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Differential regulation of a Thy-1 gene in transgenic mice

(gene regulation/brain/lymphoid tissue)

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ABSTRACT **We have generated Thy-1.1-transgenic Thy-1.2 mice to study the developmental expression of the Thy-1 gene in detail by transcriptional and immunological methods. In brain, the expression of the injected gene was identical to that of the endogenous gene in a tissue- and development-specific manner. In lymphoid tissue, the transferred gene was also expressed correctly in the early phases of T-cell lineage development; however, as the T cells matured, the transcription of the transferred gene, but not the endogenous gene, was suppressed. This result shows that different regulatory elements are used to express the Thy-1 gene in early and late lymphoid development.**

Specific changes in the composition of the cell surface are thought to play a crucial role in development. It is therefore important to understand how the expression of surface molecules is regulated. A good example is Thy-1, a small glycoprotein that appears on a number of cell types at particular stages of development (see ref. 1 for review). It is the simplest well-characterized member of the immunoglobulin superfamily of molecules (2–4). No biological role has yet been demonstrated for Thy-1, although it is capable of modulating intracellular Ca^{2+} levels (5). It appears at high levels on nervous tissue and is a substantial component of the neuronal surface (1). In other tissues, it shows marked differences between species; e.g., in mice Thy-1 is a marker for mature T lymphocytes (6), whereas in humans it is not found on these cells (7).

The Thy-1 gene from mouse (8, 9) and humans (10, 11) has been cloned and completely characterized. The mouse gene contains two promoters with two alternative exons, which are spliced onto the same second exon (Fig. 1). Both promoters are G+C-rich, lack the "TATA box" (8, 12), and are part of a methylation-free "island" (unpublished data). Such islands are characteristic of "housekeeping" genes but have also been found in some tissue-specific genes (13).

The pattern of expression of the Thy-1 gene suggests that different regulatory elements may be involved in its tissue- and development-specific regulation. In this paper we describe Thy-1 expression in transgenic mice and show that the gene is under the control of different regulatory elements in brain and lymphoid tissue.

MATERIALS AND METHODS

Microinjection and Nucleic Acid Analysis. F_2 fertilized eggs of CBA × C57BL/10 parents were injected with ≈2 pl of 10 mM Tris Cl, pH 7.5/0.1 mM EDTA containing DNA at ≈2 μg/ml. The microinjected eggs were transferred into pseudopregnant females (14, 15). Ten days after birth, the pups were analyzed by Southern blot analysis of tail DNA (16). RNA isolation and nuclease S1-protection analysis were carried out as described (15). Transcription assays in nuclei from transgenic (Tg12) brain, thymus, spleen, and liver and normal (CBA × C57BL/10)F_1 thymus were performed according to Linial *et al.* (17).

Immunological Assays. Thy-1.1 was detected by means of monoclonal antibody OX7 (18), and Thy-1.2, by means of monoclonal antibody 30-H12 (19). Radioimmunoassays were done (20) with tissue homogenates (brain, kidney, liver) or suspensions of viable cells (thymocytes), with the same tissues from age-matched A/Thy-1.1 mice (for Thy-1.1) or nontransgenic CBA × C57BL littermates (for Thy-1.2) as controls. In some experiments, thymocytes were divided into two aliquots, to one of which 15% Triton X-100 was added to 1% (wt/vol), and the lysed cells were diluted in 0.1% Triton X-100 in phosphate-buffered saline for assay (21). For immunohistochemistry, acetic acid/alcohol-fixed sections of brain (22) and acetone-fixed cryostat sections of lymphoid tissue were used, with horseradish peroxidase (HRP)-conjugated OX7 $F(ab')_2$ to visualize Thy-1.1 or 30-H12 followed by HRP-conjugated rat immunoglobulin-specific antibodies as a second layer for Thy-1.2. Endogenous peroxidase was blocked by a 30-min preincubation in 0.5% H_2O_2 in methanol. T-lymphocyte subsets were identified by using rat monoclonal antibodies YTS 191 (to L3T4) and YTS 169.4 (to Lyt-2) (23). B lymphocytes were identified using HRP-conjugated rabbit anti-mouse IgG (22). Immunofluorescence-labeled lymphoid cells were counted under a fluorescence microscope, fixed in 1% formalin in phosphate-buffered saline at 4°C, and analyzed on an EPICS-C fluorocytometer (Coulter). For purification of T lymphocytes, a spleen-cell suspension in RPMI 1640 medium was centrifuged over Histopaque-1077 (Sigma), cultured on plastic for 2 hr to remove adherent cells, and then passed down a nylon wool column.

RESULTS

Production and Characterization of Transgenic Mice. The Thy-1 gene (Fig. 1) that we introduced by microinjection into pronuclei of mouse zygotes was "marked" in two ways to enable us to distinguish its nucleic acid and protein products from those of the host. First, we used the Thy-1.1 gene, since the host mouse strain (CBA × C57BL) is Thy-1.2 in type. This allelic difference arises from a single amino acid substitution (4) and allows distinction by monoclonal antibodies. To produce a larger difference recognizable at the nucleotide level without changing the mature protein product (avoiding possible problems from a hybrid protein) a 6-kb *Eco*RI–*Apa* I fragment of the mouse Thy-1.1 gene containing the first four exons (including exons Ia and Ib) and part of exon IV was linked to a 3.1-kb *Apa* I–*Eco*RI human DNA sequence containing the remaining noncoding part of exon IV plus 3′ human flanking sequences.

Four of the 13 offspring (Tg6, Tg9, Tg11, and Tg12) were transgenic as determined by Southern blots; the copy num-

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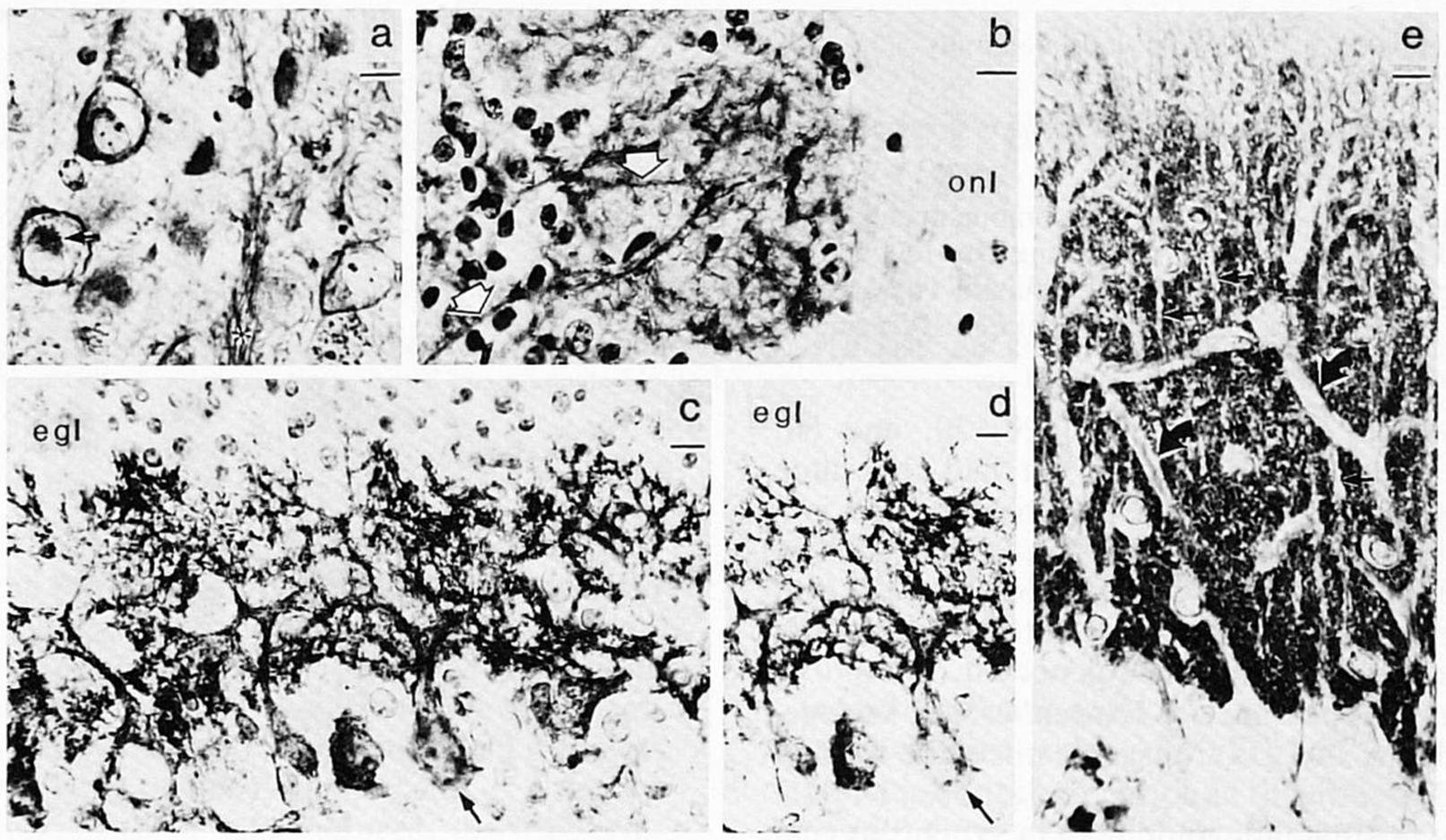

FIG. 4. Immunoperoxidase staining for Thy-1.1 on sections of brain of Tg12 mice. The peroxidase substrate was diaminobenzidine, which gives a brown reaction product, and sections have been counterstained blue with thionin to identify nuclei. (*a*) Heavy immunolabeling of the plasma membrane around three large neurons in the medial nucleus of the trapezoid body of a 28-day-old mouse, with weaker cytoplasmic labeling above one pole of the nucleus (e.g., arrow). Immunolabeled axons can be seen in longitudinal (asterisk) and cross (dots, lower right corner) sections. (*b*) Olfactory bulb, 28-day-old mouse. Two Thy-1.1-labeled dendrites of mitral cells (arrows) branch extensively to form a synaptic glomerulus with the olfactory nerve axons, which occupy the completely Thy-1.1-negative layer (onl, olfactory nerve layer). Thionin-stained nuclei of Thy-1-negative glial cells are in onl. (*c*) Cerebellum, 11-day-old mouse, showing heavy labeling of the Purkinje cells (e.g., arrow) and their dendrites. Above the dendrites are the thionin-stained nuclei of the neuroblasts of the external granule cell layer (egl); these cells migrate to beneath the Purkinje cell bodies (some of these thionin-stained nuclei can be seen), and their axons are at this stage growing between the Purkinje dendrites. (*d*) Same field as part of *c*, photographed using a blue filter to suppress thionin counterstaining to allow absence of immunolabeling of granule cells to be seen. (*e*) Cerebellum, 8-week-old mouse. Three Purkinje cell bodies (bottom) and their dendrites (e.g., curved arrows) lack appreciable antigen, whereas the granule cell axons (cut in cross-section) now stain heavily to provide dense labeling between the Purkinje dendrites. These axons demonstrate the correct (1) gradient in staining intensity, from the more mature (lower) axons to the less mature (upper) ones. The fine vertical Thy-1-negative processes (small arrows) belong to the Bergmann glial cells. (All scale bars = 10 μm.)

cells (interneurons) or their axons (Fig. 4*c*). Later in development, the Purkinje cells lacked detectable Thy-1.1; instead, it appeared on the granule cell axons lying between the Purkinje dendrites (Fig. 4*d*).

Despite a transferred-gene copy number of 20 rather than 60, the brains of adult (56–84 days old) Tg11 mice resembled Tg12 mice in having 7- to 9-fold elevated Thy-1.1 levels, normal Thy-1.2 levels, and a normal cell type distribution of both antigens. Two mice of the Tg9 strain (the same copy number as Tg11) had identical, normal levels of Thy-1.1 and Thy-1.2 in brain. Thus, there was no quantitative correlation between copy number and degree of gene expression, but there was a strict correlation in all tissues between the rate of transcription, the steady-state level of mRNA, and the amount of Thy-1 protein.

Lymphoid Expression of Thy-1. *Thymocytes*. In contrast to the postnatal rise of Thy-1 in brain, the average level of Thy-1 remains constant on thymocytes (3), a phenomenon found with the endogenous Thy-1.2 in the transgenic animals (Fig. 2). However, the level of exogenous Thy-1.1 varied, being higher in younger animals [compare enhanced immunogenicity at this stage (26)] and declining to the normal level by 56 days (Fig. 2). Within the thymus, both antigens showed the appropriate distribution by immunohistochemical analysis (Fig. 5)—i.e., more intense on the majority cortical population than on the medullary thymocytes. Analysis of thymocyte suspensions by immunofluorescence confirmed that essentially all cells carried both Thy-1.1 and Thy-1.2 (data not shown). Thymus suspensions and sections from two adult Tg11 mice showed a level and a distribution of both Thy-1 antigens that were identical to those in the Tg12 mice. In contrast, thymocytes of two Tg9 mice showed normal levels of Thy-1.2 but only 20–25% the normal level of Thy-1.1. Lysis of thymocytes from all strains with detergent prior to assay (21) failed to reveal any additional antigen.

The lower level of Thy-1.1 in adult thymocytes compared to brain was confirmed by the steady-state mRNA levels in

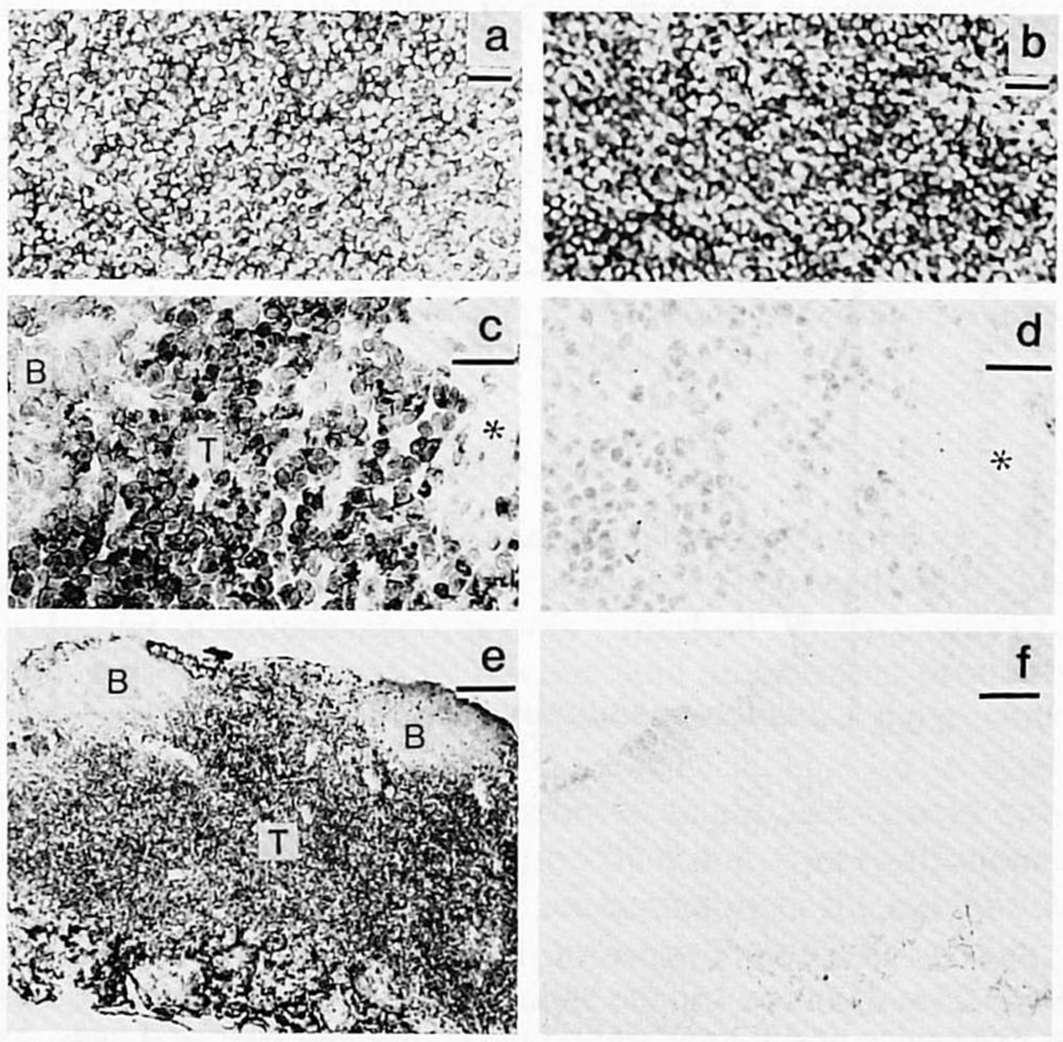

FIG. 5. Immunoperoxidase staining for Thy-1 of adjacent serial sections of thymus (*a* and *b*), spleen (*c* and *d*), and lymph node (*e* and *f*) from an 8-week-old Tg12 mouse labeled with anti-Thy-1.2 (*a*, *c*, and *e*) and anti-Thy-1.1 (*b*, *d*, and *f*) antibodies. Nuclei were counterstained with hematoxylin/eosin. The areas shown of spleen and lymph node span the border between T and B lymphocyte areas (marked T and B), where the T lymphocytes are demonstrated by their reaction with anti-Thy-1.2, but not anti-Thy-1.1, antibodies. Central arteriole is indicated by an asterisk (spleen only). [Scale bars = 20 μm (*a–d*) and 200 μm (*e–f*).]

indirect support to this second possibility (E.S., unpublished data). Consequently, Thy-1 expression in murine T-lineage cells might be regulated by at least two cis-acting DNA sequences, one (or more) contained within the *Eco*RI fragment and one upstream of this fragment. Expression in the murine thymus might use both these elements to obtain maximal levels of expression. When the cells mature and move to the periphery, only one of the elements (missing from the mouse–human construct) would be used, resulting in the normal drop in the levels of transcription of the endogenous gene. Transcription of the transferred gene would cease altogether because it lacks this element.

The elevated levels of Thy-1.1 on thymocytes in young mice were measured on the cell surface (using viable cells), and the immunohistochemical evidence strongly suggests that in brain also, the 7- to 16-fold elevated Thy-1.1 levels were incorporated on the neuronal surface. Despite this, the mice showed no obvious developmental defects, such as neurological symptoms or abnormalities in the disposition of cells within brain. Thy-1 is thought to play a role in binding on the cell surface (1, 2), in which case the concentration of its natural ligand would be limiting and such a high level of Thy-1 expression would not make any difference to normal neural (or any other) development or function. It should be noted, however, that the line Tg12 does have one physiological abnormality: many mice of this strain develop renal dysfunction in the third month of life. Tg12 mice have a high level of Thy-1.1 antigen on their kidney glomeruli, which might (indirectly) interfere with kidney function. However, Tg11 mice have similarly high levels but do not develop any abnormalities. This raises the possibility that the lethal condition in the Tg12 homozygous strain arises from an insertional mutation.

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