

Thy-1 Supports Adhesion of Mouse Thymocytes to Thymic Epithelial Cells through a Ca²⁺-independent Mechanism

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Summary

The aim of this study was to explore whether Thy-1, like other members of the Ig-like superfamily (e.g., CD2 and neural cell adhesion molecule), participates in cell-cell adhesion. This was investigated by measuring the binding of Thy-1⁺ probe cells (thymocytes or AKR1 T lymphoma cells) to Thy-1⁻ cloned mouse thymic epithelial (MTE) cells using a quantitative cell adhesion assay. The results were as follows: (a) the thymo-epithelial cell interaction was found to be inhibitable (by 25–40%) by soluble Thy-1 molecules purified from phosphatidylinositol-specific phospholipase C-treated mouse thymocytes as well as by Fab' fragments of a Thy-1-specific mAb; (b) the binding of the Thy-1⁻ AKR1 (*Thy-1^{-d}*) mutant to MTE cells was found to be reduced (by 50%) as compared with that of the wild type T lymphoma; (c) the Thy-1-mediated adhesion pathway did not require Ca²⁺ and promoted the initial thymo-epithelial binding measured at 4°C. These data provide the first direct evidence of an adhesive function of Thy-1 and suggest that this molecule, in addition to its T cell triggering properties, might play a role during the early T cell maturation by promoting thymocyte adhesion to thymic stroma.

Thy-1 is a glycoprotein abundantly expressed by neurons and murine thymocytes (1). It is composed of an IgV domain-like polypeptide of 112 amino acids which is anchored in the plasma membrane through a glycosyl-phosphatidylinositol (G-PI) tail (2). Although the Thy-1 gene appears to be extremely conserved during evolution, its pattern of expression, while under strict tissue-specific control, does vary to some extent between species (3). Thus, Thy-1 becomes a major neuronal surface protein in rodents several weeks after birth, whereas its expression on thymocytes reaches a maximum level around day 15 of gestation, i.e., immediately after the entry of prothymocytes into the thymus; Thy-1 surface expression decreases significantly when mouse thymocytes migrate to the periphery and is absent from rat peripheral T cells (3). Despite these studies on Thy-1 structure, expression, and gene organization, the physiological role of this molecule has remained a matter of speculation. Thy-1, like several other G-PI-anchored surface molecules, has been implicated in T cell activation (4).

There is increasing evidence that members of the Ig-like superfamily (e.g., neural cell adhesion molecule [NCAM], CD2, and lymphocyte function-associated antigen 3 [LFA-3]), participate in cell adhesion (2, 5). The fact that thymocytes upregulate Thy-1 surface expression while maturing in the vicinity of the thymic stroma led us to explore the possi-

bility that this molecule could participate in lympho-epithelial cell interactions. Use of normal thymocytes and T lymphoma cells rather than transfected cells was chosen to evaluate this issue. However, the likely participation of other adhesion systems such as CD2/LFA-3, LFA-1/intercellular adhesion molecule 1 (ICAM-1), CD8/MHC class I molecules shown by us and others (6–8) to play a role in these cell interactions required the use of a quantitative cell adhesion assay initially described by McClay et al. (9). We report here experimental evidence that Thy-1 is involved in lympho-epithelial cell adhesion through a heterophilic and Ca²⁺-independent mechanism.

Materials and Methods

mAbs. The rat mAbs H155-124 (γ 2c, κ), H154-200 (γ 2b, κ), and H140-150 (γ 2b, κ) recognize monomorphic Thy-1 determinants in the epitope regions C and B, respectively; 5A2 is a rat γ 1, κ anti-mouse IL-2R antibody; H129-296 is a rat γ 2a, κ mAb defining the epitope C of mouse LFA-1 heavy chain; H35-17 is a rat γ 2b, κ mAb to mouse CD8; YN1 is a rat anti-mouse ICAM-1. The origin and specificity of these mAbs have been described elsewhere (8, 10).

Cells. Viable thymocytes were obtained from neonatal Swiss mice by centrifugation on a Ficoll (Pharmacia, Uppsala, Sweden) Telebrix 38 (Guerbet, Paris, France) gradient. The wild-type (AKR1)

and the class D Thy-1⁻ mutant AKR1 (*Thy-1^{-d}*) T lymphoma cells were provided by R. Hyman (The Salk Institute, San Diego, CA).

Cell Adhesion Assay. The cell adhesion assay was modified (8) from that described by McClay et al. (9). In brief, MTE cell monolayers were grown to confluency in type I collagen-coated (Sigma Chemical Co., La Verpilliere, France), flat-bottomed, 96-well flexible plates (Dynatech Laboratories, Marnes-La-Coquette, France) for 48 h. Probe cells were labeled with ⁵¹Cr (25 μCi/ml in complete culture medium for 3 h at 37°C). Cells were washed (×2) and resuspended in culture medium containing 5 mM EDTA when appropriate. Labeled thymocytes (10⁶) or T lymphoma cells (5 × 10⁴) were added to each well of the plates containing the MTE cells and spun onto the monolayer at 50 g for 3 min at 4°C. The wells were filled with medium up to the rim and sealed with plastic sheets. The plates were immediately inverted and centrifuged upside down for 5 min. The optimal radial centrifugal force (RCF) for the detection of the Thy-1-dependent cell adhesion was evaluated to be between 100 and 200 g. In all but one (shown in Fig. 4) experiment, a force of 100 g was used. Plates were quickly frozen, and their bottom 3 mm were cut off and counted. The percentage of binding was defined as the ratio between the bound cpm and the total cpm input. Results were expressed as the arithmetical mean of quadruplicate measurements; standard errors were generally <5%. mAb-mediated inhibition of cell adhesion was assayed by preincubating radiolabeled probe cells with Fab' fragments for 30 min at 4°C. In some experiments, purified Thy-1, or the supernatants containing glypiated molecules, were preincubated with MTE cell monolayer for 2 h at 4°C. In both cases, cells were washed at 4°C before the adhesion assay.

Solubilization, Immunodepletion and Purification of Thy-1 Molecules. Glypiated cell-surface molecules were solubilized by treating thymocytes with *Bacillus thuringiensis* PI-PLC (0.1 U/ml in DMEM) (provided by G. Rougon, CNRS, URA 179, Marseille). The enzyme was removed from supernatants by immunodepletion using PI-PLC-specific rabbit Ig (prepared by G. Rougon). For antigen depletion, solubilized molecules were incubated overnight at 4°C with Thy-1 (H155-124)- or IL-2R (5A2)-specific mAb coupled to Sepharose-4B (Pharmacia). Purification of Thy-1 mAb was performed by applying the PI-PLC-solubilized molecules to an anti-Thy-1 (H155-124) affinity column as described (2).

Analysis of Thy-1 Cell Surface Expression. Membrane proteins from thymocytes of 2–5-d-old mice or MTE cells were labeled with sulfo-NHS-biotin (Pierce Europe BV., Oud-Beijerland, The Netherlands) (11) and subjected to immunoprecipitation.

Results and Discussion

The role played by Thy-1 in the lympho-epithelial cell interactions was demonstrated by studying mouse thymocyte binding to MTE cell monolayers using the adhesion assay described above. Microscopic analysis of lympho-epithelial cell interaction showed that MTE cells grew as a tight monolayer and that thymocytes were homogeneously and strongly bound to the cells (8). No Thy-1 expression could be detected on MTE cells, either by immunoprecipitation (see Fig. 1 A) or by a sensitive surface radioimmunoassay (data not shown) with two distinct Thy-1 epitope-specific mAbs. In contrast, PI-PLC-solubilized, immunopurified Thy-1 molecules (Fig. 1 B), which were shown to retain their immunoreactivity, were found to bind to MTE cell monolayer (data not shown). Preincubation of purified Thy-1 molecules

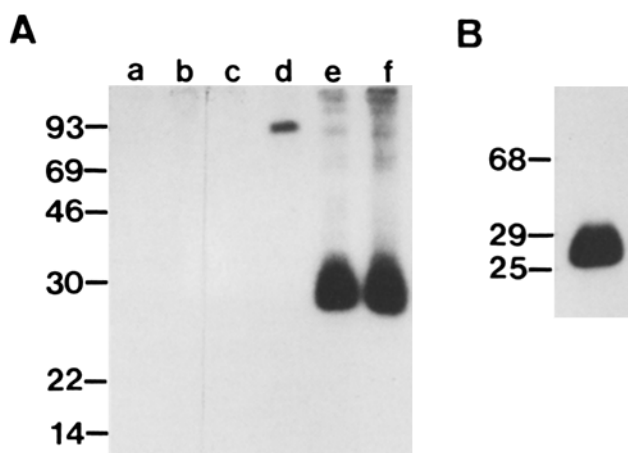


Figure 1. Thy-1 expression on mouse neonatal thymocytes and MTE cells. (A) Mouse thymocytes and MTE cells were biotinylated and lysed in Triton X-100. Labeled proteins from MTE cells (lanes a–d) and from thymocytes (lanes e and f) were immunoprecipitated by either the Thy-1-specific mAb H154-200 (lanes a and e) or H140-150 (lanes b and f). In the case of MTE cell detergent extracts, the YN-1 (anti-ICAM-1, lane d) and H35-17 (anti-CD8, lane c) mAbs were used as positive and negative controls, respectively. Immune complexes were separated by SDS-PAGE and transferred to nitrocellulose sheets. Biotinylated proteins were visualized with ¹²⁵I-labeled streptavidin and radioautography. The positions of the mol wt standards [M_r ($\times 10^{-3}$)] are indicated. (B) Purification of a hydrophilic form of the Thy-1 molecules from mouse neonatal thymocytes. Cells (5×10^6) were treated with PI-PLC (0.1 U/ml) for 2 h at 37°C. The solubilized Thy-1 molecules were purified from the supernatant by affinity chromatography on an anti-Thy-1 mAb column. The purified molecules were iodinated, subjected to SDS-PAGE and radioautography.

with MTE cells reduced (by 25%) thymocyte binding to the monolayers (Fig. 2 A). To further establish the Thy-1-specific inhibition of cell-cell adhesion, supernatants of PI-PLC-treated thymocytes (from which PI-PLC had been previously depleted) were tested for inhibition of cell adhesion before or after removal of soluble Thy-1 molecules. The results shown in Fig. 2 B support the notion that Thy-1 accounts for most of the inhibiting capacity of the supernatant. Finally, preincubation of thymocytes with Fab' fragments of a Thy-1-specific mAb (H140-150) specifically reduced thymocyte binding to MTE cells (with a plateau of 25% inhibition reached at a concentration of 30 μg/ml). No inhibition was observed with the isotype-matched mAb H129-296 which recognized the LFA-1 epitope C (nonfunctional under the conditions of initial binding at 4°C) (8).

The lack of complete inhibition of the lympho-epithelial cell interaction by purified Thy-1 molecules was not surprising. First, the thymocytes were heterogeneous, and Thy-1 might only be involved in the binding of a thymocyte subpopulation to MTE cells. Second, other molecular interactions such as CD2/LFA-3, LFA-1/ICAM are likely to participate in the cell adhesion (6–8). To optimize the detection of the Thy-1-mediated adhesion, we used the AKR1 mouse T lymphoma (which expresses similar amounts of surface Thy-1 as neonatal Swiss thymocytes) and its Thy-1⁻ variant AKR1(*Thy-1^{-d}*), in which the loss of Thy-1 expression was previously

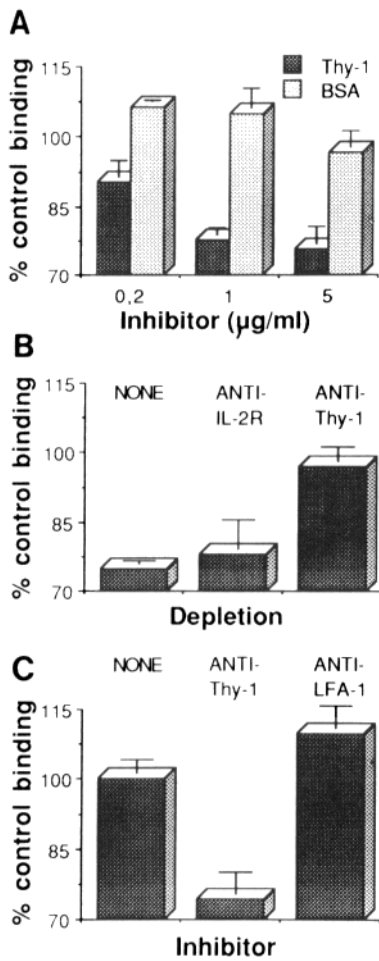


Figure 2. Thy-1 is involved in mouse thymocyte binding to MTE cell monolayer. (A) Purified Thy-1 molecules inhibit thymo-epithelial cell interactions. MTE cell monolayers were preincubated for 2 h at 4°C with different concentrations of purified Thy-1 or BSA (control) and washed with cold medium before running the adhesion assay (see Materials and Methods). Control binding ($11,504 \pm 485$ cpm) represents 54.9% of cell input. (B) Removal of Thy-1 from PI-PLC-solubilized thymocyte surface molecules reverses their capacity to inhibit thymocyte binding to MTE cell monolayer. Preincubation of MTE cell monolayers with solubilized glypiated molecules reduced their ability to bind thymocytes; this inhibition was no longer observed after depletion of the Thy-1 molecules but not of the IL-2R molecules from the supernatant. Control binding was $16,098 \pm 391$ cpm. (C) Fab' fragments of Thy-1-specific mAb (H140-150) inhibit thymo-epithelial cell interaction. ^{51}Cr -labeled thymocytes were pretreated with Fab' fragments of Thy-1 (H140-150)- or LFA-1C (H129-296)-specific mAb for 1 h at 4°C before running the adhesion assay. Control binding was $9,687 \pm 405$ cpm.

shown to result from a subchromosomal deletion encompassing the Thy-1 gene (12). Results summarized in Fig. 3 A indicate that under nonsaturating conditions (i.e., $<10^5$ thymocytes per assay), the Thy-1⁻ variant exhibited a binding level reduced by 50% as compared with the wild-type parental cells. In contrast, both T lymphoma cell lines bound with a similar efficiency to the epithelial MDCK cells (8.6% for AKR1 and 10.3% for AKR1 [Thy-1^{-d}], respectively). We next evaluated the Thy-1-dependent adhesion of AKR1

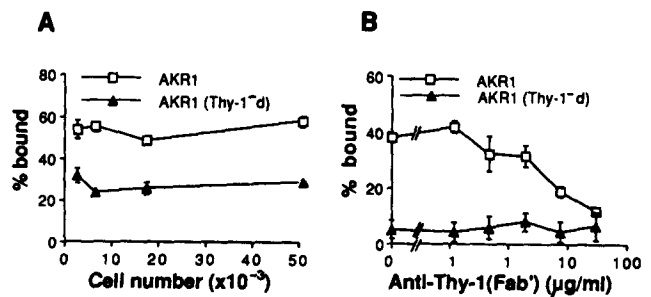
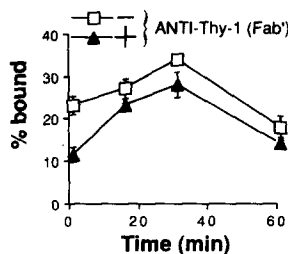


Figure 3. Thy-1 mediates adhesion between the AKR1 T lymphoma and MTE cells. (A) Binding of various numbers of AKR1 cells (□) or of its Thy-1⁻ variant AKR1(Thy-1^{-d}) (▲) to MTE cell monolayer. Control binding values were in the range of 1,600–43,000 cpm depending on the cell input. (B) AKR1 binding to MTE cells in the absence of divalent cations. AKR1 (□) and AKR1(Thy-1^{-d}) T lymphoma cells (▲) were pretreated with or without various concentrations of Fab' fragments of Thy-1-specific mAb (H140-150) for 1 h at 4°C before the adhesion assay, which was performed in the presence of 5 mM EDTA. Control binding values were $11,772 \pm 589$ cpm, and $1,801 \pm 126$ cpm for AKR1 and AKR1(Thy-1^{-d}), respectively.

in the presence of EDTA. Divalent cations (i.e., Ca^{2+} and Mg^{2+}) are known to influence the adhesiveness of cell adhesion molecules such as the integrin and cadherin (13) systems. In contrast, the adhesion processes depending upon members of the Ig-like superfamily generally appear to be Ca^{2+} independent. In the absence of divalent cations, the binding of AKR1 to MTE cells was found to be sixfold higher than that of the AKR1(Thy-1^{-d}) mutant cells and to be totally inhibitable in a dose-dependent manner by H140-150 Fab' fragments (Fig. 3 B). Under such experimental conditions, the Thy-1-mediated adhesion appeared predominant in the AKR1-MTE cell interaction. These results indicated that the Thy-1-dependent adhesion was Ca^{2+} independent.

Cell adhesion can be operationally divided into an initial contact followed by a subsequent enhancement of the interaction through energy-dependent processes (5, 14). The quantitative cell adhesion assay used in this study permits the measurements of both the initial binding and the enhancement (8, 14). In a previous report (8), a LFA-1-dependent enhancement of the thymocyte-MTE cell adhesion was observed after 15–30 min incubation at 37°C. By performing the assay at 4°C and detaching the probe cells immediately after their

Figure 4. Thy-1 is involved in the initial binding of thymocytes to MTE cells. Thymocytes were pretreated with (▲) or without (□) Fab' fragments of the Thy-1-specific mAb H140-150 (30 µg/ml) and spun onto the MTE cell monolayers at 4°C. Plates were then incubated at 37°C for various periods of time, inverted and centrifuged again at 4°C (200 g, 5 min). No Fab' fragments were dissociated from the thymocytes during the 37°C incubation steps (data not shown). Control binding value (at time 0) was $8,432 \pm 317$ cpm.



initial contact with the MTE cell monolayer, evidence was obtained that Thy-1 was involved in the initial binding. To determine whether this molecule was also involved in the enhancement of the thymo-epithelial cell adhesion, ^{51}Cr -labeled thymocytes were detached from MTE cell monolayers after warming the plates to 37°C for various periods of time. As can be seen in Fig. 4, the presence of the Thy-1-specific Fab' fragments globally reduced the percentage of bound thymocytes to MTE cells but did not abolish the enhancement of cell adhesion. Fab' fragments of Thy-1-specific mAb had a maximal inhibitory effect at the initiation of the assay. These results support the notion that Thy-1-mediated adhesion is distinct from that occurring more progressively in a LFA-1-dependent way during thymo-MTE cell interactions.

The nature of the ligand or receptor with which Thy-1

interacts at the surface of MTE cells is unknown. It seems unlikely that the ligand would be Thy-1 itself, because we failed to detect this structure at the epithelial cell surface. Our data, however, do not rule out a *cis* interaction between Thy-1 molecules on the same thymocyte membrane. Along this line, it has been suggested that Thy-1 exists in a dimeric form at the cell surface (15).

The involvement of Thy-1 in mouse lympho-epithelial cell interactions is consistent with the notion that this molecule might be structurally related to the primordial domain of the Ig-like superfamily, which has been proposed to serve as a basic unit mediating cell-cell interactions (2). In this context, it is of interest that the Thy-1 molecules expressed in nonlymphoid tissues might also be functionally important in these processes.

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References

1. Reif, A.E. 1989. The discovery of Thy-1 and its development. *In* Cell Surface Antigen Thy-1, A.E. Reif and M. Schlesinger, editors. Marcel Dekker, Inc., New York. 3-45.
2. Williams, A.F. 1989. The structure of Thy-1 antigen. *In* Cell Surface Antigen Thy-1. A.E. Reif and M. Schlesinger, editors. Marcel Dekker, Inc., New York. 49-69.
3. Silver, J. 1989. Structural organization of the Thy-1 gene. *In* Cell Surface Antigen Thy-1. A.E. Reif and M. Schlesinger, editors. Marcel Dekker, Inc., New York. 241-269.
4. Norcross, M.A., and R.T. Smith. 1989. Mitotic triggering of T-cells by anti-Thy-1 antibodies. *In* Cell Surface Antigen Thy-1, A.E. Reif and M. Schlesinger, editors. Marcel Dekker, Inc., New York. 397-410.
5. Springer, T.A. 1990. Adhesion receptors of the immune system. *Nature (Lond.)* 346:425.
6. Vollger, L.W., D.T. Tuck, T.A. Springer, B.F. Haynes, and K.H. Singer. 1987. Thymocyte binding to human epithelial cells is inhibited by monoclonal antibodies to CD-2 and LFA-3 antigens. *J. Immunol.* 138:358.
7. Singer, K.H., S.M. Denning, L.P. Whichard, and B.F. Haynes. 1990. Thymocyte LFA-1 and thymic epithelial cell ICAM-1 molecules mediate binding of activated human thymocytes to thymic epithelial cells. *J. Immunol.* 144:2931.
8. Lepesant, H., H. Reggio, M. Pierres, and P. Naquet. 1990. Mouse thymic epithelial cell lines interact with and select a CD3lowCD4⁺CD8⁺ thymocyte subset through an LFA-1-dependent adhesion-de-adhesion mechanism. *International Immunology* 2:1021.
9. McClay, D.R., G.M. Wessel, and R.B. Marchase. 1981. Inter-cellular recognition: quantitation of initial binding events. *Proc. Natl. Acad. Sci. USA.* 78:4975.
10. Pont, S., A. Regnier-Vigouroux, P. Naquet, D. Blanc, A. Pierres, S. Marchetto, and M. Pierres. 1985. Analysis of the Thy-1 pathway of T-cell hybridoma activation using 17 rat monoclonal antibodies reactive with distinctive Thy-1 epitopes. *Eur. J. Immunol.* 15:1222.
11. Lisanti, M.P., M. Sargiacomo, L. Graeve, A.R. Saltiel, and E. Rodriguez-Boulan. 1988. Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line. *Proc. Natl. Acad. Sci. USA.* 85:9557.
12. Evans, G., R. Hyman, and K. Lewis. 1987. A mutant lymphoma cell line with a defective Thy-1 glycoprotein gene. *Immunogenetics.* 25:24.
13. Edelman, G.M. 1988. Morphoregulatory molecules. *Biochemistry.* 27:3533.
14. Lotz, M.M., C.A. Burdsal, H.P. Erickson, and D.R. McClay. 1989. Cell adhesion to fibronectin and tenascin: quantitative measurements of initial binding and subsequent strengthening response. *J. Cell Biol.* 109:1795.
15. Mahanthappa, N.K., and P.H. Patterson. 1989. Thy-1 dimerization, Thy-1-derived peptides, and neurite outgrowth. *Soc. Neurosci. Abstr.* 15:651a. (Abstr.)