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Isolation, Biochemical Analysis, and *N*-Terminal Amino Acid Sequence of a Cell Surface Glycoprotein that Binds to the "Erythrocyte Receptor" of T-Lymphocytes *

T. Hünig, R. Mitnacht, G. Tiefenthaler, and F. Lottspeich

A. Introduction

Recent findings have provided strong evidence in support of a functional role of the "erythrocyte receptor" of human T-lymphocytes in T-cell activation. Thus, binding of appropriate ligands (erythrocytes or monoclonal antibodies) to the CD2 molecule, as this receptor is now called, can either block (Palacios and Martinez-Maza 1982; Martin et al. 1983; Krensky et al. 1983) or stimulate (Larsson et al. 1978; Meuer et al. 1984) T-cell proliferation and expression of T-cell function. We have previously suggested that CD2 is a cell interaction molecule, the natural ligand of which is expressed on cells with which T-lymphocytes interact during immune responses (Hünig 1985, 1986). Since the CD2 molecule had previously been characterized by the T11 antigen(s), we have named this ligand T11 target structure or T11TS. In our search for T11TS, we have assumed that the molecule recognized by CD2 on white blood cells during the cellular interactions involved in T-cell activation is identical to the one recognized on autologous and xenogeneic red cells in E-rosette formation. Consequently, a monoclonal antibody (mAb) to sheep red blood cells (SRBC) was selected that completely blocks the attachment of SRBC to either human or sheep T-lymphocytes (Hünig 1985). This mAb detects a cell surface glycoprotein of about 42 k MW which is, as we had postulated, expressed on both red and white blood cells (Hünig 1986). Furthermore, this anti-T11TS mAb inhibits the mixed lymphocyte reaction between outbred sheep, thus suggesting an involvement of T11TS in T-cell activation (Hünig 1986). Here we report some biochemical properties of T11TS, including its N-terminal amino acid sequence.

B. Materials and Methods

The E-rosette inhibition assay has been described (Hünig 1985). Affinity purification of T11TS from detergent lysates of SRBC was performed with mAb L180/1 (anti-T11TS) coupled to glutaraldehyde-activated glass beads. Preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed using slab gels of 12% acrylamide content. Trace-labeled T11TS was identified by autoradiography and electroeluted according to Goding (Goding 1984). Purified T11TS was radioiodinated employing immobilized lactoperoxidase. The N-terminal amino acid sequence was determined on an Applied Biosystems gas-phase sequencer.

C. Results

Figure 1 illustrates the complete inhibition of SRBC binding to human T cells by Fab fragments of mAb L180/1 (anti-T11TS). The specificity and concentration dependence of this effect has been studied and described in detail (Hünig 1985).

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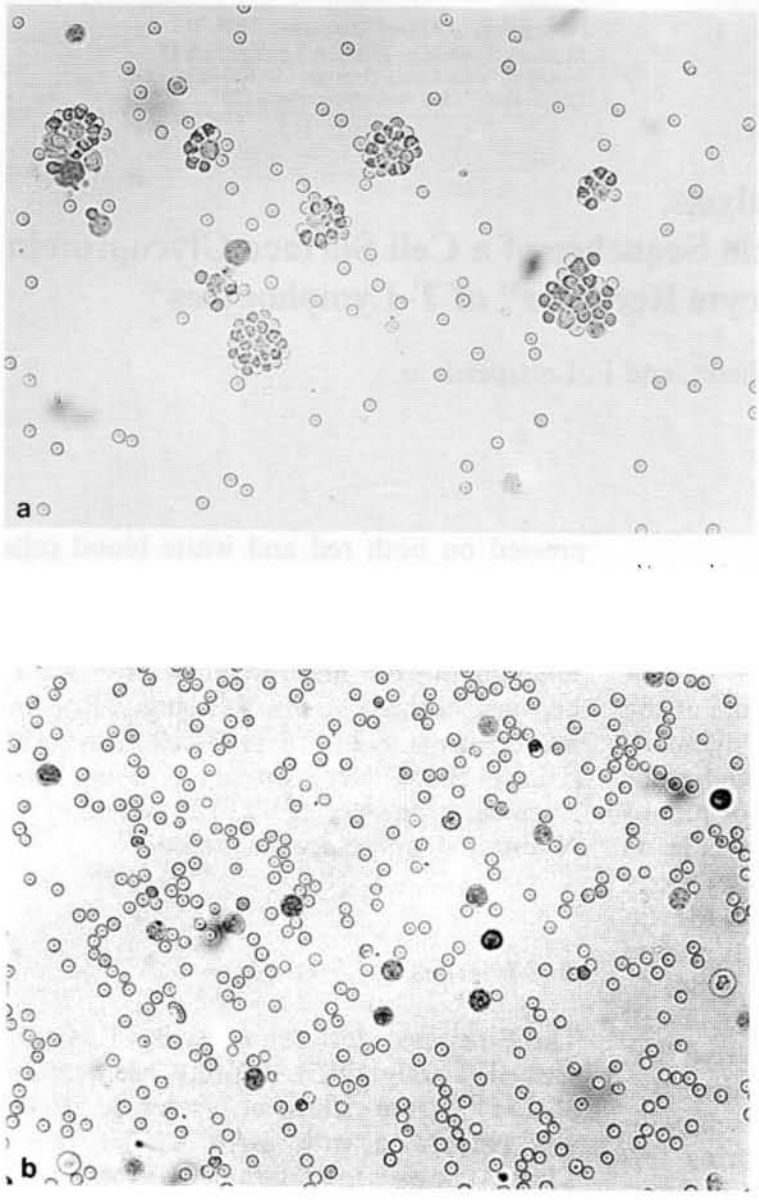

Fig. 1 a, b. Inhibition of rosette formation between SRBC and human T-lymphocytes by mAb L180/1. Nylon-wool-passed human PBL were incubated with SRBC in the absence (*panel a*) in the presence (*panel b*) of 5 μg/ml of purified Fab fragments of mAb L180/1

In order to obtain further information on the structure of T11TS, we purified the molecule to homogeneity. This was achieved in a two-step procedure using affinity chromatography on mAb L180/1 coupled to glass beads and preparative SDS-PAGE (Fig. 2). A sample of the purified material was radioiodinated and digested with endoglycosidase F, an enzyme that specifically removes N-glycosidically linked carbohydrate side chains (Fig. 3). From the intermediary products observed before the end product of about 32 k apparent MW is obtained, one can conclude that T11TS contains either three N-linked carbohydrate chains of roughly even size or two side chains of different size. From the absence of galactosamine observed during amino acid analysis (data not shown), it can be concluded that T11TS contains no O-glycosidically linked carbohydrate. In addition, a sample of radioiodinated T11TS was subjected to isoelectric focusing (Fig. 4). T11TS is an acidic membrane glycoprotein with a pI of 4.5.

The first 27 N-terminal amino acids of T11TS were determined by gas-phase microsequencing. The sequence is: FSQDIY GAMNGS(?)VTFYVSESQ PFTEIM. A search of the protein database from the Protein Identification Resource (George et al. 1985) has indicated that we are dealing with a previously unsequenced molecule with no obvious homologies of the sequenced portion of T11TS to known protein sequences.

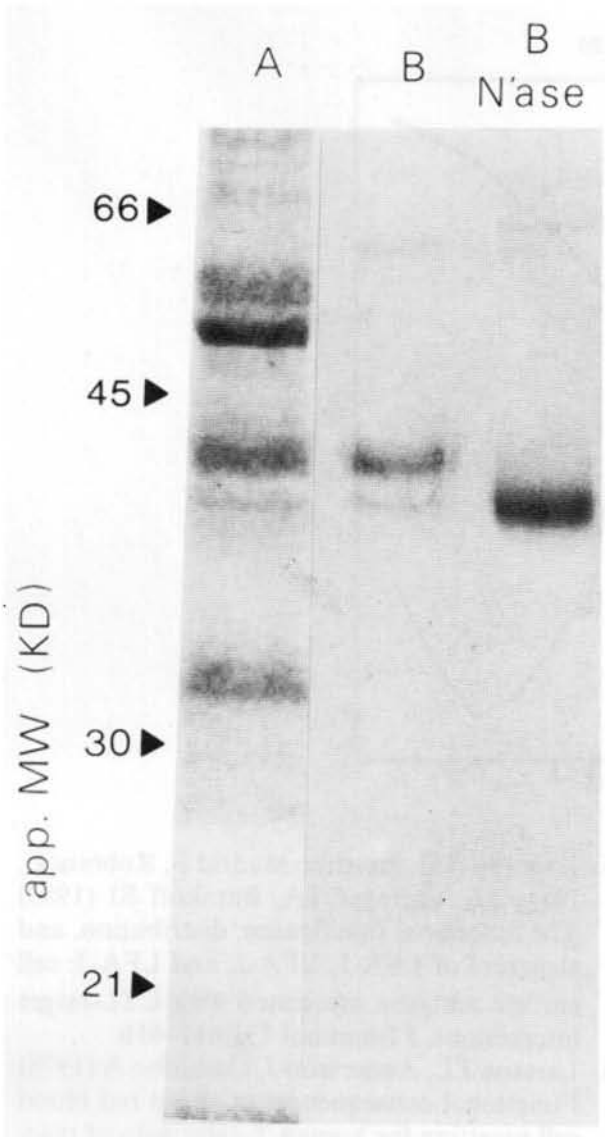

Fig. 2. Purification of T11TS. Detergent-solubilized SRBC were passed over a mAb L180/1 affinity column. In the high-salt eluate (*A*), the band at 42 k was identified as T11TS by trace labelling and autoradiography (data not shown). *B*, T11TS after electroelution from preparative slab gel of material A. *B N'ase*, material B pretreated with neuraminidase to reveal possible contaminants comigrating with T11TS. Coomassie blue stain of SDS-PAGE (12% acrylamide, reduced)

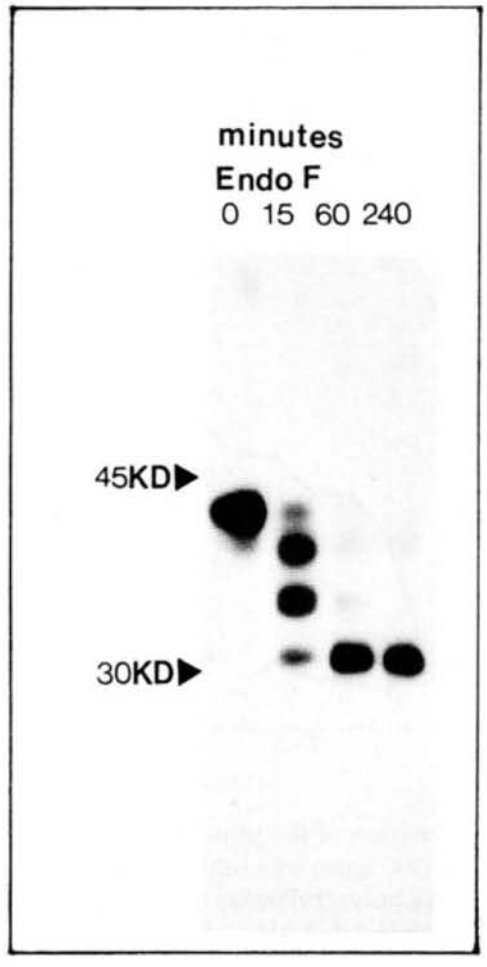

Fig. 3. Endoglycosidase F digestion of T11TS. ^{125}I-labeled purified T11TS was treated with 2U endoF for the number of times indicated, subjected to SDS-PAGE (12% acrylamide, reduced), and autoradiographed

This sequence and several short stretches of sequence determined from internal peptides of T11TS provide the basis for our current efforts to isolate the T11TS gene.

D. Conclusions

The present report and our published work show that a previously undefined glycoprotein expressed on sheep red and white blood cells is recognized by the E receptor in rosette formation. This molecule is a good candidate for the hypothetical cell interaction molecule that binds to the CD2 structure during cellular interactions of T-lymphocytes with cells of the immune system. The latter point will be difficult to prove in the sheep system. However, the known binding of human erythrocytes to human T cells via CD2 (Scheffel et al. 1982; Hünig 1985) is a strong indication that a human homolog of T11TS must exist. Indeed, recent experiments by Springer and colleagues have identified this human ligand of CD2 as the lymphocyte function-associated (LFA)-3 molecule (Selvaraj et al. 1987).

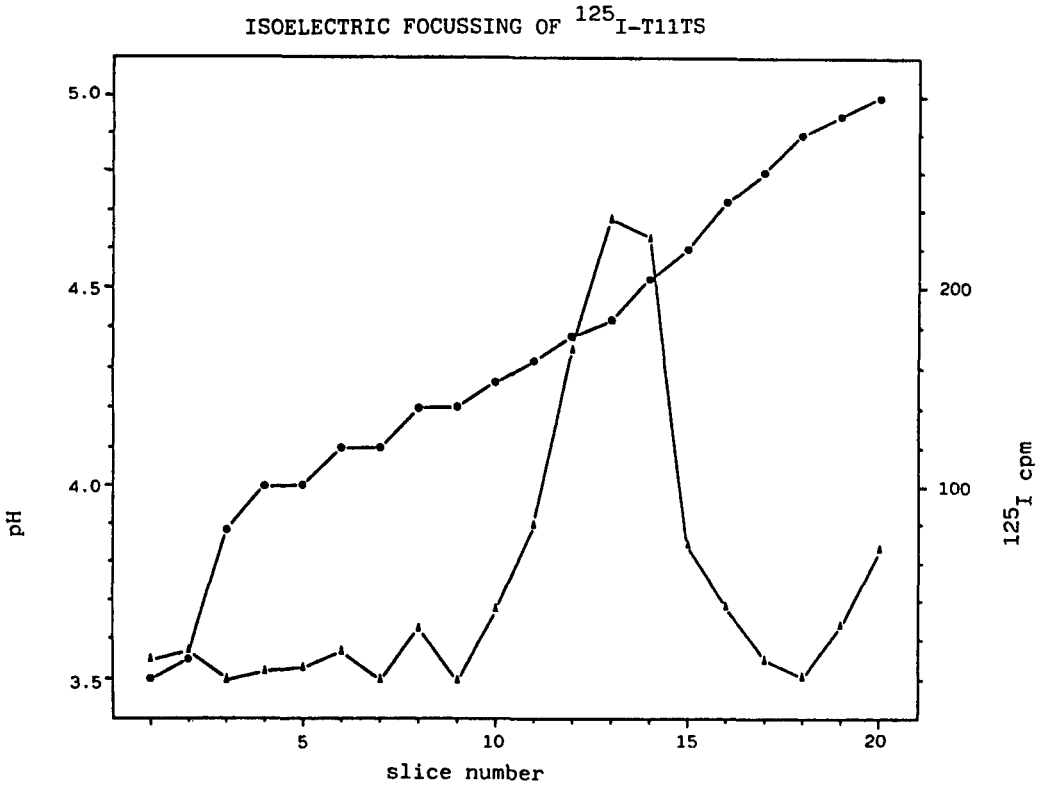

Fig. 4. Determination of the pI of T11TS. ^{125}I-labeled T11TS (3000 cpm) was subjected to isoelectric focusing in a polyacrylamide tube gel containing ampholines pH 2.5–5 pH and radioactivity in serial slices are shown

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References

1. George DG, Orcutt BC, Dayhoff MO, Barker WC (1985) PIR report REL-0185, version 2.0. National Biomedical Research Foundation, Georgetown University Medical Center, Washington
2. Goding JW (1984) A simple and efficient device for electrophoretic elution of proteins from SDS-polyacrylamide gels. Annu Rev Walter and Eliza Hall Inst 1983–1984:49–50
3. Hünig T (1985) The cell surface molecule recognized by the erythrocyte receptor of T lymphocytes. Identification and characterization using a monoclonal antibody. J Exp Med 162:890–901
4. Hünig T (1986) The ligand of the erythrocyte receptor of T-lymphocytes: expression on white blood cells and possible involvement in T-cell activation. J Immunol 136:2103–2108
5. Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ (1983) The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. J Immunol 131:611–616
6. Larsson EL, Andersson J, Coutinho A (1978) Functional consequences of sheep red blood cell rosetting for human T-cells: gain of reactivity to mitogenic factors. Eur J Immunol 8:693–696
7. Martin PJ, Longton G, Ledbetter JA, Neumann W, Braun MP, Beatty PG, Hansen JA (1983) Identification and functional characterization of two distinct epitopes on the human T-cell surface protein tp50. J Immunol 131:180–185
8. Meuer SC, Hussey RE, Fabbi M, Fox D, Acuto O, Fitzgerald KA, Hodgdon JC, Protentis JP, Schlossman SF, Reinherz EL (1984) An alternative pathway of T-cell activation: a functional role for the 50 KD sheep erythrocyte receptor protein. Cell 36:897–906
9. Palacios R, Martinez-Maza O (1982) Is the E-receptor on human T-lymphocytes a "negative signal receptor"? J Immunol 129:2479–2485
10. Scheffel JW, Swartz SJ (1982) Inhibition of autologous rosette formation by monoclonal antibody to the sheep erythrocyte receptor. J Immunol 128:1930–1932
11. Selvaraj P, Plunkett ML, Dustin ME, Sanders ME, Shaw S, Springer TA (1987) The t-lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3. Nature 326:400–402