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SEROLOGIC CROSS-REACTIVITY OF T11 TARGET STRUCTURE AND LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN 3

Evidence for Structural Homology of the Sheep and Human Ligands of CD2[1]

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T11 target structure (T11TS) and lymphocyte function-associated antigen (LFA) 3 are the cell-surface glycoproteins on sheep and human erythrocytes (E) binding to cluster differentiation 2 (the E-receptor) on T cells in E rosette formation. Here we show that this functional cross-reactivity is most likely due to a structural homology of these molecules. A rabbit antiserum to sheep T11TS is shown to cross-react with LFA-3 in several independent assays: (a) rabbit anti-T11TS antiserum blocks the formation of E rosettes by human T cells with both autologous and xenogeneic (sheep) E by binding to the respective E; (b) the antiserum blocks the binding of anti-LFA-3 monoclonal antibody to human E; and (c) it reacts with purified LFA-3 in Western blotting. Together, these findings demonstrate that T11TS on sheep E and LFA-3 on human E are serologically related, providing further support for the notion that T11TS and LFA-3 are the sheep and human forms of the same cell interaction molecule.

Until the introduction of monoclonal antibodies (mAb)[3] into surface phenotyping of human lymphoid cells, the main tool for the identification of T lymphocytes was their capacity to form spontaneous aggregates with sheep erythrocytes (E) (1–4). Under appropriate experimental conditions, human E also bind to human T cells (5, 6). Although this phenomenon of "E rosetting" has been invaluable for both analytical and preparative purposes, the functional importance of the receptor-ligand system visualized in this standard assay has only very recently been appreciated. A role for the E receptor on T lymphocytes, now known as the cluster differentiation 2 (CD2) molecule, in T cell activation became likely when several T cell-specific mAb that blocked E rosette formation were also shown to inhibit T cell proliferation and function (7–11). In addition, it was found that certain combinations of mAb to distinct epitopes on the E receptor molecule can polyclonally activate resting T cells (12–16). Together these findings provided very strong evidence for a role of CD2 in T cell activation and prompted the search for its naturally occurring ligand. The cell-surface molecules on sheep and human E responsible for rosetting have recently been identified. Thus, a mAb to sheep E was generated that completely blocks E rosette formation (17). This antibody, called L180/1, detects a cell-surface glycoprotein of about 42,000 m.w. on all types of blood cells and some other tissues that was called T11 target structure (T11TS) (17–19). Although mAb L180/1 is specific for T11TS on sheep cells, a functional human equivalent has recently been discovered when it was found that mAb to the lymphocyte function-associated antigen 3 (LFA-3) (9) block the binding of human E to T cells (20). This finding has raised the possibility that T11TS and LFA-3 are homologous molecules in sheep and man.

In the accompanying report (21), isolated CD2 was used to formally prove that T11TS and LFA-3 are the molecules recognized by the E receptor on sheep and human E, respectively. Indeed, it was found that the human E receptor binds to sheep T11TS and human LFA-3 in a highly specific fashion and with similar affinity. This functional cross-reactivity of T11TS and LFA-3 suggested that they are homologous molecules. A structural relationship between T11TS and LFA-3 is, however, not supported by the species-specificity of the two mAb that have been used to identify these ligands. Thus, mAb L180/1 to sheep T11TS does not bind to human cells, and mAb TS2/9 to LFA-3 reacts with human, but not with sheep cells. If sheep T11TS and human LFA-3 are indeed structurally homologous it may, however, be expected that in addition to the species-specific determinants detected by these mAb, they also express shared determinants that could be revealed with the help of a polyclonal antiserum to purified T11TS or LFA-3. In the present report, we describe results obtained with a rabbit antiserum to T11TS that demonstrate serologic cross-reactivity of the ligands of the E receptor in man and sheep.

MATERIALS AND METHODS

Cells. Sheep E were obtained weekly from sheep kept in the animal facility of the Max Planck Institut für Biochemie, Martinsried, FRG. Human blood cells were obtained from healthy donors;

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[3] Abbreviations used in this paper: mAb, monoclonal antibody; E, erythrocyte; CD2, cluster differentiation 2; MNC, mononuclear cells; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; LFA, lymphocyte function-associated antigen; T11TS, T11 target structure.

peripheral blood mononuclear cells (MNC) were isolated by centrifugation over Ficoll-Paque (Pharmacia GmbH, Freiburg, FRG) and enriched for T cells by nylon wool filtration (22).

Antibodies. mAb were TS2/9 (anti-LFA-3) (9), anti-$T11_1$ (12), L180/1 (anti-T11TS) (17), and I284/1 (anti-mouse lymphocyte; control IgG1).

Purification of T11TS. Purified T11TS was prepared from sheep E as described (19). Briefly, the antigen was isolated from Nonidet P-40 (Sigma GmbH, Deisenhofen, FRG)-solubilized sheep E by immune affinity chromatography with the mAb L180/1 attached to glutaraldehyde-activated glass beads (Boehringer Mannheim GmbH, Mannheim, FRG). The eluted antigen was purified to homogeneity by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), by using radiolabeled T11TS as a tracer to detect the antigen-containing band. The band was cut from the frozen gel, and the protein was electroeluted.

Purification of LFA-3. LFA-3 solubilized with Triton X-100 from human E was purified by absorption to a TS2/9 mAb-Sepharose column and acid-eluted as described (23).

Preparation of the polyclonal anti-T11TS antiserum. A rabbit antiserum was prepared by injecting 50 μg of purified T11TS at 3-wk intervals. The material was incorporated into an aluminum hydroxide gel for the first injection, and was administered in soluble form in the subsequent injections. A blood sample was taken on day 7 after each injection and the antibody titer to sheep E was observed by using indirect hemolysis. The antiserum used in this study was taken after the sixth immunization.

Radiolabeling of sheep E. Sheep E were surface-labeled with ^{125}I (Amersham Buchler GmbH, Braunschweig, FRG) as described (17) with the lactoperoxidase method. Cells were then lysed in phosphate-buffered saline (PBS) containing 0.5% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride (Sigma).

Radioimmunoprecipitation. The ^{125}I surface-labeled sheep E lysate was precleared with normal rabbit serum and protein A-Sepharose (Pharmacia). Appropriate dilutions of the rabbit anti-T11TS antiserum or preimmune serum in lysis buffer were added. After 2 hr on ice, immune complexes were precipitated with protein A-Sepharose. After extensive washing, the samples were boiled in reducing Laemmli sample buffer, and run on SDS slab gels containing 10% acrylamide (24). Gels were subsequently dried and autoradiographed.

Formation of E rosettes. E rosetting was carried out as described (17). In brief, E were treated with neuraminidase (Calbiochem GmbH, Frankfurt, FRG), washed, and adjusted to 10^8/ml in balanced salt solution containing 10% heat-inactivated autologous human serum. Peripheral blood MNC were adjusted to 8×10^6/ml. To form rosettes with sheep E, 50 μl of sheep E were mixed with 50 μl of peripheral blood MNC, left for 10 min at room temperature, centrifuged for 5 min at 200 × G, and left for another 30 min at room temperature. To form autologous human rosettes, 50 μl of human E were mixed with 50 μl of neuraminidase-treated peripheral blood MNC, incubated for 20 min at 37°C, centrifuged for 5 min at 200 × G, and left for 60 min on ice. Nucleated cells were stained with crystal violet, and rosettes were counted. Cells with three or more E bound were considered as rosettes.

E rosette inhibition assay. The E rosette inhibition assay was performed as described (17). Briefly, 50 μl of the sheep E, human E, or peripheral blood MNC were incubated with the antibody dilutions to be tested for 30 min at room temperature before carrying out the rosetting procedure. To determine whether lymphocytes or E were the target of inhibition, the cells were washed twice after antibody treatment before adding the untreated partner.

Indirect immunofluorescence and flow cytometry. A total of 5×10^5 human E or 10^6 sheep E were preincubated on ice with either 100 μl of PBS containing 0.2% bovine serum albumin (BSA) and 0.02% sodium azide (PBS/BSA/azide) or PBS/BSA/azide containing the rabbit anti-T11TS antiserum or preimmune serum at various dilutions. After 30 min, the antibody TS2/9 or L180/1 was added when staining human or sheep E, respectively. Both antibodies were used at a concentration of 1 μg/ml. The anti-mouse lymphocyte mAb I284/1 (like TS2/9 and L180/1 of the IgG1 subclass) was used as a negative control at a dilution of 1/100 of ascitic fluid. After another 30 min on ice, cells were washed twice in PBS/BSA/azide and stained with fluorescein isothiocyanate-conjugated Fab_2 fragments of rabbit anti-mouse Ig antibodies (Dakopatts GmbH, Hamburg, FRG) diluted 1/50 in PBS/BSA/azide. Analysis was performed on a fluorescence-activated cell sorter flow cytometer (Becton Dickinson GmbH, Heidelberg, FRG) with a forward angle light scatter gate for individual E and collecting green fluorescence signals from a logarithmic amplifier.

Western blotting. Sheep E and human E membranes prepared from 50 μl of packed erythrocytes, or 0.2 μg of purified LFA-3, were subjected to electrophoresis on 10% polyacrylamide Laemmli slab gels. Proteins were electrotransfered onto nitrocellulose membranes and reacted with the antibodies given. Blots were developed as described (19). Second step reagents were alkaline phosphatase-conjugated rabbit anti-mouse Ig (Dakopatts), and alkaline phosphatase-conjugated goat anti-rabbit Ig (Promega, Madison, WI).

RESULTS

Production of a rabbit anti-T11TS antiserum. We have recently purified sheep T11TS to homogeneity by immune affinity chromatography and preparative SDS-PAGE (19). Repeated immunization of a rabbit with this purified material resulted in a 30-fold increase in the hemolytic serum titer to sheep E (data not shown). Thus, despite the use of denatured immunogen, antibodies were apparently produced that recognized native cell-surface structures on sheep E. The presence of antibodies to T11TS was verified by radioimmunoprecipitation of surface-iodinated sheep E lysates (Fig. 1*A*). A band of the expected 42,000 m.w. was precipitated along with several other bands. These additional bands disappeared at higher dilutions of the antiserum, leaving only the one suspected to represent T11TS. The identity of this band with T11TS was confirmed by competing the reaction with unlabeled T11TS (Fig. 1*B*). For this purpose, the radiolabeled sheep E lysate was not precleared with rabbit nonimmune serum before precipitation with the anti-T11TS antiserum. Among the several bands precipitated under these conditions, only the 42,000 band was eliminated by the addition of 1 μg of unlabeled T11TS to the reaction.

Inhibition of E rosettes by anti-T11TS antiserum. We tested whether the rabbit anti-T11TS antiserum con-

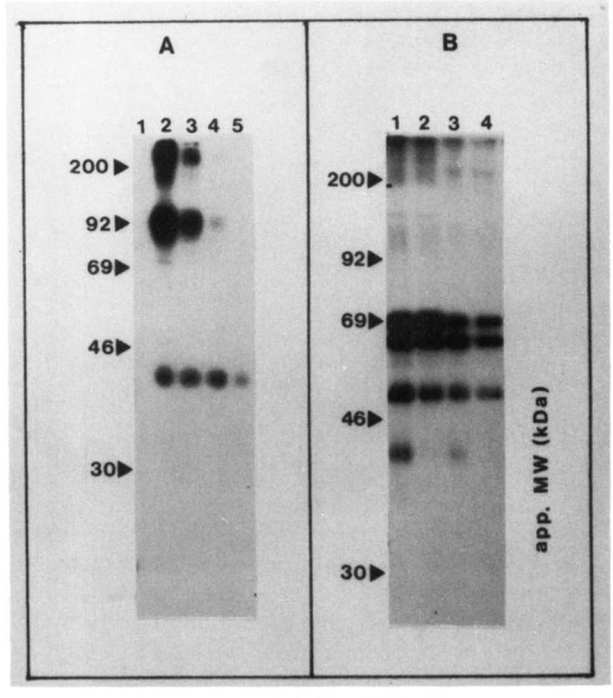

Figure 1. Radioimmunoprecipitation of T11TS with the rabbit anti-T11TS antiserum. *A*, Radioimmunoprecipitation from precleared lysate of surface-iodinated sheep E: *Lane 1*, rabbit preimmune serum (1/5 final dilution); *Lanes 2 to 5*, rabbit anti-T11TS antiserum (serial dilution from 1/5 to 1/625). *B*: Competition of radioimmunoprecipitation with unlabeled T11TS: ^{125}I-labeled sheep E lysate was reacted, without preclearing, with the rabbit anti-T11TS antiserum in the absence (*lanes 1* and *3*) or presence (*lanes 2* and *4*) of 1 μg of unlabeled purified T11TS. In *lanes 1* and *2*, the antiserum was used at a dilution of 1/25; in *lanes 3* and *4*, at a dilution of 1/125.

tained antibodies related to the binding site recognized by the E receptor on sheep and human E. In the experiment shown in Figure 2, inhibition of E rosettes formed between human T cells and sheep E or human E was studied. As expected, a mAb to the $T11_1$ determinant known to be associated with the erythrocyte-binding site of CD2 blocked the formation of both autologous and xenogeneic E rosettes. On the other hand, mAb L180/1 to sheep T11TS blocked the binding of sheep E, but not of human E to human T cells. In contrast to the species-specificity displayed by the anti-T11TS mAb, Fab fragments prepared from polyclonal rabbit anti-T11TS Ig completely blocked E rosette formation of human T cells with both sheep E and human E. Fab fragments prepared from preimmune serum had no effect. As expected the inhibitory titer of the anti-T11TS antibodies was higher in sheep E than in human E rosetting, because only those antibodies recognizing determinants shared between the two species react with the human T11TS equivalent. Next, it was determined whether the E rosette-inhibiting activity of the polyclonal anti-T11TS antibodies could be attributed to their binding to the red cell surface, as had previously been shown for the anti-T11TS and anti-LFA-3 mAb. To this end, human peripheral blood MNC or E from either humans or sheep were preincubated with mAb to $T11_1$, T11TS, LFA-3, or rabbit anti-T11TS Fab, washed, and tested for their ability to form E rosettes. As shown in Table I, the anti-$T11_1$ mAb blocked E rosetting with both human and sheep E by binding to the T lymphocytes. On the other hand, mAb L180/1 to sheep T11TS and TS2/9 to human LFA-3 blocked E rosette formation with sheep and human E, respectively, by binding to the erythrocytes. This site of inhibition was shared by the polyclonal rabbit anti-sheep T11TS antibodies, which inhibited both autologous and sheep E rosetting. Thus, by this functional criterion, the rabbit anti-T11TS antiserum reacted with the human ligand of the E receptor. It was now studied whether it also recognized the LFA-3 molecule as defined by mAb TS2/9.

Rabbit anti-sheep T11TS blocks binding of anti-LFA-3 to human E. Sheep E do not express the determinant defined by mAb TS2/9 to human LFA-3. Therefore, a rabbit antiserum to purified sheep T11TS is not expected to contain antibodies directed at this determinant. Nevertheless, if sheep T11TS and human LFA-3 are structurally homologous molecules, such an antiserum may be expected to react with shared epitopes that could block binding of anti-LFA-3 to human E by steric hinderance. To study this possibility, we investigated the ability of the rabbit anti-T11TS antiserum to block the binding of anti-LFA-3 to human E. In the experiment shown in Figure 3, indirect immunofluorescence and flow cytometry were used to demonstrate specific staining of human E with mAb TS2/9 to LFA-3 (Fig. 3*A*) and of sheep E with mAb L180/1 to T11TS (Fig. 3*B*). In Figure 3*C*, human E were incubated with anti-LFA-3 in the presence of 20% rabbit preimmune serum or rabbit anti-T11TS antiserum. Figure 3*D* shows the corresponding experiment for sheep E stained with anti-T11TS mAb L180/1. (Because the fluorescein isothiocyanate-conjugated anti-mouse Ig antibody used was produced in rabbits, the rabbit anti-T11TS antibodies used for blocking were themselves not detected by indirect immunofluorescence.) Although rabbit preimmune serum had no effect on staining by anti-LFA-3, rabbit anti-T11TS serum blocked this staining down to levels seen with the non-specific control antibody. A significant reduction in staining of human E with anti LFA-3 and of sheep E with anti-T11TS mAb was found up to a serum dilution of 1/625 (Fig. 3, *E* and *F*).

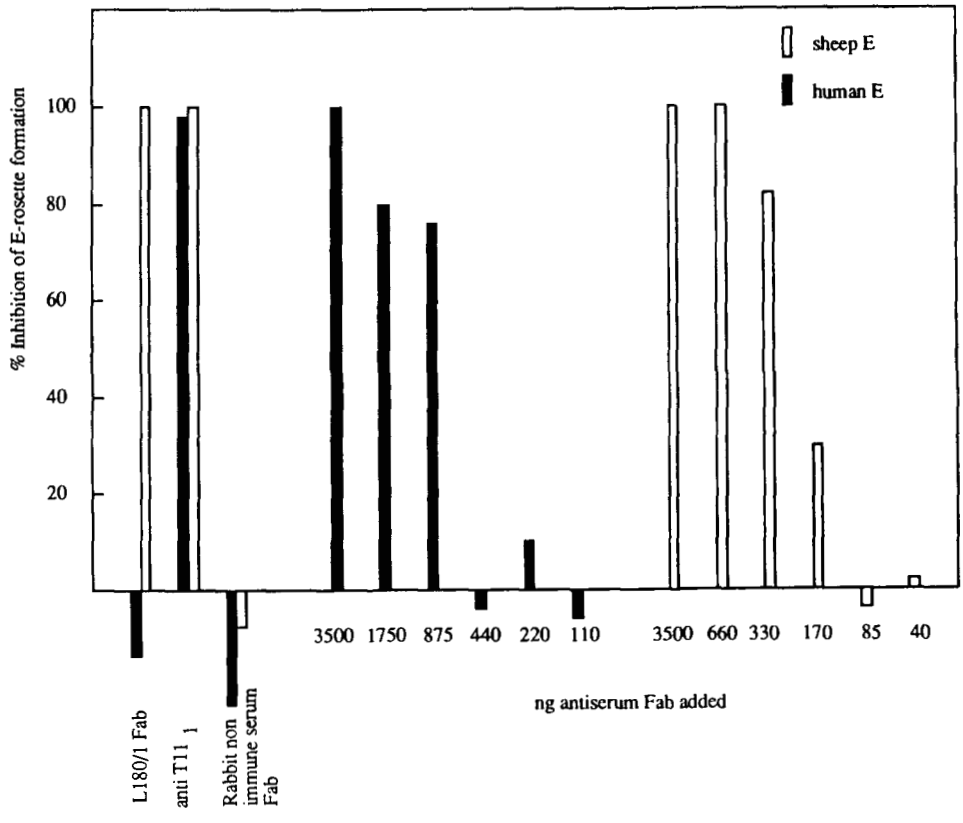

Figure 2. Inhibition of E rosetting by rabbit anti-T11TS. Human nylon wool-enriched blood T lymphocytes were incubated with neuraminidase-treated sheep or human E in the presence of the reagents given on the figure. L180/1 Fab and rabbit non-immune serum Fab fragments were used at 5 μg/ml, and anti-$T11_1$ mAb as an ascitic fluid at a final dilution of 1/300.

TABLE I

Inhibition of sheep E and human E rosetting by rabbit anti-T11TS Fab[a]

Pretreatment of		E Rosette Inhibition (%)	
Peripheral Blood	E	Human E	Sheep E
—[b]	—	0	0
Anti-$T11_1$	—	100	92
—	Anti-$T11_1$	25	−18
Anti-LFA-3	—	12	−8
—	Anti-LFA-3	94	−18
Anti-T11TS Fab	—	19	−29
—	Anti-T11TS Fab	25	87
Rabbit anti-T11TS Fab	—	19	−10
—	Rabbit anti-T11TS Fab	87	100

[a] Cells were incubated for 30 min with the respective antibodies at room temperature and washed two times with balanced salt solution before adding the untreated partner. Sheep and human E were treated with neuraminidase before rosetting. Rosetting was carried out as described in *Materials and Methods*. Antibody concentrations were: anti-$T11_1$/ascites, 1/300; TS2/9 (anti-LFA-3), 0.5 μg/ml; L180/1 (anti-T11TS) Fab, 10 μg/ml; rabbit anti-T11TS antiserum Fab, 3.5 μg/ml. In positive controls, 16% of the peripheral blood MNC formed E rosettes with autologous E and 61 with sheep E.

[b] —, No pretreatment.

Detection of T11TS and LFA-3 by rabbit anti-T11TS serum in Western blotting. The above blocking studies on intact cells indicated that determinants of the T11TS and LFA-3 molecules involved in E rosetting are recognized by the anti-T11TS antiserum. In the next set of experiments, it was investigated whether anti-LFA-3 and the rabbit anti-T11TS antiserum react with the same membrane protein on human E. As shown in Figure 4, both the anti-T11TS mAb L180/1 and the rabbit anti-T11TS antiserum detect a broad band around 42,000 in Western blots of solubilized sheep E membranes. In blots of human E membranes a broad smear between 45 and 66 kDa molecular mass is stained both by the anti-LFA-3 mAb TS2/9 and by the rabbit anti-T11TS antiserum. The antiserum reveals several distinct bands within this area that are not as prominent when the mAb is used to detect LFA-3. In order to obtain definite proof for the reactivity of the rabbit anti-T11TS antiserum with LFA-3, 0.2 μg of pure LFA-3 (prepared as given in *Materials and Methods*) was subjected to electrophoresis, blotted, and stained with the rabbit anti-T11TS antiserum. As seen in Figure 4, mAb TS2/9 to LFA-3 and rabbit anti-T11TS detect a broad smear in the same range of molecular mass, providing direct evidence for the serologic cross-reactivity of T11TS and LFA-3.

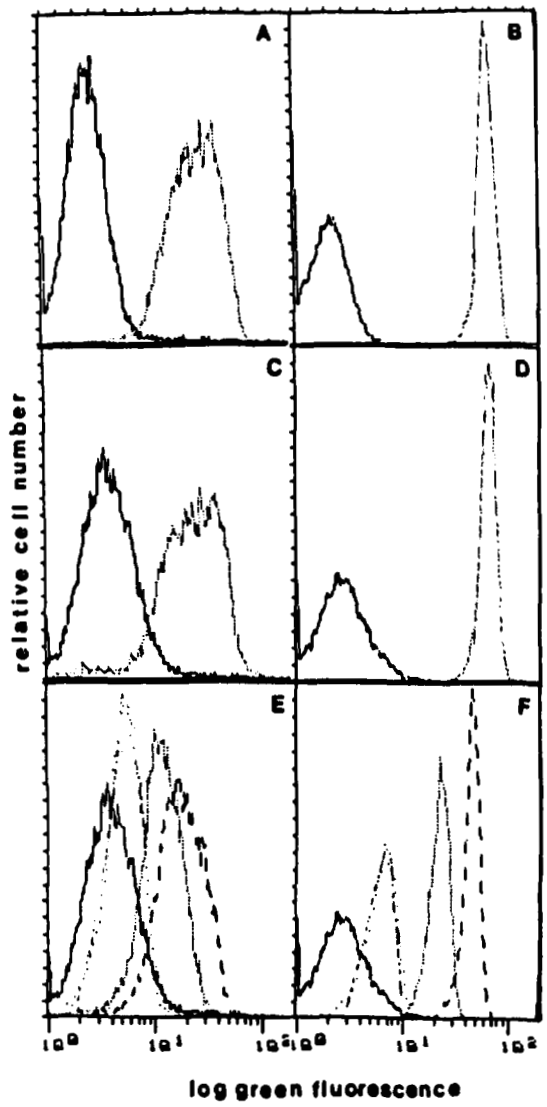

Figure 3. Rabbit anti-T11TS antiserum blocks the binding of anti-T11TS mAb to sheep E and of anti-LFA-3 mAb to human E. Human E (*A*, *C*, and *E*) or sheep E (*B*, *D*, and *F*) were analyzed by indirect immunofluorescence and flow cytometry with rabbit anti-mouse IgG as fluorescein isothiocyanate-labeled second step reagent. *A* and *B*: *continuous lines*, control antibody; *dotted lines*, anti-LFA-3 (*A*) or anti-T11TS (*B*) mAb. *C* and *D*: anti-LFA-3 (*C*) or anti-T11TS (*D*) mAb in the presence of 20% preimmune serum (*dotted lines*) or rabbit anti-T11TS antiserum (*continuous lines*). *E* and *F*: anti-LFA-3 (*E*) or anti-T11TS (*F*) mAb in the presence of rabbit anti-T11TS antiserum diluted 1/5 (*continuous lines*), 1/25 (*widely dotted lines*), 1/125 (*densely dotted lines*), or 1/625 (*dashed lines*).

DISCUSSION

In the accompanying paper, it was shown that the isolated CD2 molecule binds to LFA-3 and to T11TS on human and sheep E, respectively (21). This finding suggested that LFA-3 and T11TS are the sheep and human form of the same molecule. Here we have provided further evidence for this notion by demonstrating serologic cross-reactivity between these two ligands of the E receptor as well. Together, these results very strongly suggest that LFA-3 and T11TS are structural and functional homologues in humans and sheep.

Serologic cross-reactivity between the sheep and human ligands of the E receptor was studied by examining the reactivity of a polyclonal rabbit antiserum to sheep T11TS with the human ligand of the E receptor. Reactivity of this antiserum with the human counterpart of T11TS was demonstrated in three independent assays: a) E rosette formation of human T cells with human E was blocked at the level of the erythrocytes; b) the binding of mAb TS2/9 to LFA-3, the functional human homologue of T11TS, was competively inhibited; and c) the antiserum reacted with the human LFA-3 molecule as detected by Western blotting.

We previously developed a protocol that allowed the preparation of sufficient amounts of pure T11TS (50 to 100 μg from 1 liter of packed sheep E) to immunize a rabbit (19). This protocol, however, involves preparative SDS-PAGE as a second purification step after immune affinity, and therefore denaturation of T11TS by boiling in the presence of 2-mercaptoethanol and SDS. It was uncertain, therefore, whether antibodies to the native molecule could be induced with this immunogen. Such

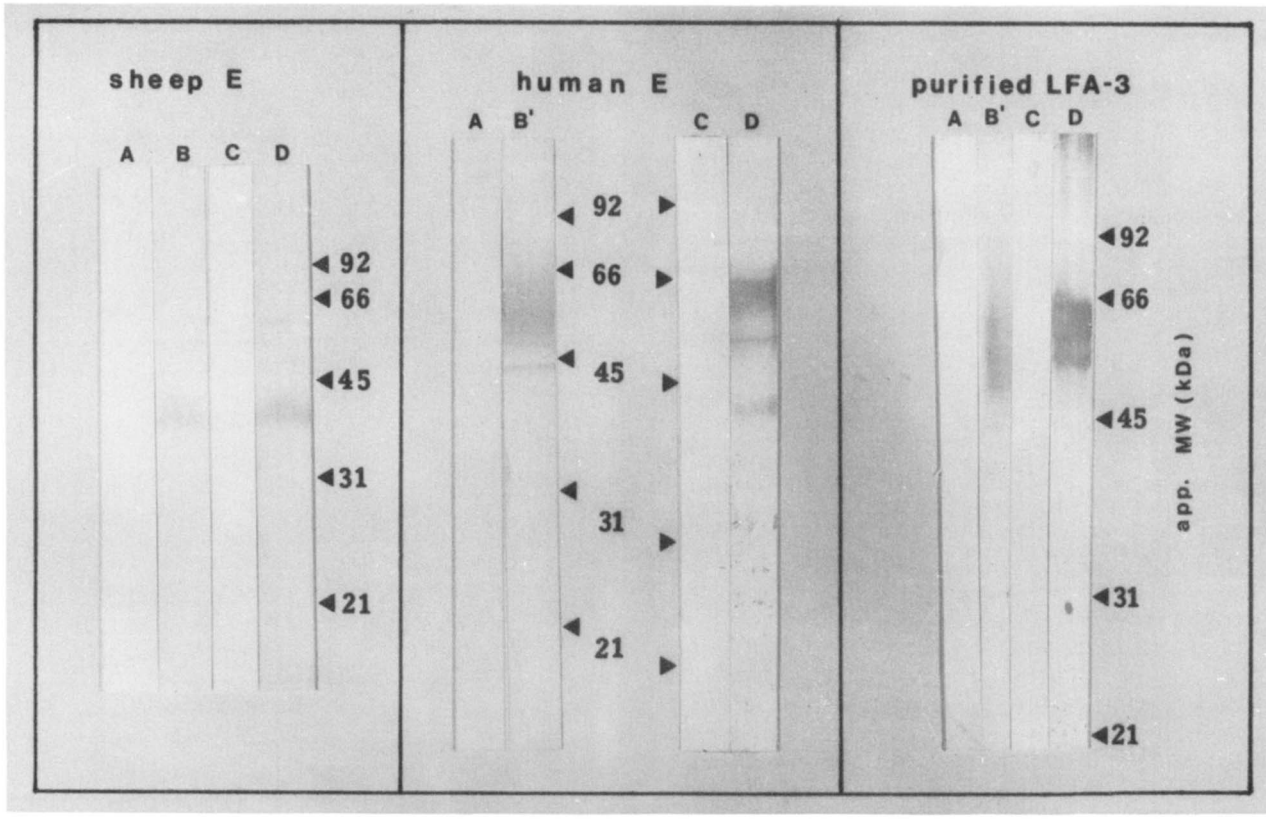

Figure 4. Western blot of human and sheep E membranes and of purified LFA-3 sheep E membrane proteins were reacted with I284/1 (*A*), L180/1 (anti-T11TS) (*B*), rabbit preimmune serum (*C*), or rabbit anti-T11TS antiserum (*D*). Human E membrane proteins and purified LFA-3 were reacted with I284/1 (*A*), TS2/9 (anti LFA-3) (*B*), the rabbit preimmune serum (*C*), or rabbit anti-T11TS antiserum (*D*). I284/1 was used as ascitic fluid at a dilution of 1/200, L180/1 was purified antibody at 10 μg/ml, and mAb TS2/9 was used at 1 μg/ml. The rabbit sera were used at a dilution of 1/10,000.

antibodies were produced, however, as was indicated by the reactivity of the anti-T11TS antiserum with T11TS solubilized by the nondenaturing detergent Nonidet p-40 (Fig. 1). More importantly for the aim of the present studies, the antiserum contained antibodies directed at epitopes related to the site recognized by CD2. Thus, binding of rabbit anti-T11TS antibodies to sheep or to human E blocked rosetting with human T cells (Fig. 2). In addition, reaction of the monoclonal anti-T11TS and anti-LFA-3 antibodies with the respective erythrocytes was blocked by the antiserum (Fig. 3). At first sight it appears paradoxical that the antiserum to sheep T11TS blocked binding of TS2/9 to human LFA-3 because T11TS, the immunogen used to induce the anti-T11TS antiserum, does not carry the epitope defined by this mAb. It must be assumed, therefore, that this blocking activity is due to steric hinderance exerted by antibodies directed at epitopes closely linked to the one recognized by TS2/9.

The most convincing demonstration of epitope sharing between T11TS and LFA-3 was obtained in Western blotting experiments (Fig. 4). The suitability of the anti-T11TS antiserum for Western blotting was first established by using sheep E membranes, where the expected band at 42 kDa apparent molecular mass is detected (Fig. 4) up to an antiserum dilution of 10^{-5} (data not shown). In lysates of human E membranes, the antiserum detects a smear of bands very similar to that revealed by mAb TS2/9 to LFA-3. The notion that the polyclonal anti-T11TS antiserum detects human LFA-3 in this assay was substantiated by using purified LFA-3 as antigen (Fig. 4). Again the antiserum, but not the preimmune serum, stained a smear of diffuse bands migrating with an apparent molecular mass of 50 to 66 kDa. This corresponds to the region of most intense staining observed with the anti-LFA-3 mAb. Staining with the antiserum yielded, however, more distinct bands than staining with the mAb. One possible explanation is a preference of the antiserum for certain forms of LFA-3 with regard to glycosylation. Thus, although every LFA-3 molecule binds only one TS2/9 mAb, differentially glycosylated forms of the antigen may express different numbers of epitopes recognized by the polyclonal antiserum.

The serologic cross-reactivity between T11TS and LFA-3 demonstrated in the present experiments strongly suggests that the functional similarities of these two molecules are due to their structural homology. These functional similarities may be summarized as a) CD2 binds both molecules with similar affinity (21); b) both are membrane glycoproteins with similar tissue distribution (9, 18, 19); and c) both appear to be involved in T cell activation (9, 18, 25).

The one striking dissimilarity between LFA-3 and T11TS is their different apparent molecular mass (9, 17). This difference appears, however, not to reside in the protein portion of these molecules. Thus, the apparent molecular mass of deglycosylated LFA-3 is around 25 kDa on E (M. L. Dustin and T. A. Springer, unpublished observations), similar to that of the T11TS protein moiety (32 kDa) (19). The specific interaction of CD2 with LFA-

3 and T11TS (21), which are expressed at only around 5,000 to 10,000 molecules per E (19, 21), indicates that CD2 binds to a glycoprotein contributing only a minute fraction to the total membrane carbohydrate. If, as was proposed some years ago (26, 27), the E receptor binds to carbohydrate on E, one has to assume a unique carbohydrate shared by T11TS and LFA-3 but absent from the remainder of the glycocalix in both species. Because this is highly unlikely, the 30-kDa protein moiety of T11TS and LFA-3 appears to be the binding site for CD2. The reactivity of mAb L180/1, which blocks E rosette formation, with deglycosylated T11TS (19) provides indirect evidence in support of this notion. It may be added that pretreatment of E with neuraminidase facilitates the formation of E rosettes (5). The apparent molecular mass of T11TS is reduced by this treatment from 42 kDa to about 38 kDa (17), indicating that at least this major constituent of the carbohydrate portion of T11TS plays no role in E rosetting. Taken together these findings provide very strong evidence for a similarity in the protein structure of LFA-3 and T11TS.

Evidence for the functional importance of the CD2-T11TS system has been obtained in vitro by using mAb to CD2 or its ligand. These experiments have indicated that the E receptor is a cell-adhesion molecule (28) with the capacity to provide an activation signal to T lymphocytes without engaging the antigen-receptor complex (12–16). It is likely that the anti-CD2 mAb inhibit T cell activation by blocking the physiologically occurring receptor-ligand interactions or stimulate by mimicking the effects of the natural ligands on the receptor molecule. In support of the latter notion, we have recently shown that binding of T11TS expressed on sheep E to CD2 synergizes with anti-CD2 mAb in inducing polyclonal T cell proliferation (25).

The high degree of evolutionary conservation of the CD2-T11TS/LFA-3 system further argues for the importance of its function. This conservation is illustrated by the expression of the same mAb-defined epitopes on CD2 in distantly related primates (29, 30), by the expression of an E receptor in sheep (17), and, most impressively, by the specific binding of human CD2 to the sheep form of its ligand (21). It is expected that elucidation of the complete primary structures by molecular cloning will confirm the present conclusion that LFA-3 and T11TS are not only functionally but also structurally homologous molecules in man and sheep.

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