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*J Immunol* 1998; 161:4591-4598; http://www.jimmunol.org/content/161/9/4591

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A Mouse Carrying Genetic Defect in the Choice Between T and B Lymphocytes

Yayoi Tokoro,* Takehiko Sugawara,* Hiroyuki Yaginuma,† Hiromitsu Nakauchi,*§ Cox Terhorst,¶ Baoping Wang,‖ and Yousuke Takahama**

Transgenic mice with human CD3ε gene have been shown to exhibit early arrest of T cell development in the thymus. The present study shows that, instead of T cells, B cells are generated in the thymus of a line, tge26, of the human CD3ε transgenic mouse. The accumulation of mature B cells in the thymus was found only in tge26 mice, not in other human CD3ε transgenic mouse lines or other T cell-deficient mice, including CD3-ε knockout mice and TCR-β/TCR-δ double knockout mice. Hanging drop-mediated transfer into 2-deoxyguanosine-treated thymus lobes showed that lymphoid progenitor cells rather than thymus stromal cells were responsible for abnormal B cell development in tge26 thymus, and that tge26 fetal liver cells were destined to become B cells in normal thymus even in the presence of normal progenitor cells undergoing T cell development. These results indicate that lymphoid progenitor cells in tge26 mice are genetically defective in thymic choice between T cells and B cells, generating B cells even in normal thymus environment. Interestingly, tge26 thymocytes expressed GATA-3 and TCF-1, but not LEF-1 and PEBP-2α, among T cell-specific transcription factors that are involved in early T cell development, indicating that GATA-3 and TCF-1 expressed during thymocyte development do not necessarily determine the cell fate into T cell lineage. Thus, tge26 mice provide a novel mouse model in that lineage choice between T and B lymphocytes is genetically defective. The Journal of Immunology, 1998, 161: 4591–4598.

T and B lymphocytes are both originated from multipotent hemopoietic stem cells (1–3). It is generally believed that hemopoietic stem cells first differentiate into either lymphoid progenitor cells or myeloid progenitor cells, and that lymphoid progenitor cells further differentiate into T cells and B cells as well as NK cells and dendritic cells (4–6). Identification of lymphoid progenitor cells in primary hemopoietic organs such as fetal liver and adult bone marrow has still been an issue of controversy (7–9), although most immature lymphoid cells in the thymus are shown to exhibit a differentiation potential that corresponds to lymphoid progenitor cells, being capable of becoming T cells, B cells, NK cells, and dendritic cells (10–13). It is also controversial whether lineage commitment of the progenitor cells into T cells takes place before or after migrating into the thymus (8, 14). Even less is understood how lymphoid progenitor cells are committed to either T cells or B cells.

We have found recently that B220, a CD45R determinant that is generally appreciated as a B cell-specific marker, is expressed by fetal liver progenitor cells that can generate T cells upon migration into the thymus, suggesting a possibility that some B cell-specific molecules are expressed by immature T-lymphopoietic progenitor cells perhaps before their commitment to T cell lineage (9). During further analysis of B cell-specific molecules expressed by immature T-lineage cells from various genetic backgrounds, we have found that B220 is highly expressed by most thymocytes in a human CD3ε transgenic mouse line, tge26. Transgenic mice with high copy numbers of human CD3ε gene, including tge26, have been shown to exhibit early arrest of T cell development as well as of NK cell development (15, 16), and it has been suggested that the block in T cell development is caused by the overexpression of human CD3ε proteins (15, 16). The present study describes that mature B cells expressing IgM are generated only in the thymus of tge26 mice, not in other human CD3ε transgenic mouse lines or in other T cell-deficient mice. Our results show that lymphoid progenitor cells in tge26 mice are responsible for abnormal B cell development in the thymus, and that tge26 fetal liver cells are destined to become B cells even in normal thymus environment. Interestingly, tge26 thymocytes express GATA-3 and TCF-1, T cell-specific transcription factors that are involved in early T cell development, indicating that the expression by thymocytes of GATA-3 and TCF-1 does not necessarily determine their destination into T cells. Thus, the present study describes a mutant transgenic mouse in that lymphoid progenitor cells exhibit the defect in the choice between T and B cells.

Materials and Methods

Mice
Human CD3ε transgenic mouse strains, tge26, tge600, and tge2978, were described previously (15). C57Bl/6 mice were purchased from SLC (Hamamatsu, Japan). TCR-β/TCR-δ double knockout mice (17) and RAG-1 knockout mice (18) were obtained from The Jackson Laboratory (Bar Harbor, ME). CD3-ε knockout mice will be described elsewhere (Wang et al., manuscript in preparation). These CD3-ε knockout mice are also deficient in the expression of CD3-γ and CD3-δ genes, similar to the recently described CD3-εΔεκας mice (19). B6-Ly-5.1 mice were bred in a pathogen-free animal facility of Laboratory Animal Research Center at University of Tsukuba (Tsukuba, Japan).
Thymus organ cultures
Neonatal thymus organ culture was described previously (20). Briefly, thymus lobes obtained from newborn tge26 mice on the day of birth were cultured on sponge-supported filter membranes at an interface between 5% CO2-humidified air and RPMI 1640-based culture medium containing 10% FBS (Life Technologies, Gaithersburg, MD), 50 μM 2-ME, 2 mM l-glutamine, 1× nonessential amino acids, 10 mM HEPES, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

Hanging drop-mediated transfer and organ culture of either fetal thymocytes or fetal liver cells in 2-deoxynosoguanosine (dGuo)-treated fetal thymus lobes were conducted as described (21, 22). Briefly, day 14 fetal thymus lobes from indicated mice were cultured for 5 to 7 days in the presence of 1.35 mM dGuo (Yamas, Chiba, Japan) on sponge-supported filter membranes at an interface between 5% CO2-humidified air and RPMI 1640-based culture medium described above. The dGuo-treated thymus lobes (1 lobe/well) were transferred to freshly prepared sponge-supported filter membranes, and or-
fentially cultured for indicated period.

Immunofluorescence staining and flow cytometry
Single cell suspensions were washed in PBS, pH 7.2, containing 0.2% BSA and 0.1% NaN3. Cells were first incubated with 2.4G2 or FcγR mAb (23) to block binding of Ig to FcγR, and stained with FITC-labeled Ab and biotinylated Ab for 30 min at 4°C. Cells were then stained with phyco-
erythrin (PE)-streptavidin for 10 min at 4°C. Following Abs were obtained from PharMingen (San Diego, CA): FITC anti-IgM (R6-60.2), FITC anti-
B220 (RA3-6B2), FITC anti-Thy-1.2 (30-H12), FITC anti-CD4 (Rm4-5), FITC anti-BP-1 (6C3), FITC normal IgG, biotinylated anti-B220, biotinyl-
ated anti-CD8 (53-6.7), and biotinylated normal IgG. Anti-Ly-5.1 Ab (clone A20 (24)) was purified and biotinylated in our laboratory. Multi-
color flow-cytometry analysis was performed using FACSORT (Becton Dickinson, San Jose, CA). Data were obtained using either LYSYS II or Cellquest software on viable cells, as determined by forward light scatter intensity and propidium iodide exclusion. Cell sorting of thymocytes was conducted using FACS Vantage (Becton Dickinson).

Immunohistochemistry
Adult thymus lobes from tge26 mice or normal C57BL/6 mice were fixed in 4% paraformaldehyde and sliced for 7-μm sections. Fixed sections were incubated with biotinylated anti-B220 or anti-Thy-1.2 Ab, followed by streptavidin-peroxidase. Slides were developed in diaminobenzidine and counterstained in methyl green.

RT-PCR analysis of mRNA expression levels
Total cellular RNA was prepared by Isogen solution (Nippon Gene, Tokyo, Japan), followed by isopropyl alcohol precipitation. Poly(A) RNA was reverse transcribed to cDNA by oligo(dT) primers and M-MLV reverse transciptase (Takara Biomedicals, Shiga, Japan) in the presence of either one of Vβ8.2 or Dβ2 primer, as previously described (22, 29). Amplified DNA products were electrophoresed on 5% polyacrylamide gel, denatured in 0.4 M NaOH, and electrotransferred to Gene Screen Plus membranes (DuPont, Boston, MA). Membranes were hybridized with biotinylated Jβ2 probe (22). Hybridization was visualized by the PolarPlex chemoluminescence detection reagents (Millipore, Tokyo, Japan).

Results

B cells are generated in tge26 human CD3 transgenic thymus
To better understand molecular basis for early arrest of thymocyte development in tge26 human CD3 transgenic mice (15), we first examined the expression of various surface molecules by tge26 thymocytes. Consequently, we found that majority of tge26 thymocytes expressed high levels of B220-CD45R determinant (Fig. 1A). These B220+ thymocytes in tge26 mice were IgM+ IgD+ CD19+ and Thy-1+ CD3+ CD4- CD8- , the phenotypes of mature B cells (Fig. 1A). Although tge26 thymus was disorganized, lacking cortex and medulla architectures (30), B220+ thymocytes were broadly localized within tge26 thymus lobes (Fig. 1B), ruling out a possibility that B cells in tge26 thymocyte preparations might be derived from contaminated blood or proximal lymph nodes.

It has been shown that small numbers of B cells exist even in normal thymus (31; also shown in Fig. 1A). To test whether B cells in tge26 thymus reflect normal thymic B cells because of the lack of T cell development, we next measured the absolute numbers of B cells in tge26 thymus and normal B6 thymus, as well as thymocytes from TCR-β/TCR-δ double knockout mice, CD3-ε knockout mice, and RAG-1 knockout mice in which T cell development is arrested at early CD4+ CD8- stage. As summarized in Table I, IgM+ B220+ B cells in tge26 thymus were 1.2 × 10^6/10^6 thymocytes, ~6 times more than the numbers of thymic B cells in normal B6 mice and ~60 times more than B cell numbers in TCR-β/TCR-δ double knockout mice and CD3-ε knockout thymuses (Table I). Thus, B cells are unusually accumulated in tge26 thymus, neither simply reflecting the lack of T cell development nor reflecting the over-
representation of normal thymic B cells.

To examine the origin of thymic B cells in tge26 mice, we analyzed the ontogeny of B cells in tge26 thymus. As shown in Figure 2A, IgM+ B220+ B cells in tge26 thymus were generated after birth by 1 wk old, and the frequency and numbers of B cells in tge26 thymus reached to adult levels by 2 wk after birth. Transient increase of BP-1+ B220+ cells, resembling pre-B cells, in tge26 thymus at 1 wk old and their subsequent decrease suggested that temporal development of B cells occurs in tge26 thymus. To di-
rectly examine whether B cells are generated from progenitor cells in tge26 thymus, we performed organ culture of newborn tge26 thymus lobes (Fig. 3). In contrast, normal B6 fetal thymocytes in hanging drop-mediated transfer of tge26 progenitor thymocytes into normal fetal thymus lobes that had been treated with dGuo. We found that tge26 fetal thymocytes generated B220+ cells in normal B6 thymus cultures as well as in tge26 thymus lobes (Fig. 3). In contrast, normal B6 fetal thymocytes generated Thy-1+ CD4/CD8- cells in tge26 thymus lobes as well as in normal B6 thymus lobes (Fig. 3). Thus, lymphoid progenitor cells rather than thymus environment are responsible for B cell generation in tge26 thymus. It is interesting to note that many B220+ cells in tge26-derived thymocytes expressed low levels of

Abbreviations used in this paper: dGuo, 2-deoxyguanosine; PE, phycoerythrin.
Thy-1, as exemplified in the 26 B6 cells (Fig. 3), although B6 environment in the 26 condition did not especially enrich Thy-1 low cells or always retard growth and development (data not shown). Thy-1 low expression by B220

1

t26 thymocytes appeared to be more pronounced in immature B220

1

B cells (Fig. 2, A and B), consistent with previous findings that immature B-precursor cells express Thy-1 even during normal B cell development (32).

To analyze whether t26 progenitor cells generate B cells even in the presence of normal T cell development in the thymus, fetal liver progenitor cells from t26 mice were mixed at graded ratios with fetal liver cells from normal B6-Ly-5.1 mice, and transferred into dGuo-treated B6-Ly-5.1 thymus lobes (Fig. 4). T26 cells (Ly-5.1

2

) could be distinguished from B6-Ly-5.1 cells (Ly-5.1

2

) by allele-specific detection of CD45 (Ly5) molecules. As shown in Figure 4, Ly-5.1

1

t26-derived cells became B220

1

even in the presence of normal Ly-5.1

1

thymocytes. It is interesting to note that the ratio of input cell numbers between t26 and B6-Ly-5.1 fetal liver cells correlated with the ratio between Ly-5.1

1

t26-derived B cells and Ly-5.1

1

normal thymocytes generated in the thymus, suggesting that t26 progenitor cells are capable of generating B cells at an efficiency comparable

FIGURE 1. B cell accumulation in t26 thymus. A, Surface molecules expressed by t26+/+ thymocytes. Adult thymocytes from indicated mice were two-color stained with FITC-labeled Ab (x-axis) and biotinylated Ab (y-axis) with indicated specificity. Biotin staining was visualized with PE-streptavidin. Numbers within each box indicate the frequency of cells within that box. B, Immunohistochemical analysis of t26+/+ thymus. Fixed sections from adult thymus of t26 mice or normal C57BL/6 mice were incubated with biotinylated Ab specific for either B220 or Thy-1,2, followed by streptavidin-peroxidase. Slides were developed in diaminobenzidine and counterstained in methyl green. Shown are representative results from four (A) and three (B) independent experiments.
with normal progenitor cells generating T cells in the thymus. These results indicate that tg26 progenitor cells efficiently generate B cells even in the thymus in which normal T cell development occurs, and that immature lymphoid progenitor cells in tg26 fetal liver are abnormal in generating B cells upon migration in the thymus environment.

Other human CD3ε transgenic mice do not exhibit excessive B cell development in the thymus

To gain insights into the mechanism by which tg26 progenitor cells develop into B cells in the thymus, we examined whether two other strains of human CD3ε transgenic mice may exhibit the similar accumulation of thymic B cells. Tg600 mice carry the same human CD3ε transgene as in tg26 mice, whereas tg2978 mice carry the transgene that encodes transmembrane and cytoplasmic regions of human CD3ε proteins lacking extracellular regions (15). Nonetheless, both tg600 and tg2978 mice exhibit severe defect in early T cell development (15). As shown in Table I, however, both tg600 and tg2978 human CD3ε transgenic mice showed no increases in B cells in the thymus; in contrast, tg26 thymocytes showed clear B cell accumulation. It should be noted that tg2978/+/− mice, which carry higher copy numbers (80–100 copies) of the transgene than those in tg26+/− mice (40–60 copies), did not exhibit any accumulation of B cells in the thymus, suggesting that B cell generation in tg26 mice is not a direct consequence of high copy numbers of human CD3ε transgene. Thus, thymic accumulation of B cells in tg26 mice is a phenotype specific for tg26 transgenic mice, not a phenotype commonly observed in other human CD3ε transgenic mice, including tg600 and tg2978 strains.

We next examined whether B cells are predominantly generated even in the thymus of F1 hybrid mice crossed between tg26 homozygous transgenic mice and normal C57BL/6 (B6) mice. Unlike tg26 homozygotes, (tg26 × B6) F1 heterozygous mice exhibited T cell development in the thymus, although they had the thymus of 10–20% cellularity of normal B6 mice (Table I). Despite the generation of many T cells, however, tg26+/− heterozygous thymocytes contained increased numbers of B cells as compared with normal B6 thymocytes (Table I). Thus, the combination of complete T cell deficiency and increased B cell development in the thymus was found only in tg26 transgenic homozygotes, not in tg26 heterozygotes, whereas tg26 heterozygotes showed a compromised T cell development and increased B cell generation in the thymus.

Expression of GATA-3 and TCF-1, but not PEBP-2α and LEF-1, in Tg26 thymus

We finally examined whether tg26 thymus, which generated B cells instead of T cells, may express modulated expression of transcription factors that are involved in early development of T cells. To do so, RT-PCR analysis was performed for the expression of T cell-specific transcription factors such as GATA-3, TCF-1, PEBP-2α, PEBP-2βB, LEF-1, and Sox-4 (Fig. 5A). We have found that tg26 thymocytes expressed GATA-3, TCF-1, and Sox-4, but failed to express PEBP-2α, PEBP-2βb, and LEF-1 (Fig. 5A). The expression of Sox-4 in B cell-generating tg26 thymocytes is consistent with previous findings that Sox-4 is expressed in immature B cells as well as in T-lineage cells (33, 34). On the other hand, it has been shown that the expression of GATA-3 and TCF-1 transcription factors is restricted in T cell lineage (35–38). It is therefore interesting to point out that GATA-3 and TCF-1 transcripts were detected even in IgM+B220− mature B cells as well as in IgM+B220− cells within tg26 thymus (Fig. 5B). The detection of GATA-3 and TCF-1 transcripts in IgM+B220− B cells in tg26 thymus is not due to contaminated T-lineage precursor cells, since these transcripts were not detected in 10-fold or 100-fold diluted cDNA from IgM+B220+ purified cells that contained equivalent amount of cDNA from IgM+B220+ cells contaminated in IgM+B220+ preparations (Fig. 5C). Moreover, in contrast to B cells in tg26 thymus, GATA-3 and TCF-1 transcripts were not detected in IgM+B220− B cells in the spleen of tg26 mice (Fig. 5D), supporting the possibility that the expression of GATA-3 and TCF-1 by B cells in tg26 thymus reflects abnormal switch toward B cells of thymus-migrated progenitor cells that are otherwise directed into T-lineage development. Collectively, these results indicate that 1) GATA-3 and TCF-1 expressed by thymocytes are not sufficient for their final decision to enter T cell lineage, and that 2) the defect in tg26 thymocytes is associated with the failure for thymocytes in expressing PEBP-2α, PEBP-2βb, and LEF-1.

It is also interesting to note that tg26 thymocytes expressed TCR-Cβ transcripts, although they did not express endogenous
CD3-ε, another T cell-specific molecule, and instead they expressed VpreB, a B cell-specific molecule (Fig. 5A). To examine whether TCR-β gene locus is rearranged in tge26 thymocytes, DNA prepared from tge26 thymocytes was PCR amplified with either Db- and Jb-specific primers or Vb8.2- and Jb-specific primers. Unlike normal thymocytes, tge26 thymocytes did not contain any detectable rearrangement of TCR-β gene locus, including D-J rearrangement (Fig. 6). These results suggest that TCR-Cβ transcripts expressed by tge26 thymocytes represent germline Cβ transcripts of unrearranged TCR-β gene.

Discussion

The present study shows that, instead of T cells, B cells are generated in the thymus of tge26 human CD3ε transgenic mice. Selective B cell generation in the thymus was found only in tge26 mice, not in other T cell-deficient mice, including two other human CD3ε transgenic mouse lines. The transfer of fetal liver progenitor cells into thymus lobes showed that tge26 lymphoid progenitor cells were destined to become B cells even in normal thymus environment, indicating that lymphoid progenitor cells in tge26 mice are genetically defective in thymic choice between T cells and B cells. Interestingly, tge26 thymocytes expressed GATA-3 and TCF-1, indicating that GATA-3 and TCF-1 expressed during thymocyte development do not necessarily determine the cell fate into T cells. Thus, tge26 mice provide a novel mouse model in that...

FIGURE 3. B cell generation in normal thymus stromal cells from tge26 progenitor cells. Fetal thymocytes (10^5) from either tge26/1/1 (ε26) mice or normal B6 mice were transferred in hanging drops to dGuo-treated fetal thymus lobes from indicated mice, and organ cultured for 12 days. Cells recovered from thymus cultures were stained with FITC-labeled anti-Thy-1.2 or anti-CD4 Ab. Cells were also stained with biotinylated anti-B220 or anti-CD8 Ab, followed by PE-streptavidin. Numbers indicate the frequency of cells within the defined area. Shown are representative results from three independent experiments.

FIGURE 2. B cell generation in tge26 thymus. A, Ontogeny of tge26 thymocytes. Thymocytes from tge26/1/1 mice at indicated age were two-color stained with FITC-labeled Ab (x-axis) and biotinylated Ab (y-axis) with indicated specificity. Biotin staining was visualized with PE-streptavidin. Numbers within each box of contour diagrams indicate the frequency of cells within that box. Numbers in parentheses indicate the numbers of thymocytes from one mouse at indicated age. B, Neonatal thymus organ culture of tge26 thymocytes. Shown are representative results from two (A) and three (B) independent experiments.
The abnormal B cell development in tg26 thymus is due to neither high copy numbers of the transgenic insertion into a gene locus that is crucial for the commitment to either T cells or B cells, although it is still possible that the phenotype is caused by a unique pattern of expression of transgene insertion into a gene locus that is crucial for the commitment to either T cells or B cells. Moreover, the results show that the aberrant switch from T cells to B cells in the thymus occurs only in tg26 mice, not in other two lines of human CD3ε transgenic mice generated in the same laboratory, including a transgenic line carrying higher copy numbers of the transgene. The aberrant switch from T cells to B cells in the thymus may be due to several T cell-specific transcription factors such as Lef-1, PEBPa2A, and PEBP2Bα in tg26 thymocytes suggests that 1) the failure in expressing these transcription factors may be involved in early arrest of T cell development in tg26 mice, and 2) the expression of these transcription factors may not be required for the expression of germline TCR-β transcriptions. How are lymphoid progenitor cells in tg26 mice destined to become B cells even in thymus environment? Our results show that the switch from T cells to B cells in the thymus is limited only in the tg26 line among CD3ε transgenic mouse strains. Thus, we think that the defect of lymphoid progenitor cells in the choice between T and B cells may be a consequence of transgene insertion into a gene locus that is crucial for the commitment to either T cells or B cells, although it is still possible that the phenotype is caused by a unique pattern of expression of transgenic CD3ε protein in early progenitor cells in tg26 mice. Our results also show that other tg26 mice still show the accumulation of B cells in the thymus, compatible with the possibility that abnormal B cell development in tg26 thymus is a genetically dominant phenotype. It is thus possible that the enhancer and promoter in the human CD3ε transgene may drive the expression of a nearby gene in thymus-migrated lymphoid progenitor cells, which turn results in the aberrant conversion of the developmental direction from T cell lineage to B cell lineage. Alternatively, it is also possible that a gene at the site of transgenic insertion may be disrupted, resulting in the switch from T cell development to B cell development in the thymus. To
better understand the molecular mechanism causing this defect in the choice between T and B cells, we are currently attempting to identify the transgene-inserted gene locus in tg26 mice.

In conclusion, the present study describes a mouse strain in that lymphoid progenitor cells are destined to become B cells in the thymus. These mice serve a unique model for the genetic defect in the choice between T and B lymphocytes. Understanding molecular basis causing the defect in tg26 mice will provide a useful

**FIGURE 5.** RT-PCR analysis of mRNA expression by tg26 thymocytes. Oligo(dT)-primed cDNA prepared from indicated cell populations was employed for RT-PCR analysis using indicated primers. Water alone (−) or cDNA from adult B6 thymocytes (+) was also PCR amplified for negative and positive controls. Molecular weight marker (M) was 100-bp DNA ladder (Life Technologies). A, cDNA prepared from tg26+/+ thymocytes was PCR amplified. For the positive control of VpreB detection, cDNA from day 14 fetal liver cells from B6 mice was used. Signals with the expected size derived from correctly spliced RNA are indicated with arrows. B and C, Thymocytes from tg26+/+ mice were two-color stained for IgM and B220. IgM+ B220− cells and IgM− B220+ cells were sorted by FACS-Vantage flow cytometry. Purity of sorted IgM+B220− cells and IgM− B220+ cells was 95.9 and 96.6% in B, and 99.6 and 98.1% in C, respectively. Equal numbers of IgM+B220− cells and IgM− B220+ cells were used for cDNA preparation, giving rise to largely comparable β2-microglobulin signals between the two groups. C shows that, unlike IgM+B220− purified cells, 10-fold- or 100-fold-diluted cDNA from IgM− B220+ purified cells failed to give rise to GATA-3 signals, ruling out the possibility that the GATA-3 signal from IgM− B220+ purified cells could be derived from <10% of contaminated IgM+B220+ cells. D, Spleen cells from tg26+/− mice were purified for IgM+B220+ (99.2% purity). Shown are representative results from six (A), three (B), two (C), and two (D) independent experiments.

![FIGURE 5](image-url)
clue to reveal the molecular mechanism underlying the lineage commitment between T cells and B cells.

Acknowledgments

We thank Drs. Masanobu Satake and Masayuki Yamamoto for polymerase chain reaction primers; Dr. Hiroyuki Ichijo for helping immunohistochemical analysis; and Drs. Willem van Ewijk, Masanobu Satake, and Alfred Singer for helpful discussion.

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