THE CELL SURFACE MOLECULE RECOGNIZED BY THE ERYTHROCYTE RECEPTOR OF T LYMPHOCYTES
Identification and Partial Characterization Using a Monoclonal Antibody

BY THOMAS HÜNIG

From the Institute for Virology and Immunobiology of the University of Würzburg, D-8700 Würzburg, Federal Republic of Germany

Until the recent advent of monoclonal antibodies (mAb)¹ specific for human T cells, the most widely used marker for human peripheral T cells and thymocytes was their ability to bind sheep red blood cells (SRBC) (1–4). Early studies using polyvalent anti-T cell sera suggested that the interaction resulting in E rosettes is mediated by specific receptors on the human T cells (5), a notion fully confirmed by the discovery that mAb against the T cell surface molecule carrying T11 and related markers block rosette formation (6–9). Nevertheless, E rosetting remained a curious, if useful, phenomenon until a functional involvement of the E receptor in T cell activation was suggested by a number of findings: First, it was shown that the rosetting procedure rendered T cells reactive to growth factor(s) (10). More recently, anti-T11 mAb were reported to either inhibit T cell activation (11–15) and T cell–mediated cytolysis (12, 13), or, if certain combinations of mAb were used, to lead to polyclonal T cell activation (14). Accordingly, a “physiological ligand” of the E receptor with a role in T cell activation was postulated (11–13, 16), which could be envisaged as either a soluble mediator or a cell surface structure on cells with which T cells interact.

We have pursued the latter hypothesis and have developed an experimental approach based on the following assumption: The binding of RBC to the E receptor is not due to fortuitous crossreactivity with the unknown physiological ligand. Since human T cells also bind autologous RBC (17), the target for T11 on RBC may be the physiological ligand itself; this ligand may be expressed on other cell types as well, where it is not as easily visualized by rosetting but may play its role in cellular interactions.

In the present report, the identification, isolation, and partial biochemical characterization of the target structure for T11 (T11TS) is described. In contrast to earlier attempts to define this molecule by conventional biochemistry (18–

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¹ Abbreviations used in this paper: BSA, bovine serum albumin; BSS, balanced salt solution; Con A, concanavalin A; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HuRBC, human red blood cells; IL-2, interleukin 2; mAb, monoclonal antibody(s); NMHgG, normal mouse IgG; NP-40, Nonidet P-40; PAGE, polyacrylamide gel electrophoresis; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; SRBC, sheep red blood cells; SWBC, sheep white blood cells; T11TS, T11 target structure.
20), we have affinity purified T11TS with an mAb to SRBC that abrogates their binding to human and sheep T lymphocytes.

Materials and Methods

Preparation of Cells. Freshly drawn human blood from healthy individuals was centrifuged in balanced salt solution (BSS) with heparin. The buffy coat cells were centrifuged over Ficoll-Paque (Pharmacia, Inc., Uppsala, Sweden) to prepare peripheral blood lymphocytes (PBL). Human RBC (HuRBC) and PBL were washed three times in BSS before use. Fresh SRBC were washed twice in BSS with heparin. The buffy coat was collected, spun down, and resuspended in 90% Percoll (Pharmacia, Inc.) in BSS. 70 and 50% Percoll suspensions and BSS were layered on top, and, after 20 min centrifugation with 180 g at room temperature, white blood cells were collected from the interphase between the BSS/50% Percoll and 50%/70% Percoll. Sheep white blood cells (SWBC) were stimulated with 2 μg concanavalin A (Con A) (Pharmacia, Inc.) per milliliter RPMI 1640 with 5% fetal calf serum (FCS), 5 × 10⁻⁵ M 2-mercaptoethanol, nonessential amino acids, and antibiotics. After 1 wk, Con A was washed out and the cells were recultured in the presence of 5 U/ml of recombinant human interleukin 2 (IL-2), the kind gift of Drs. Fiers and Devos, Biogent, Ghent, Belgium. Prolonged culture was performed by culturing with IL-2 and alternating the presence and absence of Con A on a weekly schedule. Before use, these T cell blasts were washed again in BSS with 20 mg/ml α-methyl mannoside to remove Con A.

Assay for E Rosettes. SRBC and HuRBC were treated with neuraminidase (Testneuraminidase; Behringwerke AG, Marburg, FRG) diluted 1:50 in BSS for 30 min at 37°C, washed, and adjusted to 10⁶/ml and 2.5 × 10⁷/ml, respectively in BSS with 5% FCS (BSS/FCS). Human PBL were either left untreated (for sheep E rosettes), or neuraminidase treated (for autologous rosettes), washed, and adjusted to 8 × 10⁶/ml in BSS/FCS. Sheep T cell blasts were also treated with neuraminidase and adjusted to 8 × 10⁶/ml BSS/FCS. 50 μl of lymphocytes were mixed with 50 μl of RBC and 50 μl of BSS/FCS. For rosettes of human PBL with SRBC, the mixture was left for 10 min at room temperature, centrifuged for 2 min at 200 g, and left for another 30 min at room temperature. Autologous human and sheep rosettes were prepared by incubating the cell mixture for 20 min at 37°C, centrifuging for 5 min at 200 g, and leaving the pelleted cells for 60 min on ice. All rosettes were microscopically enumerated after gently resuspending the cells and staining with crystal violet. 400 nucleated cells were examined per test, and nucleated cells with three or more bound RBC were scored as rosettes.

E Rosette Inhibition Assay. mAb to SRBC were assayed by preincubating 50 μl of SRBC with 50 μl of antibody preparations for 30 min at room temperature, followed by the addition of lymphocytes and the rosetting procedure as described above. Anti-T11 mAb (Coulter Immunology, Hialeah, FL) or partially purified T11TS were assayed by preincubating 50 μl of these preparations with 50 μl lymphocytes for 30 min at room temperature before proceeding with the assay. To determine whether lymphocytes or red cells are the target of inhibition, each partner was preincubated with the relevant preparation for 30 min at room temperature, washed twice with BSS/FCS followed by the addition of the untreated partner, and the assay performed as described above.

Isolation of mAb L180/1. Supernatants from a total of 86 SRBC-specific hybridomas, produced in two separate fusions from SRBC primed and boosted BALB/c mice with the nonsecreting myeloma X63-Ag 8.653, were screened for inhibition of rosettes between human PBL and SRBC. These hybridomas were kindly provided by M. Lohoff at this institute. One culture, termed L180, scored positive. It was subcloned, and antibody was purified from culture supernatant and ascites by ammonium sulphate precipitation and salt-gradient elution from DEAE-Affigel Blue (Bio-Rad Laboratories, Richmond, CA). The antibody L180/1 is of the IgG class and binds to protein A. Fab fragments were prepared by digestion with insolubilized papain (Sigma GmbH, Taufkirchen, FRG) and examined for purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). For controls, normal mouse IgG (NMIgG) was purified from serum. Protein content was determined using a protein assay (Bio-Rad Laboratories).
Preparation of Surface-labeled Cell Extracts. 100 μl of packed SRBC were washed three times in phosphate-buffered saline (PBS) containing 5 × 10^-6 M KI and resuspended in 0.9 ml of this buffer. 2.5 U of lactoperoxidase (Sigma GmbH), 1 mCi of 125I as NaI, and 10 μl of 0.3% H2O2 were added. 1 U lactoperoxidase and 10 μl of 0.3% H2O2 were added again, twice, at 4-min intervals. The reaction was stopped after another 8 min by washing the cells three times in PBS containing 5 × 10^-3 M KI. For labeling of cell surface carbohydrates, 5 × 10^8 SRBC were treated with 0.25 U neuraminidase and 25 U galactose oxidase (Sigma GmbH) in 5 ml BSS for 45 min at 37°C, washed once in cold PBS, and resuspended in 2 ml cold PBS containing 30 mCi tritiated BH4~ (NEN, Dreieich, FRG). After 30 min on ice the cells were washed three times in PBS. Surface-labeled cells were lysed in 2 ml PBS containing 0.5% Nonidet P-40 (NP-40) (Roth, Karlsruhe, FRG), 0.02% deoxycholate (Sigma GmbH), and 70 μg/ml aprotinin (Trasylol; Bayer, Leverkusen, FRG). After 15 min on ice the lysate was spun for 30 min at 20,000 rpm, and the pellet was discarded.

Radioimmunoprecipitation (RIP). Purified mAb L180/1 or NMIgG were covalently coupled to agarose beads according to the manufacturer's suggested procedure (Affigel 10; Bio-Rad Laboratories). ~3.5 mg IgG bound per milliliter of gel. Before radioimmunoprecipitation (RIP), the beads were washed three times in lysis buffer. 100–200 μl of radiolabeled lysate were kept with 50 μl of NMIgG-coupled beads for 30 min on ice. The supernatant was collected by centrifugation, and 50 μl L180/1 or NMIgG-coupled beads were added. After 60 min on ice, the beads were washed three times in lysis buffer containing 0.5 M KCl and three times in lysis buffer.

Large-Scale Enrichment of T11TS. 100 ml of PBS-washed, packed SRBC were lysed with an equal volume of PBS containing 1% NP-40, 0.04% deoxycholate, and 70 μg aprotinin. After 30 min on ice, the lysate was centrifuged for 30 min at 20,000 rpm in the cold. The supernatant was passed down over 1 ml of mAb L180/1-coupled Affigel 10. The immunosorbent column was washed with 50 ml lysis buffer containing 0.5 M KCl and 50 ml lysis buffer without KCl. The gel was removed from the column, residual buffer was aspirated, and the bound material was eluted overnight with 2 ml 3.5 M NH4SCN, pH 8.0 containing 0.2% NP-40. The eluate was passed over a Sephadex G-25 column equilibrated with 0.1% NP-40 in PBS. Two or three 1-ml fractions that were positive in the Bio-Rad protein assay were collected. For studies on the interaction of affinity-enriched material with viable cells, the gel was washed with PBS before salt elution and detergent was omitted during the following steps. Protein content was determined using the Bio-Rad protein assay.

Crude SRBC Membranes. 50 μl of packed SRBC were lysed with 1 ml H2O. Membranes were pelleted and washed three times in 0.2% NaCl by centrifugation at 20,000 rpm for 5 min.

SDS-PAGE and Autoradiography. Samples were boiled in Laemmli sample buffer (21) with or without 2-mercaptoethanol and run on SDS slab gels containing 10 or 12% acrylamide. 12C-labeled markers were: cytochrome c (12 kilodaltons [kD]), carbonic anhydrase (30 kD), ovalbumin (45 kD), bovine serum albumin (BSA) (66 kD), phosphorylase b (92 kD) (all from Amersham Buchler, Braunschweig, FRG). Unlabeled markers were: lysozyme (14.3 kD), trypsinogen (24 kD), ovalbumin (45 kD), BSA (66 kD) (Sigma GmbH). Gels were fixed, stained with Coomassie Blue, and dried. Gels containing 12H-labeled material were fluorographed using dimethyl sulfoxide/PPO. Autoradiography was performed with Cronex 2 x-ray film (DuPont Co., Wilmington, DE) preexposed to light, using enhancing screens.

Indirect Immunofluorescence and Flow Cytometry. 2.5 × 10^5 human PBL were preincubated for 30 min at room temperature with either 100 μl PBS or PBS containing 1 μg protein eluted from the L180/1 affinity column. The cells were washed once and treated with 4 μg NMIgG, anti-T11 mAb diluted 1:100, or anti-T3 mAb (OKT3; Ortho Diagnostic Systems, Inc., Raritan, NJ) diluted 1:50, in 0.1 ml PBS containing 0.1% BSA and 0.02% sodium azide (PBS/BSA/azide). After 30 min on ice, the PBL were washed in this buffer, stained with goat anti-mouse, fluorescein isothiocyanate (FITC)-coupled antibodies (Coulter Diagnostics, Hialeah, FL) diluted 1:50 for 30 min on ice, and washed three times.
in PBS/BSA/azide. Analysis was performed on an Epics V flow cytometer (Coulter Electronics, Inc., Hialeah, FL), using a forward angle light scatter gate for lymphocytes and collecting integrated green fluorescence signals from a logarithmic amplifier.

Results

Inhibition of Heterologous and Autologous E Rosette Formation by Anti-T11 and mAb L180/1. The culture supernatants from a total of 86 SRBC-specific hybridomas from two independent fusions were screened for their ability to inhibit rosette formation between human T cells and SRBC. One positive culture (L180) was identified, subcloned, and the antibody purified from culture supernatant and ascitic fluid. Fab fragments were prepared to eliminate hemagglutination and tested again for rosette inhibition.

As shown in Fig. 1A, nanogram amounts of L180/1 Fab fragments completely abrogated the formation of rosettes between human PBL and SRBC. Since mAb L180/1 does not bind to HuRBC (data not shown), it was expected that autologous human rosettes were not affected by L180/1 Fab (Fig. 1B). On the other hand, anti-T11, which is known to block binding of SRBC to human T cells (6–9) (Fig. 1A), also blocked autologous human rosette formation (Fig. 1B), suggesting that the same E receptor binds SRBC and HuRBC. The absence of the epitope recognized by L180/1 from HuRBC could either mean that the target structure recognized by the human E receptor on SRBC and HuRBC are unrelated, or that there is antigenic variation of the same molecule between species, as is commonly found.

If, as hypothesized, the putative target structure for the E receptor is a cell
interaction molecule, it must be functioning in an autologous situation such as in the binding of human T cells to human erythrocytes. Since, as discussed above, mAb L180/1 detects the putative ligand of T11 on sheep but not on human RBC, we have devised a protocol for the formation of sheep-sheep rosettes. Although freshly isolated SWBC do not form stable autologous E rosettes, ~10% of SWBC activated by Con A and propagated in human recombinant IL-2 bound numerous SRBC to form stable rosettes (Fig. 2). This rosette formation was completely inhibited by L180/1 Fab (Fig. 1C), suggesting that sheep T cell blasts have a receptor for autologous RBC that binds the same target structure also recognized by the human T11 molecule. The mAb to T11 that was used neither bound to sheep T cell blasts (data not shown) nor inhibited sheep autologous rosettes (Fig. 2C). In summary, it appears that analogous receptor-ligand systems exist in man and sheep, and that the mAb to T11 and mAb L180/1 recognize species-specific determinants on the E receptor and its target, respectively.

Isolation of T11TS from SRBC. SRBC were either surface iodinated or surface labeled by treatment with neuraminidase plus galactose oxidase followed by reduction with tritiated borohydride. Immunoprecipitates from detergent lysates were analyzed by SDS-PAGE and autoradiography. As can be seen in Fig. 3A, a band with an apparent molecular weight of ~42,000 was precipitated from $^{125}$I-labeled extracts by mAb L180/1 coupled to agarose beads under both reducing and nonreducing conditions. The additional band of higher molecular weight was, in all experiments where it was found in L180/1 immunoprecipitates, also present in control precipitates. It is therefore regarded as nonspecific. Immunoprecipitation of $^3$H-labeled glycosylated membrane molecules revealed a single band of slightly smaller apparent molecular weight than observed with iodinated extracts. Note that this band does not comigrate with the bulk of the labeled
FIGURE 3. SDS-PAGE of surface-labeled SRBC extracts radioimmunoprecipitated with mAb L180/1. (A) ^125I-labeled, 10% acrylamide; (B) ^3H-labeled, 10% acrylamide reduced; (C) ^125I-labeled, 12% acrylamide reduced. Neuraminidase (N'ase) treatment of the lysate (C) was performed for 30 min at 37°C with 1:50 dilution of Testneuraminidase.

material (Fig. 3B). The difference in molecular weight between iodinated and ^3H-labeled material is accounted for by the removal of sialic acid during the labeling procedure used for the latter, since the same shift in molecular weight was observed if ^125I-labeled extracts were neuraminidase treated before immunoprecipitation (Fig. 3C). The apparent glycoprotein nature of T11TS has been further substantiated by trypsin and endoglycosidase degradation (data not shown).

The ready availability of the starting material also permitted large-scale purification of T11TS. As an initial step, a detergent lysate prepared from 100 ml packed SRBC was passed over insolubilized mAb L180/1 and eluted with high salt. As can be seen in Fig. 4C, T11TS is a major, although not the only band visible upon Coomassie Blue staining of the eluate in SDS-PAGE. Subsequent radiolabeling of this affinity-enriched material and immunoprecipitation (data not shown) confirmed that the band indicated by arrow in Fig. 4 is the material precipitated by mAb L180/1 as described above. To determine whether mAb L180/1 precipitates a major protein of the red cell membrane, a crude SRBC membrane preparation (Fig. 4, A and B) and total detergent lysate (Fig. 4, D and E) were run in parallel. It appears that T11TS is only a minor component of the red cell membrane as detected by this method. T11TS can be further purified by adsorption to Con A-Sepharose (not shown), and our current efforts are directed towards obtaining enough homogeneous material for microsequencing.

Binding of Partially Purified T11TS to the E Receptor of Human T Cells. Initial experiments showed that after elution of detergent-solubilized T11TS from the L180/1 affinity column with high salt and transfer into PBS via salt-exchange chromatography, the yield of T11TS was greatly enhanced if the presence of detergent was maintained. However, enough material for detection by protein stains in SDS-PAGE was recovered if the high salt eluate was transferred to detergent-free PBS, permitting investigation of the interaction between the affinity-enriched material and the E receptor on viable cells. Two types of experiments were performed with such preparations. First, we investigated whether partially purified T11TS blocks formation of rosettes between human
TARGET STRUCTURE FOR THE E RECEPTOR

**FIGURE 4.** Large-scale affinity enrichment of T11TS. (A, B) 1:10 and 1:100 dilutions of crude SRBC membranes; (C) eluate from L180/1 affinity column; (D, E) 1:100 and 1:10 dilutions of detergent lysate applied to affinity column. SDS-PAGE (12% acrylamide) was run under reducing conditions and stained with Coomassie Blue.

**FIGURE 5.** Inhibition of E rosette formation by affinity-enriched T11TS. Human PBL were preincubated with the eluate from the affinity column before addition of neuraminidase-treated SRBC. In the absence of T11TS, 58% of PBL formed rosettes.

PBL and SRBC (Fig. 5). Complete inhibition was indeed observed when 500 ng of protein was added to the assay system. To assess whether the observed effect was caused by the putative ligand of the E receptor or by antibody leaking from
Effect of Preincubation of PBL or SRBC with mAb L180/1, anti-T11, or Partially Purified T11TS on E Rosette Formation

| Pretreatment of: | Percent inhibition of E rosettes after pretreatment | Human PBL | SRBC |
|-----------------|-----------------------------------------------------|---------|------|
| Anti-T11        | 95                                                  |         | 0    |
| Fab L180/1      | 0                                                   | 100     |      |
| T11TS           | 91                                                  | 18      |      |

PBL or SRBC were preincubated for 30 min with mAb to T11 (1:300 final), 2 µg Fab of mAb L180/1, or 2 µg of partially purified T11TS, washed, and tested for rosette formation. 66% of nucleated cells formed rosettes in untreated controls.

Figure 6. Interference of partially purified T11TS with the binding of anti-T11 to human PBL. PBL were preincubated with affinity-enriched T11TS, washed, and stained with anti-T11 and anti-T3, followed by FITC-coupled goat anti-mouse Ig as described in Materials and Methods. NMIgG was included as a negative control but, for reasons of clarity, is not shown.

the immunosorbet column, PBL or SRBC were separately preincubated with either anti-T11 mAb, Fab L180/1, or partially purified T11TS, then washed, mixed with the untreated partner, and examined for rosettes (Table I). As expected, T11 mAb and partially purified T11TS blocked rosette formation if the PBL were preincubated, whereas mAb L180/1 was only effective after pretreatment of red cells. It appears, therefore, that a protein was present in the column eluate that was capable of competing with the binding site for red cells on human T cells.

More direct evidence for the binding of partially purified T11TS to the E receptor is presented in Fig. 6. Human PBL were preincubated with the eluate from the affinity column or with control buffer, washed, treated with mAb to T11 or to T3, stained with FITC-conjugated second antibody, and analyzed by flow cytometry. Pretreatment with the preparation believed to contain T11TS resulted in a marked reduction of staining with the anti-T11 mAb, but had no effect on staining with anti-T3 mAb. This indicates a specific blocking of the binding site recognized by anti-T11 and thus confirms the presence of E receptor binding material in the affinity-enriched preparation.
Discussion

The results presented here show that an mAb to SRBC, termed L180/1, completely inhibits rosette formation between human or sheep T lymphocytes and SRBC. In addition, the molecule recognized by this mAb, a minor glycoprotein of the SRBC membrane with an apparent molecular weight of ~42,000 (42 K), also inhibited rosette formation and the binding of anti-T11 mAb to the E receptor on human T cells. Since these findings appear to indicate that mAb L180/1 detects the cell surface molecule recognized by the E receptor, we have given this glycoprotein the preliminary name T11 target structure, or T11TS.

This is not the first report claiming the identification of the SRBC membrane molecule involved in rosette formation, although blocking of the E receptor as detected by reduced binding of mAb to T11 (Fig. 6) has, to our knowledge, not been described. Kitao et al. (18) described a sialoglycopeptide of 10 K mol wt released from SRBC by trypsin, which blocked the formation of E rosettes; Fletcher et al. (19) demonstrated rosette inhibition with glycoproteins of 37, 26.5, and 9 K mol wt that were isolated by biochemical means from SRBC membranes. In contrast, Gürtler (20) detected rosette-inhibiting activity in the glycolipid fraction of SRBC membranes. The relationship between these preparations and the molecule described here is unclear at present, but it should be noted that in these reports the amount of glycoprotein or glycolipid required to block rosette formation was several hundred-fold higher than is reported here for the partially affinity-purified material. Thus, the active component may have comprised only a small minority of the glycoprotein or glycolipid preparations isolated biochemically. Indeed, the results in Figs. 3 and 4 show that the molecule recognized by mAb L180/1 is not one of the major glycoproteins of the well-studied red cell membrane (22), a finding corroborated by the expression of T11TS on white blood cells also (Hünig, manuscript in preparation). We are presently attempting to purify enough material for microsequencing, so that the identity of T11TS will hopefully be soon resolved.

The nature of the interaction between the E receptor and its target structure remains unclear, although several laboratories have presented evidence for the recognition of sugar moieties on the red cell membrane. Boldt and Armstrong (23) demonstrated rosette inhibition by several glycoproteins rich in sialic acid, galactose, N-acetyl glucosamine, and mannose, although at concentrations several orders of magnitude higher than were required for blocking in our experiments. Moreover, Fletcher et al. (19) reported that the glycoproteins which they isolated lost their activity in the rosette inhibition assay after enzymatic desialylation, an apparent contradiction to the enhanced rosette-forming capacity of neuraminidase-treated SRBC. We have confirmed this finding to some extent with the glycoprotein studied here (data not shown), but the poor solubility of T11TS in the absence of detergents suggests that the reduction in inhibitory activity after desialylation may not be due to destruction of the site recognized by the E receptor but rather to a further loss of solubility in aqueous buffers. It should be added that neuraminidase treatment of SRBC or iodinated lysates did not impair the binding of mAb L180/1 as determined by radioimmunoprecipitation (Fig. 3C) and quantitative flow cytometry (data not shown), indicating that sialic acid residues are not directly involved in the antigenic determinant recognized...
by this mAb. Nevertheless, it cannot be distinguished at present whether mAb L180/1, much less the E receptor, recognize the protein or the sugar moiety of T11TS. Experiments addressing this question are under way. Quite clearly, however, the complete inhibition of rosette formation by nanogram amounts of Fab fragments of an mAb specific for only one minor glycoprotein of the red cell membrane demonstrates that the interaction of the E receptor with its complementary structure is highly selective.

The expression of T11TS on white blood cells (Hünig, manuscript in preparation) points to the possible biological significance of this interaction; the present demonstration of autologous rosettes in the sheep system that appear to use the same molecule on SRBC as that recognized by human T lymphocytes (Fig. 1), is intriguing in this respect. The E receptor as defined by anti-T11 and related mAb is widely distributed among primates (24), and the present results suggest that an analogous system also exists in more distantly related mammals. In this context, it is of interest that anti-T11 mAb blocked both heterologous rosettes between human T cells and SRBC, and autologous human rosettes (Fig. 1). This suggests that the E receptor target system is highly conserved and that SRBC rosetting is more than an immunological curiosity. One may speculate that red cell rosetting involves the interaction of complementary cell interaction molecules, a notion fully consistent with published experiments on the regulation of T cell activation via the E receptor (10–16).

Summary

A monoclonal antibody (mAb) to sheep red blood cells (SRBC), termed L180/1, is described that completely blocks rosette formation between SRBC and human or sheep T lymphocytes. L180/1 precipitated a minor glycoprotein of about ~42,000 mol wt from surface-labeled SRBC. This glycoprotein was partially affinity purified and found to block E rosette formation and to compete with anti-T11 mAb for the E receptor. The molecule detected by mAb L180/1 thus appears to be recognized by the E receptor and was given the preliminary name, T11 target structure (T11TS). Since the mAb to sheep T11TS blocks the binding of SRBC to both human and sheep T cells, and mAb to T11 blocks the binding of red cells from human and sheep to the human E receptor, we concluded that analogous receptor-ligand (T11-T11TS) systems exist in man and sheep that are crossreactive over the species barrier. The possibility is discussed that the E receptor, which is known to be involved in T cell activation, and T11TS function as complementary cell interaction molecules in T cell responses.

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