# IDENTIFICATION AND UNUSUAL TISSUE DISTRIBUTION OF THE CANINE AND HUMAN HOMOLOGUES OF THY-1 ($\theta$)*

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One of the few cell membrane antigens for which homology between species has been established is Thy-1 of mouse (1, 2) and rat (3–5). Even between species as phylogenetically close as these, important differences in tissue distribution have been noted, a point of considerable interest from the point of view of elucidating the function of the molecule. Thus, whereas in both species it is found in large and approximately equal amounts on brain and thymus, in the mouse it is absent from bone marrow cells and found on all peripheral T lymphocytes, although in the rat it is found on 30–40% of bone marrow cells and absent from peripheral T lymphocytes. These results prompted us to study the tissue distribution of the canine and human homologues of Thy-1.

Liver absorbed rabbit anti-brain xenosera assayed on thymus cells have been used as serological markers for mouse (2) and rat (4, 5) Thy-1. Similar assay systems can be established in other species, but it is clearly of fundamental importance to establish that the molecules being recognized are indeed the homologues of rat and mouse Thy-1. One approach for establishing homology is to demonstrate serological cross-reactivity with purified antigen from a well-studied reference species. In this paper we have used liver absorbed rabbit anti-dog and human brain xenosera assayed on thymus cells to study the tissue distribution of canine and human Thy-1. We have taken particular care (*a*) to establish homology with rat Thy-1 by demonstrating cross-reactivity with pure rat Thy-1 (kindly supplied by Dr. A. F. Williams, Oxford [6]) and (*b*) to use quantitative absorption techniques with a large number of different tissues to detect quantitative as well as qualitative differences in tissue distribution. Our results demonstrate that canine and human Thy-1 show remarkable differences in tissue distribution when compared to the known distribution of both mouse and rat Thy-1.

## Materials and Methods

*Tissue Homogenates.* Kidneys, livers, hearts, and brains of adult dogs, kidneys and brain of a 1-d old dog, and livers and brains of 10–20 wk old DA rats were taken from freshly exsanguinated animals. Human liver, heart, and kidney were obtained fresh from cadaver kidney donors and a piece of fresh normal human brain (cerebrum) was obtained from the Neurosurgery Unit. The capsule of the organ to be homogenized was removed and the tissue minced in phosphate-buffered saline (PBS)[1] with mechanically driven blades, followed by

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; GAR, goat $F(ab')_2$ anti-rabbit $F(ab')_2$; PBS, phosphate-buffered saline; RAD, rabbit $F(ab')_2$ anti-dog $F(ab')_2$; RBC, erythrocyte.

mechanical homogenization with a Teflon pestle and manual ground glass homogenization. Large particles were removed by centrifuging at 40 *g* for 1 min. The homogenate was then washed twice in PBS by spinning at 35,000 *g* for 20 min. The second pellet was resuspended in an equal volume of PBS, aliquoted and frozen at −40°C. All procedures were carried out at 4°C.

*Cell Suspensions.* All cell suspensions used in this study were prepared fresh, immediately before use. Thymus was taken from 6–8 wk old DA rats. 2–3 mo old pups, and as 1 $cm^3$ biopsies from prepubertal patients at open heart surgery. All other tissues were from adults. Human spleen was taken from splenectomy cases for trauma or from cadaver kidney donors, and human lymph nodes, usually cervical, were obtained from patients without systemic illness at surgery. Human bone marrow came from ribs removed at thoracotomy. Dog bone marrow also came from ribs and dog lymph nodes were mesenteric. Rat lymph nodes were a mixture of cervical and mesenteric. Contaminating erythrocytes (RBC) were removed by incubating in Tris-buffered ammonium chloride (7). Viability was assessed by trypan blue exclusion and was almost always better than 80%. Relatively pure suspensions of RBC were prepared by washing heparinized blood three times and removing supernate, buffy coat, and upper layer of RBC pellet after each wash. Cell counts were performed by using a Coulter Counter model DN. Pure suspensions of platelets were prepared by removing leukocytes and RBC from blood by two sequential spins at 200 *g* for 20 min. Platelets were washed twice and counted under phase contrast microscopy on a hemocytometer.

*Antisera*

RABBIT ANTI-DOG AND HUMAN BRAIN SERA. Rabbit anti-brain sera were raised by immunizing three New Zealand white rabbits with dog brain homogenate and three with human brain homogenate. The initial injection consisted of 1 ml of brain homogenate emulsified in an equal volume of Freund's complete adjuvant, and was given intramuscularly to both thighs. Boosts of 1 ml of homogenate in Freund's incomplete adjuvant were also given intramuscularly to the thighs at 2, 4, and 6 wk after the initial injection. A further boost of 1 ml of homogenate in PBS intraperitoneally was given at week 7, and the animals were bled three times during weeks 8 and 9. The three bleeds from any one animal were pooled.

IMMUNOADSORBENT PURIFIED GOAT F(AB′)$_2$ ANTI-RABBIT F(AB′)$_2$ (GAR). Rabbit F(ab′)$_2$ was prepared by precipitating rabbit immunoglobulins from serum three times with 18% wt/vol $Na_2SO_4$ at room temperature, followed by pepsin degradation at an enzyme/substrate ratio of 4% for 20 h at 37°C in sodium acetate buffer at pH 4.5, and finally by gel filtration through G-200 (Pharmacia, Sweden). The F(ab′)$_2$ was pure as assessed by SDS polyacrylamide gel electrophoresis (8). Approximately 100 mg of rabbit F(ab′)$_2$ was coupled to 10 ml of CNBr-activated Sepharose (Pharmacia, Sweden) essentially as described by the manufacturers. Heat inactivated and ultracentrifuged goat antiserum to this F(ab′)$_2$ preparation was passed through the column until it was saturated, the column was washed, and the adsorbed antibody eluted with I molar propionic acid. The eluted fractions were immediately neutralized with 2 M Tris, and the eluted antibody pepsin degraded as above, except that a pH of 4.1 was necessary for complete degradation. After pepsin degradation the antibody was passed through G-200. GAR was iodinated by using the chloramine T method, and labeled with fluorescein isothiocyanate by using bicarbonate buffer, pH 9.5, for 3 h at room temperature.

IMMUNOADSORBENT PURIFIED RABBIT F(AB′)$_2$ ANTI-DOG F(AB′)$_2$ (RAD). This was prepared essentially as had been described for GAR, except that dog immunoglobulin was pepsin degraded at a pH of 4.1.

*Quantitative Absorption Analysis.* This was essentially as described by Morris and Williams (5). Cell suspensions, and tissue and serum dilutions, were in 0.5% bovine serum albumin (BSA) in PBS. All procedures were at 4°C or on ice.

Initial titrations of the sera were performed to choose a dilution which represented conditions of target antigen excess in the assay system. For the titration, 25-μl aliquots of doubling dilutions of the antisera were transferred in duplicate to LP3 tubes (Luckham Ltd., Labro Works, Victoria Gardens, Burgess Hill, Sussex) and to each tube was added 25 μl of thymus cells at $10^8$/ml as targets. This was incubated for 1 h on ice, and the cells were then washed twice in 0.1% BSA/PBS. To the cell button of the second wash was added 100 μl of GAR at 25 μg/ml with a trace (≈400,000 cpm/assay) of $^{125}$I-labeled GAR. The cells were incubated again for 1 h on ice, washed twice, and the cell-bound radioactivity measured. With the assay, the amount

of GAR bound per assay is directly proportional to the amount of first antibody bound, and the results of the assay are expressed as ng GAR bound per assay, which can be calculated easily.

For the absorptions, equal vol (80 μl) of antiserum at the appropriate dilution and tripling dilutions of the absorbing cell suspension or tissue homogenate were incubated for 1 h on ice. Absorbing tissue was removed by centrifugation, and the supernate frozen at −40°C until assayed. When all absorptions for a particular analysis were complete, supernates were thawed, spun at 6,000 *g* for 5 min, and 25-μl aliquots were assayed in duplicate as above.

The starting concentration of cells for absorptions was $10^9$/ml, except for RBC and platelets which were used at $3 \times 10^9$ ml and $6 \times 10^9$/ml, respectively. Homogenates were used at starting concentrations as prepared above (i.e., ≈50% solid tissue) which corresponded to a protein concentration of 25–35 mg/ml as assayed by the method of Lowry et al. (9). Lymph node lymphocytes at $10^9$/ml also had protein concentration of ≈25 mg/ml. Cell suspensions and tissue homogenates were aligned on the basis of protein content, i.e. $10^9$ cells/ml was equivalent to 25 mg protein/ml, the rationale for which has been discussed previously (10).

When rat thymus cells were used as targets, the GAR was used under trace conditions (0.1 μg/ml and ≈300,000 cpm/assay) to increase sensitivity (5).

*Fluorescence-Activated Cell Sorter.* Cells for analysis on the cell sorter were prepared exactly as in the binding assay mentioned in the previous section, except that (*a*) the rabbit anti-dog brain serum was pepsin degraded to $F(ab')_2$ to prevent binding to Fc receptors, and (*b*) the second antibody consisted of 50 μl of fluorescein-labeled GAR at 25 μg/ml.

## Results

*Canine Thy-1.* The three rabbit anti-dog brain sera were screened for the broad tissue distribution of the brain-thymus specificities present, by absorbing with liver, heart, kidney, and brain and assaying on thymus cells. All three gave the same picture, with liver and heart giving absorption plateau after removing ≈50% of the antibody, indicating the presence of brain-thymus specificities of relatively restricted tissue distribution. All subsequent analyses were performed on the one batch of serum after it had been absorbed with liver to the absorption plateau.

TISSUE DISTRIBUTION OF THE PUTATIVE CANINE HOMOLOGUE OF THY-1. In this section, we describe the tissue distribution of the antigens recognized by liver absorbed rabbit anti-dog brain serum assayed on dog thymus cells. The entire absorption analyses were done twice on separate occasions with very similar results, and the quantitations quoted represent the mean of the two experiments.

In Fig. 1 are given the results for absorptions by the tissue homogenates and thymus. It can be seen that brain gave very strong absorption, while the absorptive capacity of thymus was only 8% that of brain. The absorptive capacity of kidney was equal to that of thymus, although liver and heart had very little absorptive capacity. It should be noted that the absorption by kidney could not be nonspecific because, like liver, heart, and brain, it gave only poor absorption of rabbit anti-dog thymocyte sera.[2]

Fig. 2 gives the results for absorptions with various cell suspensions. It can be seen that both lymph node and spleen could absorb out the antibody, their absorptive capacities compared with thymus being 37 and 14%, respectively. Bone marrow gave some absorption, its absorptive capacity compared to thymus being 7%. The absorptive capacities of lymph node spleen and bone marrow compared to brain were 3, 1, and 0.5%, respectively. Platelets, RBC, and normal serum gave no absorption. In another experiment bone marrow, spleen, and lymph node were shown to be capable of absorbing out the antibody down to background levels.

[2] R. Dalchau and J. W. Fabre. Manuscript in preparation.

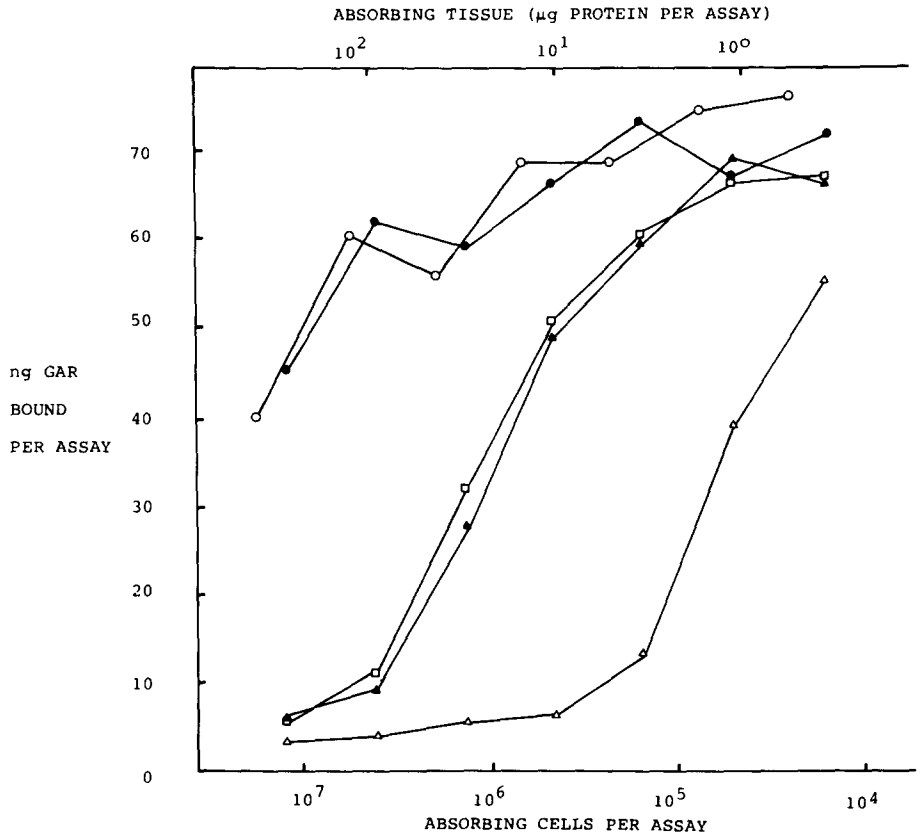


FIG. 1. Tissue distribution of canine Thy-1. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the dog tissues indicated and assayed on dog thymus cells. ▲, thymus; △, brain; □, kidney; ○, liver; ●, heart.

If the assays shown in Figs. 1 and 2 are detecting the canine homologue of Thy-1, then canine Thy-1 is present primarily on the brain, and to a lesser extent on thymus, kidney, and the peripheral lymphoid tissues.

ESTABLISHMENT OF HOMOLOGY WITH RAT THY-1. Arndt et al. (11) have shown that rabbit anti-human brain sera are cytotoxic for mouse and rat thymus cells. This prompted us to set up binding assays of the liver absorbed rabbit anti-dog brain serum on rat thymus cells, to enable us to study the component of the serum recognizing specificities shared between dog brain and rat thymus. We wished to show (*a*) that this component represented the dog-rat cross-reactive component of Thy-1 and (*b*) that this component reacted with the same antigen(s) as the full complement of antibodies studied in Figs. 1 and 2.

Fig. 3 gives the results of absorptions with rat tissues, to give the tissue distribution in the rat of the cross-reactive component, and also of absorptions with pure rat Thy-1 (kindly supplied by Dr. A. F. Williams, Oxford [6]). It can be seen that rat brain and thymus gave excellent absorption, although rat liver and lymph node absorbed poorly or not at all. The discrepancy between thymus and lymph node is especially striking, and the pattern corresponds precisely to what one would expect if the assay

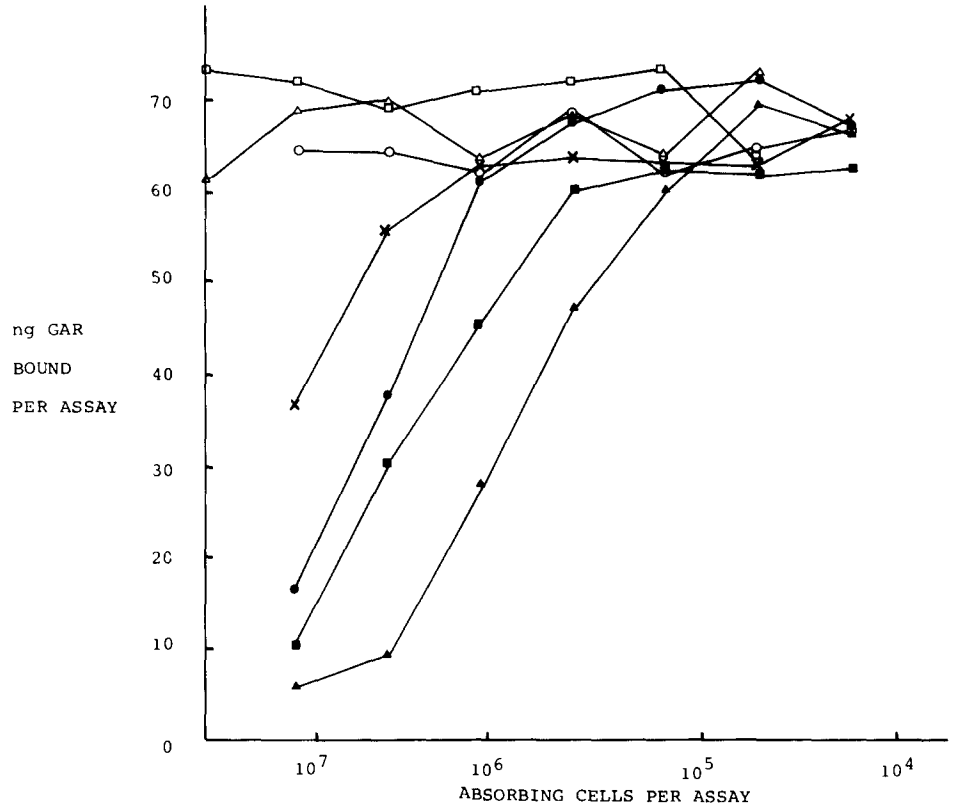


FIG. 2. Tissue distribution of canine Thy-1. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the dog tissues indicated and assayed on dog thymus cells. ▲, thymus; ■, lymph node; ●, spleen; ×, bone marrow; □, erythrocytes; ○, platelets; △, normal serum.

were detecting Thy-1 (4, 5). This is confirmed by the absorption with small quantities of pure rat Thy-1. The slope of the absorption curve is less steep with pure Thy-1 probably because the soluble molecule absorbs low affinity antibodies less efficiently than Thy-1 in matrix form on the membrane of whole cells.

The results presented in Fig. 3 establish that a component of the liver absorbed anti-brain serum is detecting the canine homologue of Thy-1, but this does not necessarily mean that all the antibodies assayed in Figs. 1 and 2 are also directed against Thy-1. To establish this point, we looked at the tissue distribution of the cross-reactive component by using dog tissues. The results are given in Fig. 4, and show that the tissue distribution in the dog of the cross-reactive component is the same as the tissue distribution of the full complement of specificities recognized in Figs. 1 and 2. This strongly suggests that the assays in Figs. 1–4 are recognizing the same molecule. In Figs. 1 and 2 the dog-specific as well as the dog-rat cross-reactive components of Thy-1 are being recognized, although only the latter component is being recognized in Figs. 3 and 4.

CANINE THY-1 ON PERIPHERAL T LYMPHOCYTES. Thy-1 is a marker for peripheral T lymphocytes in the mouse (1) but not the rat (4). To see what the situation was in the dog, the distribution of canine Thy-1 on lymph node lymphocytes was analyzed by using the fluorescence-activated cell sorter (Materials and Methods). Lymph node

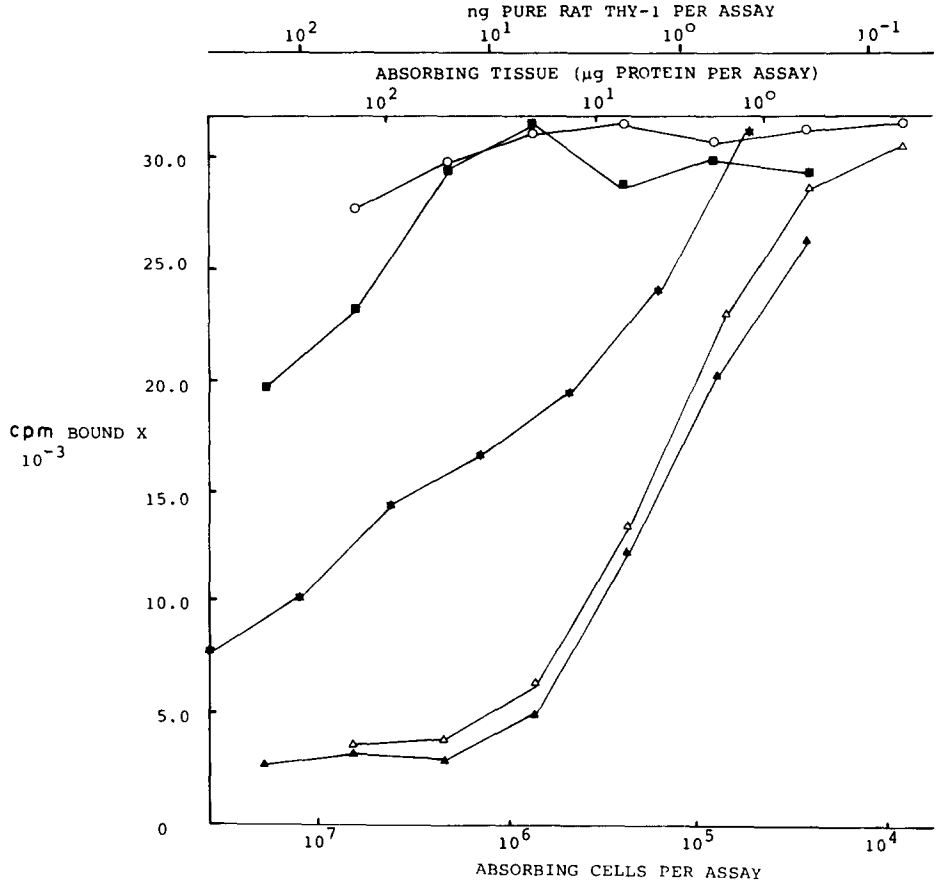


FIG. 3. Dog-rat cross-reactive component of Thy-1 on rat tissues. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the rat tissues indicated and assayed on rat thymus cells. ▲, rat thymus; ■, rat lymph node; *, pure rat Thy-1; △, rat brain; ○, rat liver.

lymphocytes were incubated with (*a*) liver absorbed, pepsin degraded rabbit anti-dog brain serum at 1/10 (*b*) immunoadsorbent purified RAD at 50 μg/ml to label B lymphocytes; (*c*) both (*a*) and (*b*) together, to give the same final concentration of both antisera; and (*d*) 0.5% BSA/PBS. The fluorescence profiles for (*a*) and (*c*) are given in Fig. 5. It can be seen that with the liver absorbed anti-brain serum there were two distinct subpopulations, with the dull peak (on the left of the Figure) containing 33% of the cells, and the bright peak 67%. The RAD labeled 36% of the cells (Figure not shown). These results were suggestive that the liver absorbed anti-brain serum was labeling the T lymphocytes but not the B lymphocytes, and to prove this point the mixing experiment (*c*) was performed. It can be seen that the addition of RAD to the liver absorbed anti-brain serum resulted in the disappearance of the dull peak, showing that this peak contained the B lymphocytes. Thus canine Thy-1, like mouse Thy-1, is found on all peripheral T lymphocytes.

CANINE THY-1 ON THYMOCYTES. Studies with the fluorescence-activated cell sorter were also performed by using thymus cells (Figure not shown). In four experiments, 95–100% of thymocytes were brightly stained by using the liver absorbed anti-brain serum, suggesting that all, or virtually all, dog thymocytes were positive for Thy-1.

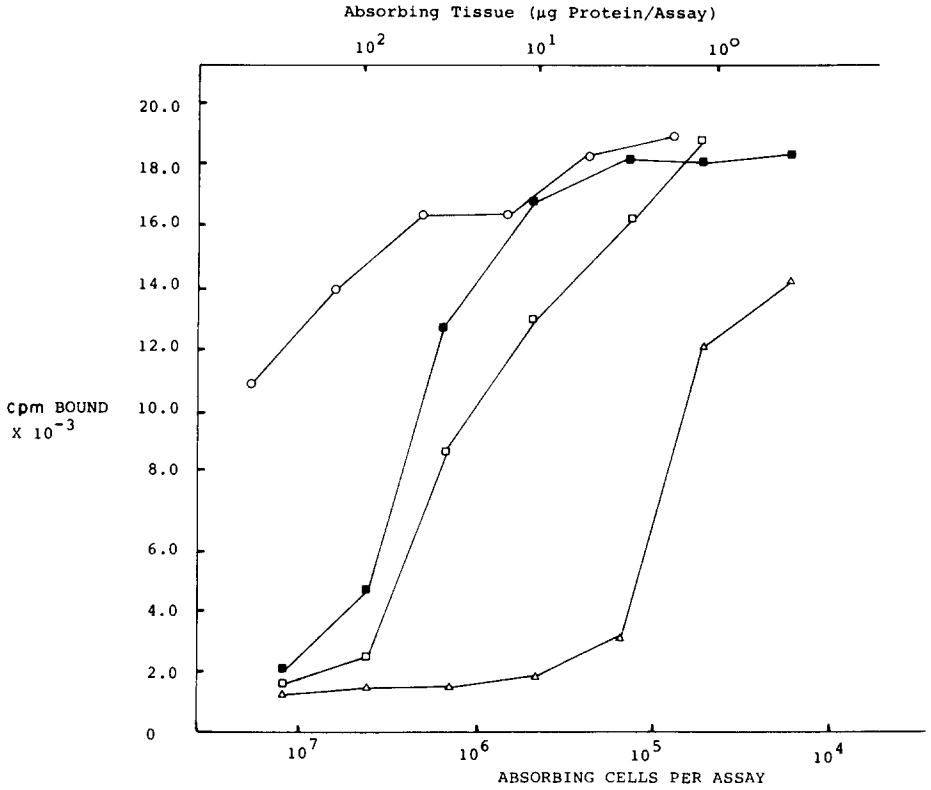


FIG. 4. Dog-rat cross-reactive component of Thy-1 on dog tissues. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the dog tissues indicated and assayed on rat thymus cells. ■, lymph node; △, brain; □, kidney; ○, liver.

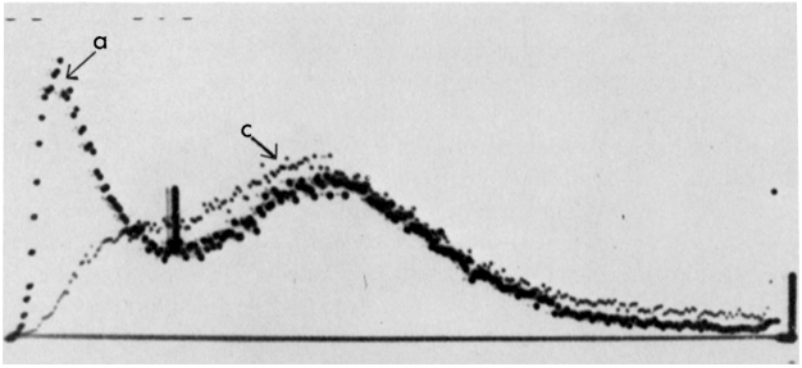


FIG. 5. Canine Thy-1 on peripheral T lymphocytes. Fluorescence profile of dog lymph node lymphocytes incubated with (a) liver absorbed, pepsin degraded rabbit anti-dog brain serum at 1/10 and (c) anti-brain serum as in (a) plus immunoadsorbent purified rabbit $F(ab')_2$ anti-dog $F(ab')_2$ at 50 μg/ml. The ordinate is a log scale for cell number and the abscissa gives the degree of fluorescence in arbitrary units.

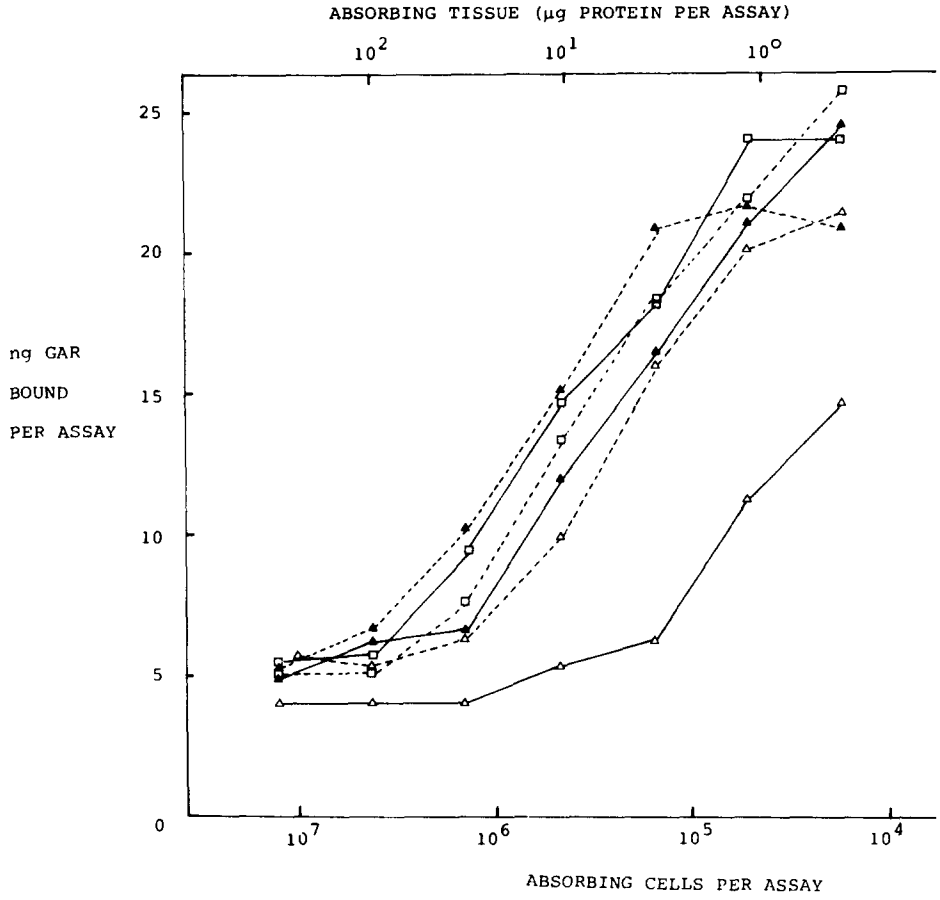


FIG. 6. Canine Thy-1 on neonatal tissues. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the dog tissues indicated and assayed on dog thymus cells. ▲——▲, thymus; ▲- - - -▲, neonatal thymus; △——△, brain; △- - -△, neonatal brain; □——□, kidney; □- - -□, noenatal kidney.

CANINE THY-1 ON NEONATAL TISSUES. Neonatal rats (4) and mice (1) have normal amounts of Thy-1 on thymus but only small amounts on brain, and it was of interest to check this point with respect to canine Thy-1. Results of absorptions with thymus, brain, and kidney from neonatal and adult dogs are given in Fig. 6. It can be seen that neonatal thymus and kidney have approximately as much Thy-1 as adult tissues. Neonatal brain, however, has only 14% as much Thy-1 as adult brain, indicating that brain Thy-1 develops mainly in postnatal life in the dog.

*Human Thy-1.* The three rabbit anti-human brain sera were screened for the broad tissue distribution of the brain-thymus specificities present, by absorbing with liver, heart, kidney, and brain and assaying on thymus cells. In all three cases, liver, heart, and kidney could absorb out all the brain-thymus specificities. This picture is quite different from that in the dog and from the well known situation in the rat (4) and suggests that the anti-human brain sera do not recognize brain-thymus specificities of restricted tissue distribution.

There are three broad interpretations of this finding in terms of a human homologue

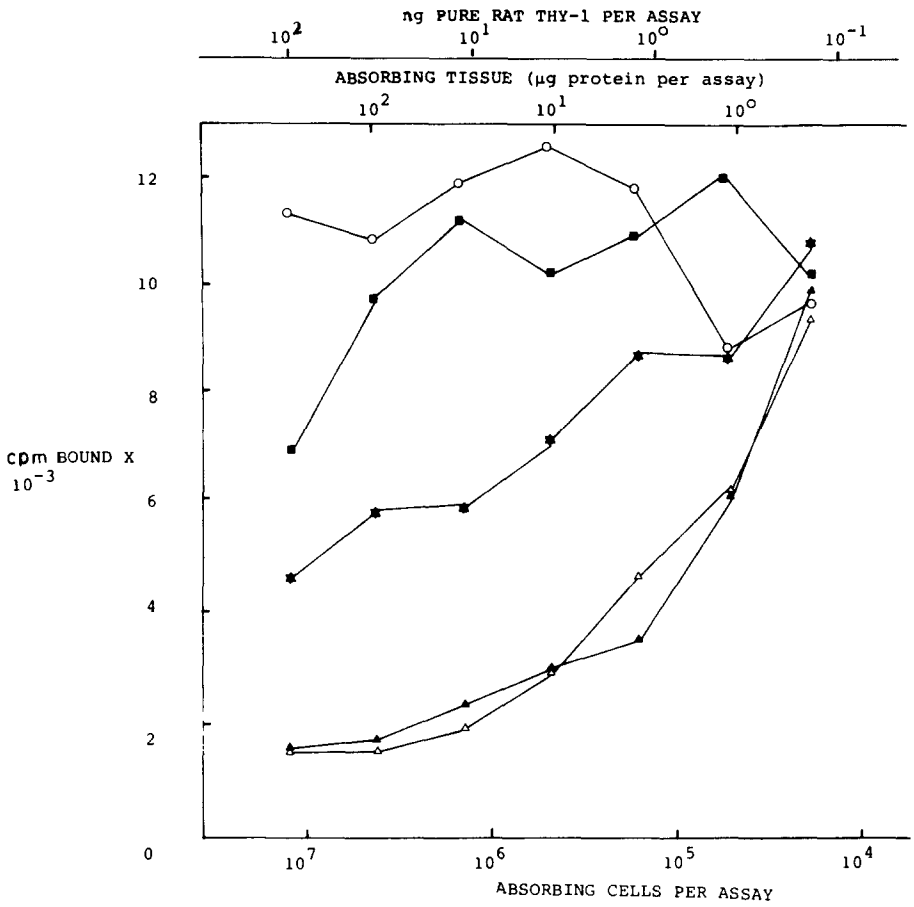


FIG. 7. Human-rat cross-reactive component of Thy-1. Rat liver absorbed rabbit anti-human brain serum at a 1/8 dilution absorbed with the rat tissues indicated and assayed on rat thymus cells. ▲, rat thymus; ■, rat lymph node; *, pure rat Thy-1; △, rat brain; ○, rat liver.

of Thy-1. First, it is possible that our antisera do not contain anti-Thy-1 antibodies, because Thy-1 might not be immunodominant on human brain, or might even be absent from human brain. Second, the antisera might contain anti-Thy-1 antibodies, and Thy-1 in man is more broadly distributed than in the rodent or dog. Finally, it is possible that Thy-1 is absent from human thymus, in which case any anti-Thy-1 antibodies would not be assayed for when human thymocyte targets are used.

ASSAYS FOR THE HUMAN-RAT CROSS-REACTIVE COMPONENT OF THY-1. We tested our anti-human brain sera for binding to rat thymus cells and found that as in the dog, this system enabled us to study antigens cross-reactive between human brain and rat thymus cells. One batch of serum was used for all the experiments, and it was given a preliminary absorption with rat liver before the subsequent assays were performed.

To establish that the rat liver absorbed rabbit anti-human brain serum assayed on rat thymus cells was detecting the human-rat cross-reactive component of Thy-1, we performed experiments similar to those described in Fig. 3 for the dog. The results are given in Fig. 7, and show that the cross-reactive component is present in equal amounts on rat brain and rat thymus, in negligible amounts on rat liver and lymph

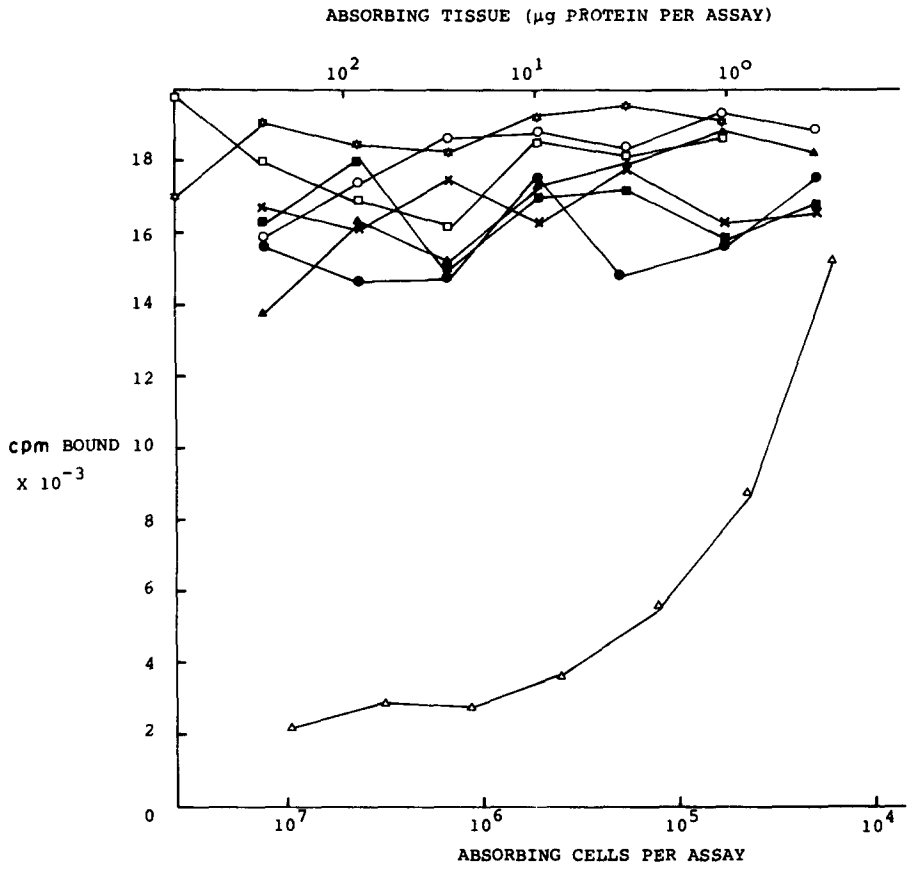


FIG. 8. Tissue distribution of human Thy-1. Rat liver absorbed rabbit anti-human brain serum at a 1/8 dilution absorbed with the human tissues indicated and assayed on rat thymus cells. △, brain; ▲, thymus; ■, lymph node; ●, spleen; ×, bone marrow; □, erythrocytes; ○, platelets; *, normal serum.

node lymphocytes, and that pure rat Thy-1 in small quantities could remove ≈75% of the antibodies with the absorption curve still on a downward slope. As previously explained, these cross-reactivity studies establish that our assay system is detecting the human-rat cross-reactive component of Thy-1. It follows, therefore, that our sera do contain antibodies to the human homologue of Thy-1.

TISSUE DISTRIBUTION OF HUMAN THY-1. In Figs. 8 and 9 are given the results of quantitative absorptions with 11 different human tissues. The analysis was performed twice on separate occasions with virtually identical results. It is clear from Fig. 8 that brain contains large amounts of Thy-1 (the inhibition curve is similar to that seen in dog and rat systems) but that thymus, lymph node, spleen, bone marrow, erythrocytes, platelets, and serum contain no detectable Thy-1. Since the assay system is sensitive enough to detect amounts of Thy-1 of the order of 0.5% or less compared with brain, and since the tissues mentioned gave no hint of absorption, a conservative estimate of the maximum amount of Thy-1 that could be present on them would be 0.2% compared with brain. It is reasonable, therefore, to say that Thy-1 is absent from these tissues. Fig. 9 shows that Thy-1 is absent from liver and heart, but present on

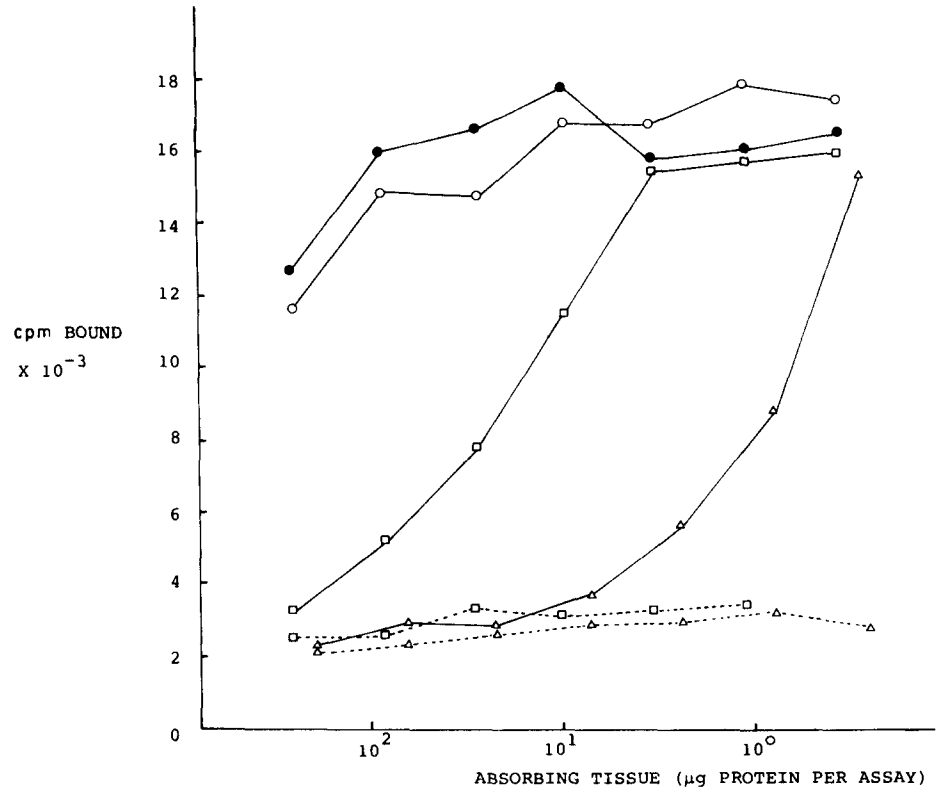


FIG. 9. Tissue distribution of human Thy-1. Rat liver absorbed rabbit anti-human brain serum at a 1/8 dilution absorbed with the human tissues indicated and assayed on rat thymus cells. Designation X 2 indicates preabsorption with the pellet after centrifugation of an equal volume of the tissue indicated. △——△, brain; △- - -△, brain × 2; □——□, kidney; □- - -□, kidney × 2; ○, liver; ●, heart.

human kidney, the amount being ≈5% compared with brain. This is similar to our findings in the dog. There is no question of the absorption by kidney being nonspecific because, like liver, brain, and heart, it gave only weak absorption of anti-thymocyte sera.[2]

CROSS-REACTIVITY STUDIES. Morris et al. (12) and Thiele and Stark (13) have studied the serological cross-reactivity between mouse and rat Thy-1. These authors have shown that rabbit antibodies to rat Thy-1 fall into two main groups, one directed at a rat-specific component, and one at a rat-mouse cross-reactive component. We were interested to examine the extent of serological cross-reactivity between the Thy-1 molecules of the relatively distant species studied in this paper, because this might give an idea of the extent to which Thy-1 has been conserved during evolution. It would also be interesting to relate serological cross-reactivity to subsequent structural studies.

The results given in Fig. 10 show that human brain can absorb out a substantial component of the rabbit antibodies against dog Thy-1, indicating a substantial degree of serological cross-reactivity between dog and human Thy-1. Rabbit antibodies against dog Thy-1 are clearly divisible into those directed at a dog-human cross-

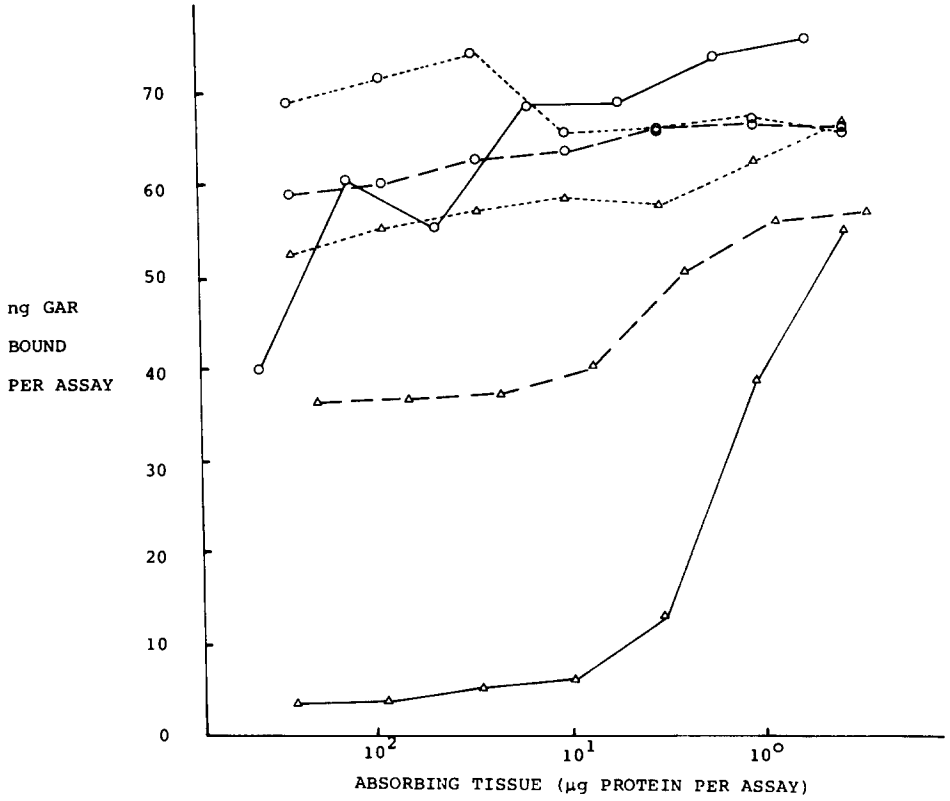


FIG. 10. Cross-reactivity among rat, dog, and human Thy-1. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the tissues indicated and assayed on dog thymus cells. Δ——Δ, dog brain; Δ— —Δ, human brain; Δ- - -Δ, rat brain; O——O, dog liver; O— —O, human liver; O----O, rat liver.

reactive component (that part absorbed by human brain) and a dog-specific component. Rat brain gave only slight absorption, showing that dog and rat Thy-1 show only a slight degree of cross-reactivity.

Fig. 11 shows that human brain can absorb out all the dog-rat cross-reactive component. The converse experiment showed that dog brain could absorb out all the human-rat cross-reactive component, the figure (not shown) being virtually identical to Fig. 11. Thus the dog-rat and human-rat components are identical and this indicates that a part of the Thy-1 molecule has been highly conserved during evolution.

## Discussion

This study shows that the tissue distribution of Thy-1 varies remarkably from species to species. A summary of the data in the mouse, rat, dog and man is given in Table I. The only tissue to give consistent results in all species studied to date is brain, which in all cases has expressed large amounts of Thy-1. The absorption curves for the cross-reactive components (Fig. 11) suggest that the amount of Thy-1 on the brains of the different species is approximately the same. Thymus contains large amounts of Thy-1 in the rat and mouse, small amounts of Thy-1 in the dog, but no

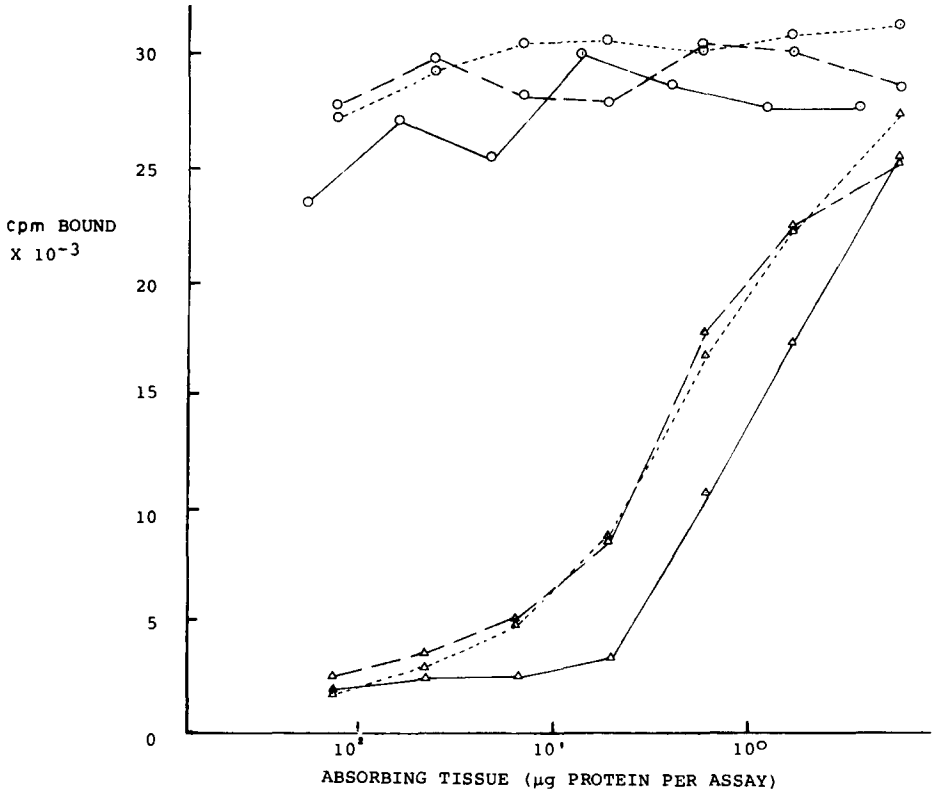


FIG. 11. Dog-rat cross-reactive component of Thy-1 on human brain. Liver absorbed rabbit anti-dog brain serum at 1/10 absorbed with the tissues indicated and assayed on rat thymus cells. Δ——Δ, dog brain; Δ— —Δ, human brain; Δ- - - -Δ, rat brain; O——O, dog liver; O— —O, human liver; O- - -O, rat liver.

detectable Thy-1 in man. Kidney has significant amounts of Thy-1 in dog and man, but does not carry Thy-1 in mouse or rat. In one study (5) rat kidney was noted in passing to have very slight absorptive capacity for Thy-1, ≈0.5% compared with rat brain) but this was not considered significant. Peripheral T lymphocytes of mouse and dog, but not rat and man, carry Thy-1. Bone marrow cells of the rat and dog, but not mouse or man, carry Thy-1.

With such unexpected tissue distributions for canine and human Thy-1, it is clear that the establishment of homology is crucial to the whole study. The use of the rat as a reference species, and pure rat Thy-1 for cross-reactivity studies establishes homology in our assay systems. In addition we have just completed biochemical studies with deoxycholate solubilized brain (J. L. McKenzie and J. W. Fabre, unpublished observations) which show that the liver absorbed rabbit anti-dog brain serum assayed on dog thymus cells and the liver absorbed rabbit anti-human brain serum assayed on rat thymus cells each detects on dog and human brain, respectively, a single specificity which behaves in every respect (molecular weight on SDS gels, Stokes radius, lectin affinity chromotography) like rat Thy-1 (6).

Thus, not only is Thy-1 present on functionally diverse tissues within a species, but its presence on any given tissue varies with the species studied. The most striking

TABLE I

*Summary of Tissue Distribution of Thy-1 in the Mouse, Rat, Dog, and Man**

| | Mouse | Rat | Dog | Man |
|---|---|---|---|---|
| Brain | + + + | + + + | + + + | + + + |
| Kidney | 0 | 0 | + | + |
| Liver, heart | 0 | 0 | 0 | 0 |
| Thymus | + + + | + + + | + | 0 |
| Lymph node | + + | 0 | + | 0 |
| Spleen | + + | + | + | 0 |
| Bone marrow | 0 | + | + | 0 |
| RBC, platelets, serum | 0 | 0 | 0 | 0 |

* Results for mouse and rat taken from (1) and (4), respectively.

expression of this phenomenon is thymus. Thy-1 is a major component of the thymocyte membrane of mouse and rat, amounting to ≈600,000 molecules per cell (5) but is not detectable on human thymus (the upper limit, given the sensitivity of the assay, being 1,000 molecules of Thy-1 per human thymocyte). Whatever function Thy-1 performs on rat thymocytes has clearly been lost or taken over by another molecule on human thymocytes. An interesting corollary of this finding is that even major membrane molecules will not necessarily have homologues in other mammalian species.

One possibility that should be considered is that the unusual tissue distribution of Thy-1 within and between species is an artifact of assaying for antigenicity. Barclay et al. (14) have shown that rat Thy-1 is a glycoprotein with a high carbohydrate content, and that the composition of the carbohydrate differs between brain and thymus Thy-1. The antigenicity, however, resides in the polypeptide chain. It is possible that quantitative or qualitative differences in glycosylation of the polypeptide chain could affect antigenicity either by steric hindrance or by changes in tertiary structure. However, the simplest and most likely hypothesis is that antigenicity is a true reflection of the tissue distribution of the molecule.

Probably because Thy-1 of rat and mouse is found in large amounts on brain and thymus, attempts have been made to define brain-thymus antigens in the dog and man. In the human studies, xenosera to fetal brain were shown not to react with thymus cells (15–17) though in two studies they did react with a subpopulation of peripheral T lymphocytes (5, 6). Of three studies with xenosera to adult human brain, two, (18, 19) could not detect any activity against thymus cells, while in one study (20) some of the sera analyzed reacted with all thymocytes and peripheral T lymphocytes. The previous study in the dog (19) showed that appropriately absorbed rabbit anti-dog brain sera were cytotoxic for 100% of thymocytes, 50% of lymph node lymphocytes, and 25% of bone marrow cells, and moreover that the specificity examined was present in low amounts on neonatal brain.

In none of the above studies were attempts made to look at the tissue distribution of the antigens recognized. In addition, where positive interactions were obtained between brain and peripheral T lymphocytes (15, 16) and between brain and thymus in man (20) and dog (19), no attempts were made to establish homology with rodent systems. This is especially important because a brain-thymus-peripheral T lymphocyte antigen quite distinct from Thy-1 has recently been described in the rat (21). Moreover, in the absence of prior knowledge as to the immunodominance of Thy-1

on human brain the negative reports for brain thymus cross-reactivity are difficult to interpret with respect to Thy-1.

Thy-1 has been detected in the rodent on skin (22) fibroblasts (23), mammary tumors (24), and striated muscle in culture (25). Whether or not canine and human Thy-1 are on these tissues remains to be seen. Antigens which are primarily on brain and to a lesser extent on kidney have been described in the mouse (26–29) and rat (30), but in all cases these antigens have been absent from thymus. The specificities examined in this paper are clearly present in large amounts on rat thymus (Figs. 3 and 7) and are therefore not related to the above-mentioned brain-kidney antigens.

Thy-1 was so named because it is a major component of rat and mouse thymus. The absence of Thy-1 from human thymus therefore raises interesting problems of nomenclature.

## Summary

The tissue distribution of the canine and human homologues of Thy-1 were studied using quantitative absorption analyses of liver absorbed anti-brain xenosera assayed on thymus cells. Cross-reactivity studies with pure rat Thy-1 established that the assays were detecting the homologues of rat Thy-1. The results showed that the tissue distribution of Thy-1 varies remarkably between species. Canine Thy-1 was found in large amounts only on adult brain. It was present in much smaller amounts on thymus (8% compared with brain), and was also found on lymph node, spleen and bone marrow (3, 1, and 0.5%, respectively, compared with brain). Surprisingly, it was found on kidney in amounts equal to that on thymus. Studies with the fluorescence-activated cell sorter established that canine Thy-1 was present on all thymocytes and peripheral T lymphocytes. Neonatal thymus and kidney had adult levels of Thy-1, but only small amounts of Thy-1 were present on neonatal brain. In man, brain was again the only tissue to contain large amounts of Thy-1. Surprisingly, it was absent from human thymus, and present on human kidney in amounts roughly equivalent to that seen in the dog. It was absent from spleen, lymph node, bone marrow, liver, heart, erythrocytes, platelets, and serum. Cross-reactivity studies showed that dog and human Thy-1 showed extensive serological cross-reaction, and that the dog-rat and human-rat cross-reactive components were identical.

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