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ROSETTING OF HUMAN T LYMPHOCYTES WITH SHEEP AND HUMAN ERYTHROCYTES

Comparison of Human and Sheep Ligand Binding Using Purified E Receptor[1]

PERIASAMY SELVARAJ,* MICHAEL L. DUSTIN,* RITA MITNACHT,† THOMAS HÜNIG,† TIMOTHY A. SPRINGER,[2]* and MARIAN L. PLUNKETT*

*From the *Laboratory of Membrane Immunochemistry, Dana Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston MA 02115 and †Genzentrum der Universität München, Am Klopferspitz, D-8033 Martinsried, FRG*

Previous studies have shown that the purified T lymphocyte glycoprotein, cluster differentiation 2 (CD2) (also known as T11, lymphocyte function-associated antigen (LFA)-2, and the erythrocyte (E) rosette receptor) interacts with the LFA-3 molecule on human E. We have examined the interaction of the purified CD2 molecule with the T11 target structure (T11TS) molecule on sheep E, and compared the two interactions. Purified, ^{125}I-labeled CD2 bound to sheep E and the binding was inhibited by anti-T11TS monoclonal antibody (mAb). Reciprocally, the binding of T11TS mAb to sheep E was inhibited by pretreatment of sheep E with purified CD2. High concentrations of purified CD2 aggregated sheep E, possibly by inserting into the membrane, and the aggregation was inhibited by T11TS mAb. The affinity and number of binding sites for purified CD2 on sheep and human E was found to be similar, with K_a of 9×10^7/M and 6×10^7/M and 9800 and 8300 CD2 binding sites/E, respectively. Thus, the human T lymphocyte CD2 molecule is a receptor that cross-reacts between LFA-3 on human E and T11TS on sheep E, suggesting that LFA-3 and T11TS are functionally homologous ligands. As measured by saturation mAb binding, there are 8100 and 3900 ligand molecules/sheep and human E, respectively. Human and sheep E have surface areas of 145 and 54 μm^2, respectively. The 3.2- to 5.6-fold higher ligand density on sheep E appears to account for the ability of sheep but not human E to rosette with certain types of human T lymphocytes.

When human T lymphocytes are held at 4°C with erythrocytes (E)[3] of certain species they are found to adhere to multiple E in rosettes (1, 2). Rosetting requires cocentrifugation of cells and resuspension with a minimum of shear for optimal results and is sensitive to cell-surface charge and other factors (3). Since the early 1970s, immunologists have used E rosetting to purify and enumerate T lymphocytes (1, 2). Sheep E form rosettes with all types of human T lymphocytes; human E form rosettes with thymocytes, activated T lymphocytes, and neuraminidase-treated peripheral blood lymphocytes but not with resting peripheral blood T lymphocytes (4). Rosetting has long been considered a curious laboratory phenomenon, even after the discovery that monoclonal antibody (mAb) to a specific T lymphocyte surface glycoprotein, cluster differentiation 2 (CD2) (T11, lymphocyte function-associated antigen (LFA)-2, E rosette receptor) completely abolishes it (5). Recent work, however, has suggested that CD2 interacts with specific molecules on E, T11 target structure (T11TS) on sheep E and LFA-3 on human E, and that these interactions are relevant to physiologically important pathways of T lymphocyte interaction with target cells and antigen-presenting cells (6, 7).

The L180/1 anti-sheep E mAb was selected for its ability to inhibit rosetting of human T lymphocytes with sheep E (8). This mAb defines a molecule termed the T11TS. The partially purified T11TS molecule inhibits human peripheral blood lymphocytes from rosetting with sheep E and inhibits binding of CD2 (T11) mAb to human T cells. T11TS mAb inhibits the mixed leukocyte reaction in the sheep, suggesting a functional importance of T11TS in T lymphocyte responses (6).

A mAb (TS2/9) against the LFA-3 molecule was identified in humans by screening for mAb that were able to block cytotoxic T lymphocyte-(CTL)-mediated killing (9). LFA-3 mAb inhibited CTL-mediated killing by binding to the target cell; whereas CD2 mAb were found to inhibit killing by binding to the CTL (10). Subsequent studies with mAb and purified molecules demonstrated that CD2 and LFA-3 interact in a receptor-ligand manner to mediate adhesion between effector cells and target cells (11, 12). Purified CD2 was found to bind LFA-3 on B lymphoblastoid target cells and on E (7, 12). Purified CD2 inhibits rosetting with both human and sheep E (7). Similarly, the pretreatment of T lymphocytes with CD2 mAb inhibits rosetting with both human and sheep E (7, 8, 13). Pretreatment of human E with LFA-3 mAb was found to abolish autologous rosetting (7). These findings showed that human autologous rosetting is mediated by the in-

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[2] Address correspondence and requests for reprints to Dr. Timothy A. Springer, Dana Farber Cancer Institute, Dept. of Pathology, 44 Binney St., Boston, MA 02115.
[3] Abbreviations used in this paper: E, erythrocytes; mAb, monoclonal antibody; LFA, lymphocyte function-associated antigen; T11TS, T11 target structure; CD2, cluster differentiation 2; FITC, fluorescein isothiocyanate; DAF, decay-accelerating factor.

teraction between CD2 and LFA-3 (7). Like sheep T11TS (14), human LFA-3 is broadly distributed on E, leukocytes, vascular endothelium, and smooth muscle (9).

These findings suggested that T11TS in the sheep and LFA-3 in humans might be related. Other characteristics of T11TS and LFA-3 neither rule out nor reinforce their relationship. T11TS isolated from sheep E is 42,000 M_r (8) although LFA-3 isolated from human B lymphoblastoid cells and human E is 70,000 (9) and 60,000 M_r (15), respectively.

In the present study, we have examined whether the sheep T11TS molecule and the human LFA-3 molecule function similarly in binding purified CD2. We find the molecules are functionally homologues. Furthermore, to obtain insight into the molecular basis of the much more efficient rosetting of human T lymphocytes with sheep E than human E, we have determined the affinity and number of binding sites of purified CD2 for human and sheep E, and the number of T11TS mAb and LFA-3 mAb binding sites. We report a substantially higher density of ligand on sheep than human E. An accompanying paper describes immunochemical evidence for a structural relationship between LFA-3 and T11TS.

MATERIALS AND METHODS

Cells. T and B lymphoma cell lines were maintained in RPMI 1640 with 10% fetal bovine serum (FBS). Sheep E were received monthly (Colorado Serum Co., Denver, CO) and human E were obtained from healthy human donors.

mAb. mAb were L180/1 (T11TS) (8); TS2/18 (CD2), TS2/9 (LFA-3), and TS1/22 (LFA-1) (9); MI/87.27.7 (Forssman) (16), control P3x63 (myeloma IgG1), E3 (human glycophorin) (17), D44 (CR1) (18), and 1A10 (DAF) (19).

Membrane proteins. CD2 was purified to homogeneity from Jurkat or SKW3 T lymphoma lines by mAb affinity chromatography as previously described (20). CD2 was eluted from TS2/18 CD2 mAb-Sepharose column with 0.1 M glycine-HCl buffer, pH 2.75, containing 0.2 M NaCl and 0.2% Triton X-100. LFA-1 was purified from the same SKW3 cell lysate by using a TS1/22-Sepharose column linked in series to the CD2 mAb-Sepharose column and was eluted under identical conditions.

All the experiments with soluble CD2 were carried out in the presence of bovine serum albumin (BSA, which binds detergent (21)) in order to prevent the damage of cells by the detergent present in the CD2 preparation. The final concentrations of Triton X-100 was <0.05%. Under the experimental conditions we have used no cell lysis was observed.

Preparation of ^{125}I-CD2. Purified CD2 was labeled with $Na^{125}I$ by using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (22) and extensively dialyzed against 10 mM Tris-HCl, pH 8.0, 0.14 NaCl, 0.02% NaN_3.

^{125}I-CD2 binding and inhibition by antibody. A total of 5×10^6 sheep or human E were incubated with 50 μl of antibody for 45 min at 4°C. Then 50 μl of ^{125}I-CD2 were added and the incubation continued for another 2 hr on ice. Cells were subsequently washed three times with 10% FBS/RPMI 1640/2 mM HEPES, pH 7.4, and counted in a gamma counter. The specific activity of ^{125}I-CD2 used was 3.8×10^8 cpm/nmol.

Saturation binding of ^{125}I-CD2. From 2.5×10^6 to 5×10^6 sheep or human E were incubated with or without 5 μl of purified anti-T11TS (300 μg/ml) or anti-LFA-3 (1.2 mg/ml) for 30 min at 4°C. Then 25 μl of varying concentration of ^{125}I-CD2 (diluted in 10% FBS/RPMI 1640/3% BSA; specific activity = 4.4×10^7 cpm/nmol) were added and the binding assay was continued as above. The dissociation constant was obtained from the slope = $-/K_d$ from Scatchard analysis. CD2 molecules bound per cell were calculated assuming the M_r of CD2 as 50,000.

Saturation T11TS mAb binding. The T11TS mAb was purified by DEAE-Affigel blue chromatography and iodinated with $Na^{125}I$ (Amersham Buchler, Braunschweig, FRG) by using H_2O_2 in conjunction with solid phase lactoperoxidase (Pharmacia Fine Chemicals, Piscataway, NJ) (14). More than 90% of the radioactivity was associated with active antibody. A total of 10^7 sheep E were incubated on ice for 1 hr with serial dilutions of ^{125}I-T11TS mAb in 0.4 ml of phosphate-buffered saline with 10% heat-inactivated sheep serum. Sheep E were centrifuged at 12,000 rpm for 1 min through 1 ml of 80% dibutyl phthalate and 20% paraffin oil. Radioactivity in the pellet and aqueous supernatant were determined. Calculations of molecules bound per cell and free antibody concentration were based on a M_r of 1.65×10^6 for the T11TS mAb. The dissociation constant was obtained from Scatchard analysis.

Immunofluorescent flow cytometry. When flow cytometry was used to analyze inhibition of T11TS mAb and LFA-3 mAb binding by CD2, cells were preincubated with CD2, LFA-1, or control buffers for 1 hr at 4°C in 20 μl of Hanks' balanced salt solution with 15% BSA. All samples receiving membrane protein were adjusted to the same detergent and buffer concentrations. mAb were added in an additional 20 μl and incubated another 15 min. Suboptimal concentrations of mAb were used (25% of saturating concentrations, 0.5 μg/ml for T11TS mAb and 2 μg/ml for LFA-3 mAb) and comparable concentrations were used for the control mAb and a nonbinding control IgG1. A mouse anti-human decay-accelerating factor (DAF) mAb and rat anti-mouse Forssman determinant mAb were used as controls for human and sheep E, respectively. The cells were washed and stained with fluorescein isothiocyanate (FITC) anti-mouse or anti-rat IgG where appropriate. For comparison by immunofluorescence of the number of LFA-3 mAb and T11TS mAb binding sites of E, the concentrations of LFA-3 mAb and T11TS mAb for saturation binding were determined by using purified mAb. Cells were stained with the primary mAb for 30 min at 4 °C, washed, and stained with FITC anti-IgG for 30 min. Cells were fixed and analyzed on a Coulter Epics V flow cytometer.

RESULTS

We examined binding of purified CD2 to sheep E and its inhibition by the mAb to T11TS. ^{125}I-labeled CD2 bound to sheep E (Table I). Binding to sheep E was specific, because it was inhibited >96% by mAb to CD2. Strikingly, anti-T11TS inhibited ^{125}I-CD2 binding to sheep E by 99%. Binding of ^{125}I-CD2 to sheep E was not inhibited by control mAb. In parallel, we examined binding of purified CD2 to human E. Anti-LFA-3 inhibited ^{125}I-CD2 binding to human E as previously described (7). Anti-LFA-3 mAb did not affect ^{125}I-CD2 binding to sheep E, and T11TS mAb did not inhibit ^{125}I-CD2 binding to human E, as expected from the lack of cross-reactivity of the mAb between species (23). When binding to human and sheep E was compared, we always observed more binding to sheep than human E (see below).

Reciprocal experiments tested whether unlabeled CD2 could inhibit binding of T11TS mAb to sheep E. Sheep E were preincubated with unlabeled purified CD2, then treated with the mAb to T11TS, washed, and subsequently stained with FITC-conjugated second antibody for analysis by flow cytometry. Purified CD2 interfered in a dose-dependent fashion with the binding of anti-T11TS to sheep E. At 400 nM, CD2 inhibited anti-T11TS

TABLE I

Inhibition of ^{125}I-CD2 binding by anti-LFA-3 and anti-T11TS antibodies[a]

Antibody Specificity	^{125}I-CD2 Bound (cpm ± SD)	
	Sheep E	Human E
X63	12,303 ± 94	6,864 ± 120
Anti-LFA-1	13,905 ± 99	7,093 ± 119
Anti-CR1	14,318 ± 371	6,206 ± 12
Antiglycophorin	13,689 ± 225	5,864 ± 51
Anti-T11TS	139 ± 12	5,898 ± 147
Anti-LFA-3	14,571 ± 243	139 ± 22
Anti-CD2	206 ± 6	167 ± 26

[a] A total of 5×10^6 sheep or human E were incubated with 50 μl of antibody (either culture supernatant TS2/9, TS2/18, L180/1, or appropriately diluted ascites X63, TS1/22, D44, E3) for 45 min at 4°C. Then 50 μl of ^{125}I-CD2 (diluted to 4000 cpm/μl with 10% fetal calf serum/RPMI/2 mM HEPES, pH 7.4/3% BSA) was added and the incubation continued for another 2 hr at 4°C. After incubation the cells were washed three times with 10% fetal calf serum/RPMI/2 mM HEPES, pH 7.4, and counted in a gamma counter.

binding to sheep E by 51% (Fig. 1*a*). Binding of control antibody to the Forssman antigen was unaffected (Fig. 1*b*). As another control, preincubation with purified LFA-1 (1000 nM) had no effect on anti-T11TS binding to sheep E. Parallel experiments with human E showed half-maximal inhibition of LFA-3 mAb binding at 14 nM CD2 (Fig. 1*a*), similar to our previous reports (7). The results strongly suggest that CD2 binds directly to T11TS on sheep E.

Previous functional studies with purified CD2 showed that it could aggregate E, and that aggregation was inhibited by anti-LFA-3 (7). The physical form of CD2 that mediates this LFA-3-dependent aggregation of E is unknown, but it may be due to integration of CD2 into the membrane via a hydrophobic domain. Sedimentation in a detergent-free sucrose gradient suggested that the CD2 was monomeric (data not shown). We examined aggregation of sheep E by CD2. Purified CD2 aggregated sheep E (Fig. 2*B*). Anti-T11TS completely inhibited CD2-mediated aggregation of sheep E (Fig. 2*C*). As a control, agglutination of sheep E induced by Forssman IgM mAb was unaffected by anti-T11TS (Fig. 2*D*). Identical experiments with human E were carried out in parallel. CD2-mediated aggregation of human E was completely inhibited by anti-LFA-3, as previously reported (7), and was unaffected by anti-T11TS (data not shown). These findings suggest that aggregation of sheep E is mediated by direct binding of CD2 to T11TS on sheep E, and illustrate functional parallels between T11TS and LFA-3 in homotypic adhesion mediated by purified CD2.

The above data show that LFA-3 and T11TS are functional homologues, but do not resolve the question of why human lymphocytes rosette much more readily with sheep than human E. To address this question, we measured the affinity and number of binding sites for purified CD2 on sheep and human E. CD2 showed saturable binding to sheep and human E that was inhibited by T11TS mAb and LFA-3 mAb, respectively (Fig. 3, *A* and *B*). At saturation 9800 ± 1600 molecules of CD2 were bound per sheep E and 8300 ± 1400 molecules of CD2 were bound per human E (averages and ranges of two independent experiments). Scatchard analysis (Fig. 3, *C* and *D*) showed the dissociation constant for CD2 binding to sheep E and human E was 10.7 ± 3.3 nM and 15.8 ± 0.6 nM, respectively (or a K_a of 9×10^7/M for sheep T11TS molecule and a K_a of 6×10^7/M for human LFA-3 molecule).

To correlate the number of CD2 binding sites per sheep E with the number of T11TS antigen sites per sheep E, saturation binding experiments were carried out with radiolabeled T11TS mAb (Fig. 4). A total of 8100 molecules of ^{125}I-T11TS mAb were bound to each sheep E with a dissociation constant of 1 nM or a K_a of 1×10^9/M. The number of sites to which CD2 bound was in good agreement with the number of T11TS mAb binding sites per cell.

The number of mAb binding sites for T11TS on sheep E and LFA-3 on human E were compared by immunofluorescence flow cytometry (Fig. 5). Both mAb are of the IgG1 subclass, allowing relative comparisons of mAb bound per cell by using a second FITC-anti-Ig reagent. Under saturating conditions, 2.07 ± 0.18 (mean ± SD of three separate experiments) fold more T11TS mAb bound per sheep E than LFA-3 mAb per human E.

DISCUSSION

Recent observations have suggested that the T11TS molecule on sheep E and the LFA-3 molecule on human E are important in CD2-dependent rosette formation (7, 8), but it was unclear whether these molecules were functionally related. We have found that sheep T11TS and human LFA-3 are functionally homologous in their ability to interact with the human T lymphocyte surface glycoprotein CD2. We found that purified CD2 binds to both sheep and human E and that this binding is inhibited by T11TS mAb and LFA-3 mAb, respectively. Reciprocally, we found that preincubation of E with purified CD2 inhibited T11TS mAb binding to sheep E and LFA-3 mAb binding to human E. These results show that CD2 binds to both T11TS in sheep and LFA-3 in human. That the interaction between CD2 and T11TS can mediate cell-cell interaction was directly demonstrated by the ability of purified CD2 to cause T11TS-dependent sheep E aggregation. Purified CD2 has been previously shown to mediate LFA-3-dependent aggregation of human E (7). Demonstration that the interaction between purified CD2 and its ligand molecules on both sheep and human E can directly mediate cell-cell adhesion further supports the conclusion that T11TS and LFA-3 are functional homologues. In addition to demonstrating functional relationships, our results with purified CD2 extend previous studies with purified T11TS that suggested an interaction between CD2 and T11TS (8). Parallel studies on the interaction between CD2 and LFA-3 included here for comparative purposes are in agreement with previous results (7).

The expression of LFA-3 and T11TS on E provides an excellent model system for studying ligand receptor interactions. Human T lymphocytes rosette more vigorously with sheep E than human E; thus, paradoxically the heterologous human-sheep interaction appears more efficient than the homologous human-human interac-

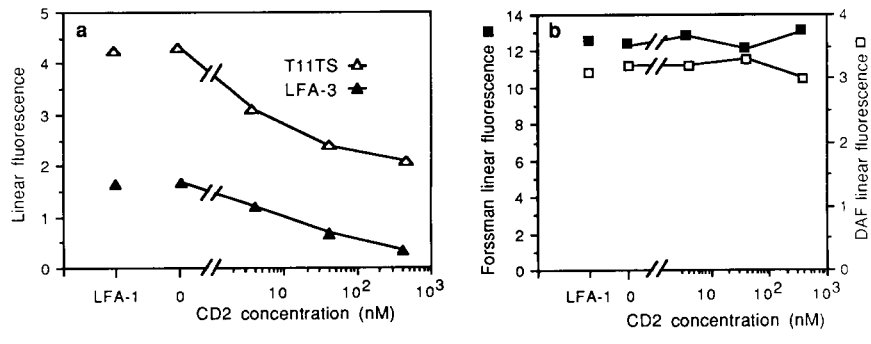

Figure 1. Inhibition of T11TS mAb and LFA-3 mAb binding to sheep E and human E, respectively, by purified CD2. Erythrocytes were pretreated with purified CD2 and analyzed for the binding of mAb as described under *Materials and Methods*.

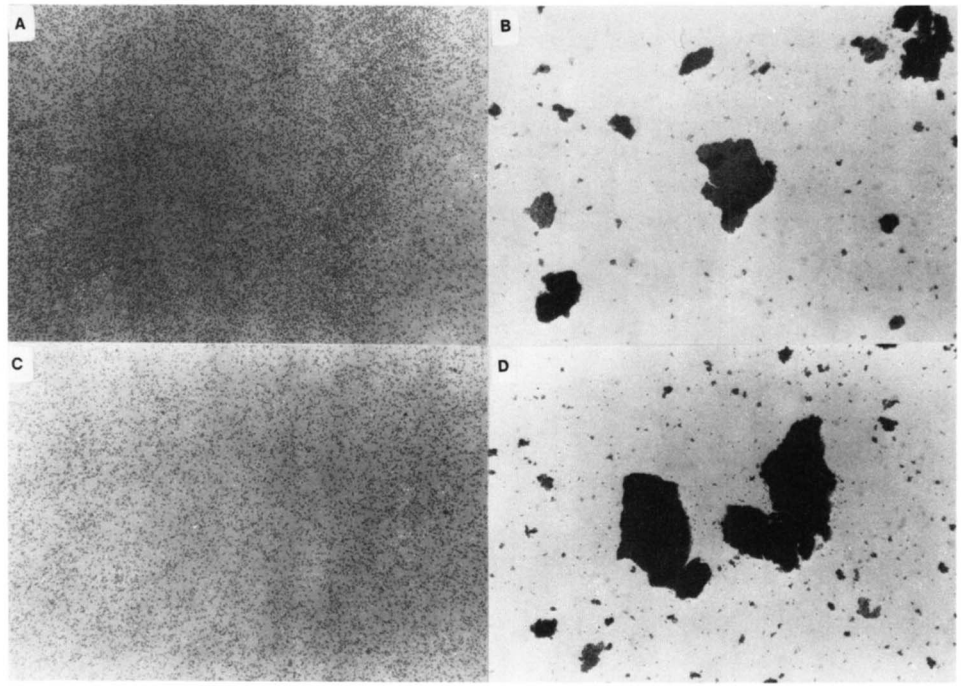

Figure 2. CD2 induced aggregation of sheep E. A total of 10^7 E were mixed with 20 μg/ml of CD2 with or without 30 μg/ml of mAb in the presence of 15% BSA. The mixture was centrifuged for 3 min at 200 × G and incubated on ice for 1 hr. The cells were gently resuspended by rotating the tube and photographed with a Nikon inverted microscope. *A*, Sheep E; *B*, sheep E + CD2; *C*, sheep E + CD2 + anti-T11TS; *D*, sheep E + antiForssman + anti-T11TS.

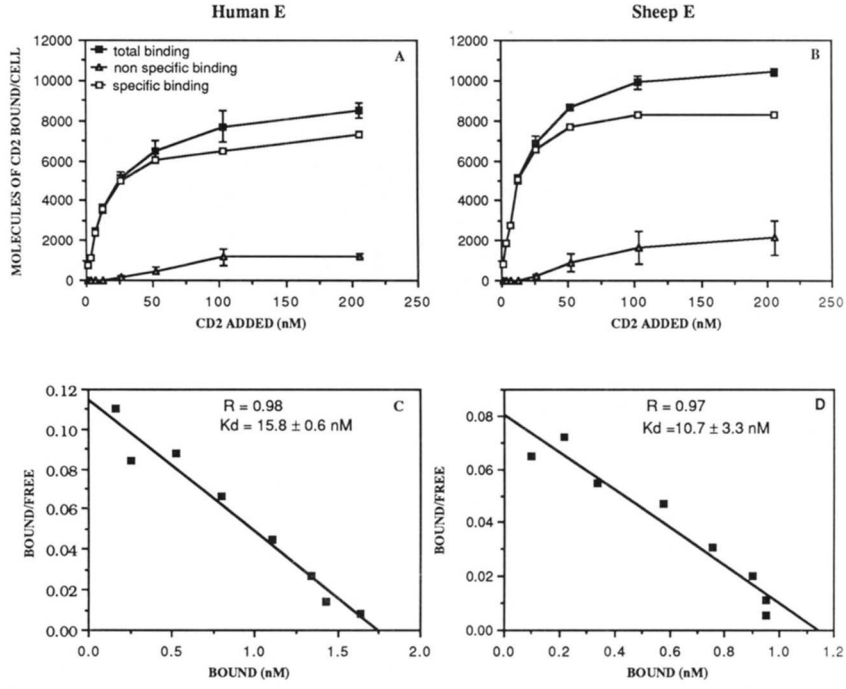

Figure 3. Saturation binding of ^{125}I-CD2 to human and sheep E. 4.7×10^6 cells or 2.6×10^6 cells of human or sheep E, respectively, were incubated with indicated concentrations of ^{125}I-CD2 and the binding assay was carried out as described under *Materials and Methods*. Binding in presence of 200-fold excess of anti-LFA-3 antibody or anti-T11TS antibody was taken as nonspecific binding. Specific binding was obtained by subtracting the nonspecific binding from total binding. Each value is average of triplicates. The figure represents the result of one experiment. Reported values are average of two such experiments. *C* and *D*, Scatchard analysis of specific ^{125}I-CD2 binding to human and sheep E, respectively. *R* is correlation coefficient; K_d is dissociation constant obtained from slope = $-1/K_d$.

tion. By using purified CD2, we measured two variables of importance in regulating this interaction: the affinity and number of binding sites for the ligand molecules on human and sheep E. Saturation binding experiments showed the affinity of purified CD2 is similarly high for both types of cellular ligands, with a $K_a = 9 \times 10^7$ M^{-1} for T11TS and $K_a = 6 \times 10^7$ M^{-1} for LFA-3. Data on CD2 and mAb binding sites per cell are summarized in Table II. Considering the experimental uncertainty in whether mAb binds monovalently or bivalently to the cell surface, the data on CD2 binding and mAb binding are in good agreement. The agreement between the number of CD2 binding sites on sheep and human E, and the number of LFA-3 and T11TS antigen sites, is consistent with our finding that CD2 binds directly to LFA-3 and T11TS. The higher number of CD2 binding sites on sheep than human E is especially significant because sheep E have 2.7-fold less surface area (24, 25) (Table II). The ligand density on sheep and human E has been estimated both as density of CD2 binding sites and density of mAb binding sites (Table II). The densities of CD2 binding sites and mAb binding sites are 3.2- and 5.6-fold higher, respectively, on sheep than human E.

Cell-surface charge must also be considered as a factor which can regulate cell-cell adhesion mediated by the CD2-LFA-3 receptor ligand interaction. Erythrocytes and lymphocytes are both highly negatively charged at physiologic pH and thus repel one another; much of this negative charge is due to cell-surface sialic acid. Peripheral blood lymphocytes, which except for a small subpop-

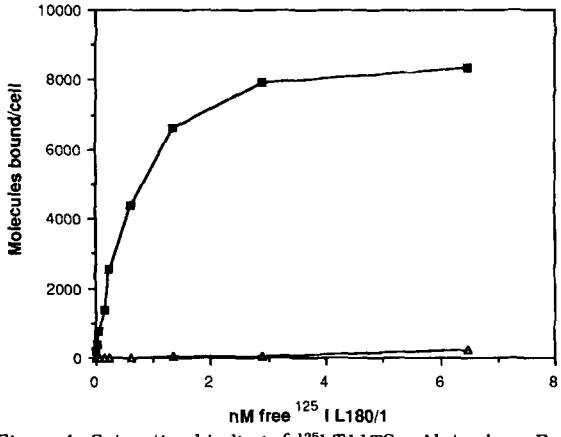

Figure 4. Saturation binding of ^{125}I-T11TS mAb to sheep E. The saturation binding assay was carried out with ^{125}I-L180/liter as described under *Materials and Methods.* ■, Total binding; Δ, binding in presence excess of unlabeled antibody.

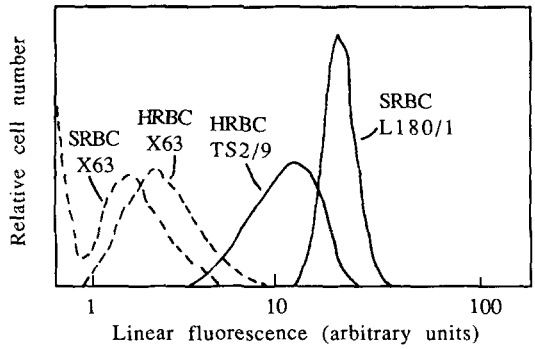

Figure 5. Immunofluorescence flow cytometry comparison of T11TS and LFA-3 expression on sheep E and human E, respectively. Erythrocytes were incubated with saturating concentrations of LFA-3 mAb (TS2/9) or T11TS mAb (L180/1) for 30 min at 4°C. Then the cells were washed, stained with FITC-labeled goal anti-mouse antibody, and analyzed by immunofluorescence flow cytometry.

TABLE II

CD2 ligand density and charge density on sheep and human E[a]

Characteristics	Sheep E	Human E
CD2 binding sites/cell	9,800 ± 1,600	8,300 ± 1,400
mAb binding sites/cell	8,100	3,900 ± 340
Surface area (μm^2/cell)	54	145
Density of CD2 binding sites (molecules/μm^2)	182 ± 29	57 ± 10
Density of mAb binding sites (molecules/μm^2)	150	27 ± 2
Sialic acid density (molecules/μm^2)	141,000	165,000
Charge/μm^2	93,000	82,000

[a] CD2 binding site and mAb binding site data is from this study. Surface area, sialic acid density, and charge density were obtained from published values (24, 25, 34).

ulation (4, 26, 27) fail to rosette with human E, have fivefold more sialic acid than thymocytes (28), which readily rosette with human E. Removal of sialic acid from T cells or E with neuraminidase (7, 13, 29, 30), or addition of positive charges with aminoethylisothiouronium bromide (31), enhances both autologous and xenogeneic rosetting (29), demonstrating the regulatory influence of cell-surface charge. Activation epitopes on CD2, which are modulated by neuraminidase treatment (32) as well as by T lymphocyte activation (33), may also regulate the CD2:T11TS and CD2:LFA-3 interactions.

Charge density differences do not appear to explain the propensity of sheep but not human E to rosette with human peripheral blood T lymphocytes. Previously published work (34) has shown that the density of sialic acid and charge is very similar on sheep and human E (Table II). This suggests that the higher density of the ligand for CD2 on sheep E, and its slightly higher or equivalent affinity for CD2, accounts for the preferential rosetting of sheep E with human T lymphocytes.

Rosetting of peripheral blood lymphocytes with heterologous erythrocytes has been found in many different species combinations (35); rosetting of activated T lymphocytes and thymocytes but not peripheral blood T lymphocytes with autologous erythrocytes is also observed in many but not all species (29, 36–39). Thus, besides the sheep and human, homologues of CD2 and LFA-3 are likely to be found in many species. Why is autologous rosetting between blood lymphocytes and E generally not observed, although heterologous rosetting is observed in certain species combinations? We propose that the cell-surface density of the CD2 and LFA-3 homologues, their affinity for one another, and cell-surface charge are evolutionarily adjusted to a threshold that does not give autologous rosetting of resting peripheral blood lymphocytes with erythrocytes. We further propose that the individual factors vary from species to species, giving heterologous rosetting in combinations in which, for example, the receptor and ligand densities are both high. Our finding that the sheep T11TS molecule is present in higher density than human LFA-3 predicts that resting sheep T lymphocytes do not rosette with sheep E because the sheep CD2 homologue has a lower density or affinity for T11TS than human CD2. The equilibrium is shifted after activation, because T lymphocyte blasts form autologous rosettes both in the human (7, 13) and sheep (8) systems.

mAb blocking experiments suggest the interaction between LFA-3 on thymic epithelial cells and CD2 on thymocytes is important in functional responses (40). Although the interaction between CD2 and LFA-3 appears important to T cell functions, it is not known if this receptor-ligand interaction is sufficient for induction of a proliferative signal in human T cells. T cell proliferation and effector function can be triggered by mAb to CD2 (33). Thus, in addition to its function in adhesion, the interaction of CD2 with its biologic ligand may be a physiologically relevant pathway of T lymphocyte stimulation. Purification of human T lymphocytes by sheep E rosetting induces T lymphocytes to proliferate in the presence of mitogenic factors (41). Recently, Hünig et al. (42) have shown sheep E binding to human T cells synergizes with anti-CD2 in activating T cells, and concluded that T11TS binding to CD2 delivers one of the signals required for the induction of T cell activation via the CD2 pathway. Recent studies suggest that LFA-3 binding to CD2 on T cells leads to similar functional consequences as that of T11TS binding (G. Tiefenthaler, T. Hünig, M. Dustin, T. A. Springer, and S. C. Meuer, unpublished observations).

Experiments of Larsson et al (41) and Hünig et al. (42) showing that sheep E can potentiate proliferation of human T cells suggest that activation by ligand is conserved across species. Our finding that CD2 has a similar affinity for ligand molecules on sheep and human E, and the common occurrence in mammals of heterologous rosetting, suggests that the receptor and ligand molecules are highly conserved. Thus, human LFA-3 and sheep T11TS

might have common structural features. Indeed, a rabbit anti-T11TS antiserum cross-reacts with human LFA-3 antigen. A detailed study of the serologic cross-reactivity between sheep E T11TS and human E LFA-3 is described in an accompanying paper (23).

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