2	70	15
Prot. A	Leu3	49	8
Prot. A	Leu4	77	11
Prot. A	anti-β_2m	81	5
Con A (also present in the medium)		99	87

* Expressed in % no. of cells allowed to settle.
† Expressed in % no. of attached cells.

mononuclear cells from separate individuals that attached to this surface. Under the same conditions, Leu3 and OKT8 induced lamellar activity in 50–80% and 20–40% of the cells, respectively. It is shown in Table 2 that the spreading capacity was slightly dependent on the concentration of antibodies used for the coating of the substratum. The presence of antibodies in the medium prevented the attachment of lymphocytes to a solid surface coated by the same antibodies. When the monoclonal antibodies first were reacted with the lymphocytes, which subsequently were allowed to settle, the number of attached cells was indistinguishable from the situation when non-antibody-coated cells were allowed to settle on an antibody-coated surface. However, under these conditions, the percentage of antibody-coated lymphocytes showing lamellar surface activity was low for all the specificities, independent of the antibody concentration. When antibody-coated lymphocytes were allowed to settle on a solid surface not coated with protein A, the number of cells showing peripheral lamellar activity was negligible.

Table 2. The effect of antibody concentration on spreading capacity

Specificity	Dilution	% Attached cells	% Spread cells
OKT3	1:10	70	61
	1:50	52	74
	1:100	30	83
	1:200	11	52
OKT8	1:10	28	31
	1:50	25	33
	1:100	22	45
	1:200	14	62

The cells were allowed to settle on a surface coated with protein A and antibodies.

Four patient sera containing antibodies to lymphocyte surface antigens as determined by indirect IF were incubated with protein A-coated surfaces whereafter lymphocytes were allowed to settle. This provoked the formation of peripheral lamellar activity in 10–70% of the attached cells, depending on the serum used. Sera from healthy individuals or from patients lacking anti-lymphocyte antibodies did not induce spreading.

The presence of cytochalasin B 10 μg/ml prevented the antibody-mediated attachment to the substratum and subsequent spreading of T-enriched lymphocytes. When the cells were allowed to settle on an antibody-coated surface at 4°, the number of fully spread cells was reduced from values above 60% to below 10%.

Cell–cell interaction and peripheral lamellar activity

The behaviour of lymphocytes in contact with surfaces coated or not coated with OKT3 antibodies or Con A was monitored by time-lapse microscopy of living cells at different cell densities. In addition, SEM was performed in order to observe details. Provided that the cell density was high enough, the number of cell–cell collisions and established contacts increased markedly during a 1 hr observation period on the ligand-coated surfaces. In contrast, the number of cell–cell contacts was small on the non-coated ones (Table 3). Cells in contact on the ligand-coated surfaces appeared to interact almost exclusively by means of lamellar projections (Fig. 1).

Table 3. Frequency of cell–cell interactions in relation to the presence of catalysing ligand and cell density

Experimental conditions	No. of cells examined	% of the cells in contact			
		$0{\cdot}5 \times 10^6$/culture after		2×10^6/culture after	
		15 min	60 min	15 min	60 min
Con A-coated surface	500	< 1	< 1	2	11
OKT3-coated surface	450	< 1	< 1	1	9
Uncoated surface	200	< 1	< 1	< 1	< 1

T-enriched human lymphocytes were allowed to settle and observed continuously during a 1 hr period.

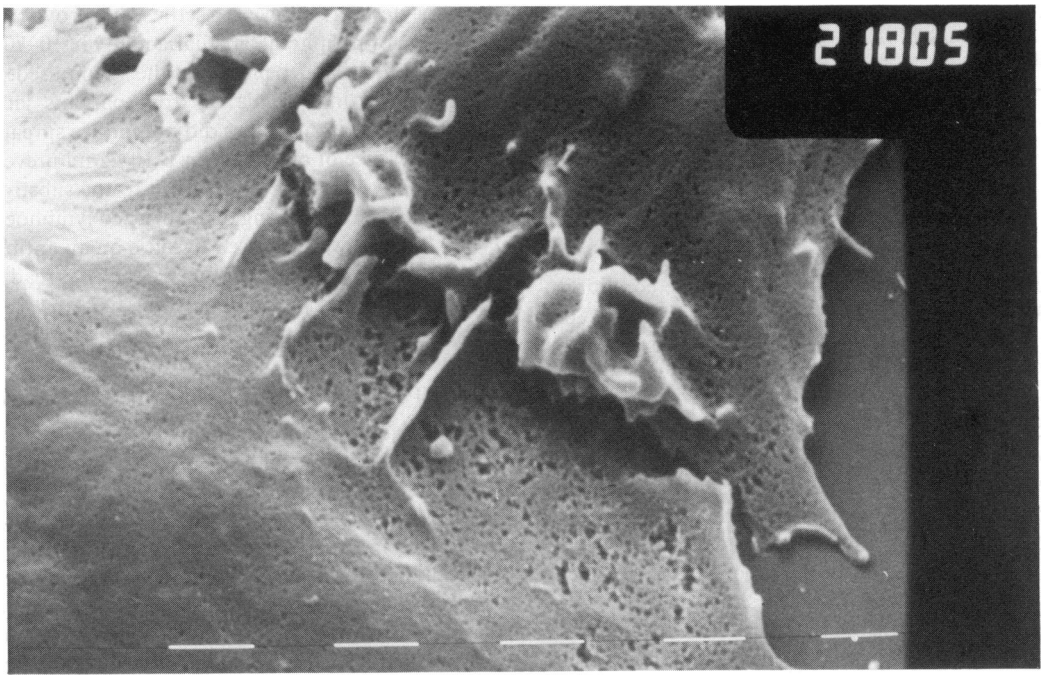

Figure 1. Contact zone between T-enriched human lymphocytes.

DISCUSSION

Lymphocytes, in contrast to fibroblasts and other spontaneously adherent cells, are non-adherent to physiological and artificial substrata. Thus, when allowed to settle on a surface, the majority of lymphocytes are spherical. The present results show that certain antibodies to antigens present on T cells in the same way as Con A can trigger a sequence of cytochalasin and temperature-sensitive motile changes in human lymphocytes. The inducing ligand is in itself insufficient for the induction of spreading. It is the contact with an adhesive surface that initiates this process, but only when the inducing ligand is on this surface before the cells. The results show that the difference in behaviour depending on ligand presentation is primary and not related to the concentration of ligand on the cells or the solid surface. The reason why precoating of cells with ligand abrogates spreading without inhibition of adhesion is not known. One plausible explanation is that antibodies attached to a solid surface exert a more effective cross-linking of lymphocyte membrane antigens. This asymmetry in the induction of spreading has an interesting similarity to ligand-mediated effector–target cell interaction, where binding of ligand to the target but not to the effector cell leads to lysis (Berke *et al.*, 1981; Berke, 1980). These results show that antibodies can function as powerful regulators of adhesion and associated motility in lymphocytes. The capacity of antibodies to provoke a spreading process may reflect specific interaction with cell surface determinants since poly-l-lysine does not cause spreading of T cells although it attaches them to substratum (Sundqvist *et al.*, 1980). The fact that antibodies can trigger inert lymphocytes to express pronounced plasma membrane activity which, as shown by the data, facilitates or is a prerequisite for cell–cell interaction, demonstrates that spreading capacity in lymphocytes may be biologically important. It is also interesting in this context that anti-lymphocyte antibodies in patient sera provoke peripheral lamellae. Thus, it is conceivable that anti-lymphocyte antibodies *in vivo* may provoke lamellar lymphocyte activity, and in this way modulate short-range interactions of the cell with its environment.

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