a genotype frequency of about 18% in the US beef cattle population.[25] Both, DNA amplified from fresh as well as paraffin-embedded tissues, showed the codon 192 polymorphism, indicating that these samples were derived from the same animal. United States BSE case 2 had a synonymous polymorphism at codon 185, reported to be variable with a genotype frequency of about 6% in the US beef cattle.[25] We conclude from these data that samples from both US BSE cases had normal, unremarkable cattle-like *Prnp* gene sequences. Polymorphisms in the sheep prion protein have been correlated with both variable incubation periods and degrees of resistance to scrapie.[19,20] This has not been the case for BSE in cattle.[29,34] However, noncoding regions of the *Prnp* gene locus of cattle (e.g., prion protein gene promoter polymorphisms) might have an influence on BSE susceptibility as reported recently.[43,44] These regions have not been analyzed in this study.

Unusual cases of BSE have been reported in the past 2 years by investigators from several countries. There have been 2 molecular types of unusual BSE isolates described in the literature: 1) a type with a lower molecular mass of the unglycosylated isoform (L-type) and 2) a type with higher molecular mass of the unglycosylated isoform (H-type). The L-type has been found in cattle in Italy,[11] Japan,[54] and Belgium.[15] In Italy, 2 cattle, older (11 and 15 years) than other bovines affected with BSE, showed an unusual molecular phenotype. Western blot analysis showed a PrP^{Sc} type with a predominance of the monoglycosylated isoform and the unglycosylated isoform fragment of lower molecular mass than usually seen with BSE.[11] The disorder was pathologically characterized by the presence of PrP^{Sc}-immunopositive amyloid plaques, as opposed to the lack of amyloid deposition in typical BSE cases, and by a different pattern of regional distribution and topology of brain PrP^{Sc} accumulation.[11] On the basis of the above features, the authors proposed to name the disease bovine amyloidotic spongiform encephalopathy, BASE.[11] A Japanese case was identified in an ELISA-positive specimen from a 23-month-old Holstein steer slaughtered in September 2003. The animal was reportedly healthy before slaughter and histology showed no spongiform changes and IHC revealed no signal of PrP^{Sc} accumulation typical for BSE. Western blot analysis of brainstem homogenate revealed a small amount of PrP^{Sc} with an electrophoretic profile different from that of typical BSE-associated PrP^{Sc} with 1) a lower content of the diglycosylated molecular isoform of PrP^{Sc}, 2) a faster migration of the unglycosylated isoform of PrP^{Sc}, and 3) less resistance against PK digestion when compared with typical BSE.[54] Another case involved a 64-month-old Belgian cow, whose obex sample was positive by ELISA, and the cow was reported healthy before slaughter. The histopathology of the obex, pons, and midbrain showed no spongiform changes and IHC of the brainstem revealed no signal of PrP^{Sc} accumulation. Western blot analysis of the obex region revealed a small amount of PrP^{Sc} with an electrophoretic profile of the unglycosylated isoform of PrP^{Sc} showing a lower migration pattern compared with that of a typical BSE case.[15]

So far, the H-type has been only described in cattle from France and Germany.[5,9] A distinct molecular phenotype was found in 1 German and 3 French BSE cattle following active surveillance of the disease at slaughterhouses or in rendering plants using rapid BSE tests. The 3 French cases did not have clinical signs suggestive of BSE during their life and the German and French animals were older cattle (8–15 years old). The unusual molecular phenotype of the German[9] and French[5] cases was characterized by 1) a higher molecular mass of the unglycosylated PrP^{Sc} isoform, 2) a strong labeling of all 3 PrP^{Sc} polypeptides with antibody P4, and 3) the French cases by a glycoform profile with a less prominent diglycosylated PrP^{Sc} isoform.[5] All of the H-type features were also observed with US BSE case 2 (Fig. 3).

Unusual cases of BSE are an unexpected finding since it was previously believed that BSE disease in cattle is caused by a single strain of infectious agent, which has been shown to be very consistent and uniform in appearance, even after transmission to other species.[7,28,46] The reports of unusual phenotypes of BSE in cattle suggest that different PrP^{Sc} phenotypes exist in cattle with BSE. There are several hypotheses which can explain these findings:[5] 1) there are manifestations of the BSE agent with different molecular features in cattle. It is known that sequence differences in the *Prnp* gene give rise to variants in electrophoretic profiles of PrP^{Sc}, as shown in cases of human CJD,[10] however, comparison of the *Prnp* alleles available from cattle with unusual BSE phenotypes (including US BSE case 2) and the general cattle population have shown normal cattle *Prnp* sequences; 2) cattle may have been infected by another source of infectious agent (e.g., scrapie or CWD). However, a survey of brain material derived from 262 high-risk, adult cattle in CWD-endemic areas in Colorado for the presence of changes indicative of a TSE infection was negative.[22] Interestingly, experimental infection of cattle with either US sheep scrapie isolates[14] or CWD sources[23,24] led to a cattle disease with clinicopathological features different from typical BSE in cattle; 3) a rare sporadic form of TSE disease could exist in cattle as described for human TSEs.[16] Further studies are needed to

determine the frequency and origin of such novel BSE phenotypes. Critical studies would elucidate the transmissibility of unusual BSE cases to cattle or other mammalian hosts; transmission has not been reported in the literature so far.

It is concluded from the studies reported here that 1) the PrP^{Sc} profile from the first US BSE case showed similar molecular properties to the typical PrP^{Sc} pattern described for the May 2003 Canadian and European BSE isolates,[4,5] and 2) the PrP^{Sc} profile from the second US BSE case showed unusual molecular properties similar to atypical high molecular weight BSE cases reported in France and Germany.[5,9] Both cases were identified by the USDA surveillance program in place and the carcasses did not enter the human or animal food chain. IHC staining in brainstem sections for an unusual high molecular BSE case is described here for the first time. A germline mutation as an etiological possibility for the disease conditions of both cases can be most likely ruled out. Future work will address the question whether brain material from both US BSE cases are infectious in cattle after intracerebral and oral inoculation.

Acknowledgements

The authors would like to thank S. Lebepe-Mazur, D. Clouser, K. Hassall, L. Manning, Shelley Ganske, Sharon Lund, Nadine Beckwith, and D. Orcutt for excellent technical support and Drs. M. Kehrli and R. Levings for their encouragement and support during the BSE diagnostic work. The authors also wish to thank the 7 State/University Veterinary Diagnostic Laboratories for their hard work screening US cattle for the presence of BSE.

Disclaimer: Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

Sources and manufacturers

a. Platelia/TeSeE™ ELISA BSE test, Bio-Rad, Hercules, CA.
b. Fisher Superfrost Plus, Fisher Scientific, Hampton, NH.
c. Dako, Carpinteria, CA.
d. Biocare, Walnut Creek, CA.
e. Ventana Medical Systems Inc., Tucson, AZ.
f. VMRD, Pullman, WA.
g. Non Ultra Dish Liquid, Original Scent, Dawn. Procter & Gamble, Cincinnati, OH.
h. Prionics, Schlieren, Switzerland.
i. Fisher Scientific, Pittsburgh, PA.
j. Invitrogen, Carlsbad, CA.
k. Immobilon-P, Amersham Biosciences, Piscataway, NJ.
l. R-Biopharm Inc, Marshall, MI.
m. Amersham Biosciences, Piscataway, NJ.
n. Novagen, Madison, WI.
o. Sigma, St. Louis, MO.
p. Beckman Coulter Inc, Fullerton, CA.
q. USB, Cleveland, OH.
r. Roche, Indianapolis, IN.
s. Kodak, St. Louis, MO.
t. Qiagen, Valencia, CA.
u. Amresco, Solon, OH.
v. Strategene, La Jolla, CA.
w. Q-BIOgene, Ivine, CA.
x. Big Dye Teminator, Applied Biosystems, Foster City, CA.

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 5月6 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains. Raymond, G. J. et al, J. Virol., 81, 4305 -4314 (2007).	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
慢性消耗性疾患（CWD）の新規げっ歯類モデルを作製するために，初めて無細胞プリオン蛋白質（PrP）変換測定法を用いて種々のハムスター及びマウスの動物種の感受性を評価した。本測定法では，3 種シカ（ヘラジカ，オジロジカ及びミュールジカ）から得られたプロテイナーゼ K 耐性 PrP による，げっ歯類動物の正常なプロテイナーゼ K 感受性 PrP から耐性 PrP への変換反応を評価した。その結果，一部のげっ歯類のみが感受性を有することが示唆された。これらの結果は CWD 罹患シカの脳組織をげっ歯類の脳内に接種した際に in vivo でも確認された。検討したげっ歯類動物のうち，遺伝子組み換えマウスがシリアンゴールデンハムスターのプリオン蛋白を発現したのに加えて，アルメニアン，チャイニーズ，シベリアン，シリアンゴールデンは限定的ではあるが感受性を示した。状態が悪化した少数の動物は，長期の潜伏期間の後に疾患の臨床徴候を発現した。ジャンガリアンハムスター及び野生型マウスは罹患しないままであった。しかし，シリアンゴールデンハムスターにおける CWD 分離株の連続継代によって，明らかな神経病理学的特徴を示し，臨床症状を呈するまでの平均潜伏期間が異なる 2 つのプリオン分離株を特定した。また，当該動物種では接種を受けた動物の全てが第 2 及び第 3 継代の後，疾患を発現した。これらの結果から，CWD は一部のげっ歯類の動物種に伝播し，様々な分離株が存在する可能性があることが示された。	BYL-2007-0281

報告企業の意見	今後の対応
この報告はげっ歯類モデルの脳内伝播で特異的な神経病理学的疾患を引き起こす CWD の様々なプリオン株の存在を強く示唆するものである。これらの CWD プリオン株は異種宿主への連続継代においてより発現率が高くなる傾向にある。この研究報告は CWD でも種の壁を崩壊する可能性があることを示唆している。	これまでに，種の壁が崩壊したという報告例はないため，現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

36

JOURNAL OF VIROLOGY, Apr. 2007, p. 4305–4314 Vol. 81, No. 8
0022-538X/07/$08.00+0 doi:10.1128/JVI.02474-06

Transmission and Adaptation of Chronic Wasting Disease to Hamsters and Transgenic Mice: Evidence for Strains▽

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Received 9 November 2006/Accepted 23 January 2007

In vitro screening using the cell-free prion protein conversion system indicated that certain rodents may be susceptible to chronic wasting disease (CWD). Therefore, CWD isolates from mule deer, white-tailed deer, and elk were inoculated intracerebrally into various rodent species to assess the rodents' susceptibility and to develop new rodent models of CWD. The species inoculated were Syrian golden, Djungarian, Chinese, Siberian, and Armenian hamsters, transgenic mice expressing the Syrian golden hamster prion protein, and RML Swiss and C57BL10 wild-type mice. The transgenic mice and the Syrian golden, Chinese, Siberian, and Armenian hamsters had limited susceptibility to certain of the CWD inocula, as evidenced by incomplete attack rates and long incubation periods. For serial passages of CWD isolates in Syrian golden hamsters, incubation periods rapidly stabilized, with isolates having either short (85 to 89 days) or long (408 to 544 days) mean incubation periods and distinct neuropathological patterns. In contrast, wild-type mouse strains and Djungarian hamsters were not susceptible to CWD. These results show that CWD can be transmitted and adapted to some species of rodents and suggest that the cervid-derived CWD inocula may have contained or diverged into at least two distinct transmissible spongiform encephalopathy strains.

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE), or prion disease, that affects mule deer (*Odocoileus hemionus*), black-tailed deer (subspecies of *Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces*) (17). Other TSE diseases include scrapie in sheep and goats, transmissible mink encephalopathy (TME), bovine spongiform encephalopathy (BSE), and Creutzfeldt-Jakob disease in humans. Some TSE diseases, such as scrapie, BSE, and Creutzfeldt-Jakob disease, have been experimentally transmitted and adapted to rodents, yielding experimental animal models that have proved useful in the study of TSE diseases (4, 10, 13). Recently, CWD was transmitted to mice transgenic for cervid PrP (16a, 24). Although CWD has been transmitted to ferrets, and then from ferrets to Syrian golden (Sg) hamsters (2), there have been no reports of direct transmissions of CWD to any hamster species or wild-type mice (25).

An important and measurable occurrence in TSE diseases is the conversion of the normal host proteinase K (PK)-sensitive prion protein (PrP-sen) to an abnormal, disease-associated isoform that is characteristically PK resistant (PrP-res). One in vitro method that has been used to assess the potential interspecies transmissibility of TSE agents is a cell-free conversion (CFC) reaction in which PrP-res from one species is tested for its relative efficiency in converting PrP-sen of other species to PrP-res (20, 23). Such CFC reactions were found in the present study to predict the susceptibility of hamsters to CWD. Accordingly, CWD isolates were inoculated into various species of rodents, and some species proved to be modestly susceptible. The resulting rodent-adapted CWD models could be useful in comparative studies of TSE strains in vivo as well as for testing potential anti-TSE therapeutic agents.

MATERIALS AND METHODS

PrP-sen ^{35}S labeling and purification. The generation and detailed analyses of the cervid and human PrP-sen molecules have been described previously (6, 20). The PrP polymorphic types used in this study were as follows: elk e-GLSE and e-GMSE (PrP amino acid residues 96, 132, 138, and 226 are designated); md/wd-GMNQ and md/wd-GMSQ, which are identical in mule deer and white-tailed deer; wd-SMSQ, found only in white-tailed deer; and human, hu-M and hu-V (residue 129 is designated). The Sg hamster PrP-sen construct lacks a glycosylphosphatidylinositol (GPI) anchor (15). The ^{35}S–PrP-sen molecules of the various species were immunopurified from various cultured cell lines labeled metabolically with [^{35}S]methionine (EasyTag; Perkin-Elmer) (8), and each cell line used expressed one of the PrP-sen types. In order to simplify analysis of PrP conversion products, the ^{35}S–PrP-sen molecules were radiolabeled in the presence of 10 μg/ml tunicamycin (Roche), an inhibitor of glycosylation. R521 antibody (Ab) (20) was used for immunoprecipitation of the various cervid ^{35}S–PrP-sen molecules, and 3F4 monoclonal Ab (11) was used for the human and hamster PrP-sen molecules.

PrP-res purification. Hamster PrP-res (ha263K) was purified from the brains of 263K-affected Sg hamsters (21). PrP-res isolates from brainstems of CWD-affected elk (eCWD), mule deer (mdCWD), and white-tailed deer (wdCWD) were the same as those used in a previous study (20) and were purified using the same method as that for ha263K.

CFC reactions. CFC reaction methods have been described previously (20, 22) and are summarized here. For each CFC reaction, 250 ng of each of the purified, nonradiolabeled PrP-res molecules isolated from brain pools (20) was incubated with 20,000 to 30,000 cpm of each of the immunopurified ^{35}S–PrP-sen molecules. PrP-res was pretreated with 2.0 to 2.5 M guanidine hydrochloride at 37°C for 1 h,

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† Deceased.
▽ Published ahead of print on 7 February 2007.

added to the ^{35}S–PrP-sen with final concentrations of 1 M guanidine-HCl, 50 mM sodium citrate, pH 6.0, 5 mM cetylpyridinium chloride, and 1.25% Sarkosyl, mixed, and incubated for an additional 72 h at 37°C. One-eleventh of each reaction mix was used for the mock (no-PK) digestion control, and the remainder was treated with 20 μg/ml PK for 1 h at 37°C. One microliter of 0.1 M Pefabloc (Roche) was added to each sample, and the samples were methanol precipitated, pelleted, boiled in polyacrylamide gel electrophoresis (PAGE) loading buffer, and run in precast 16% sodium dodecyl sulfate-PAGE (SDS-PAGE) gels (Invitrogen). A Storm phosphorimager (GE Healthcare) was used for detection, and ImageQuant software was used to quantitate radioactive PrP bands. The conversion efficiency of each reaction was the percentage of input ^{35}S–PrP-sen (determined from the no-PK aliquot) that was converted to 16- to 18-kDa PK-resistant ^{35}S-PrP bands (determined from the PK-treated aliquot).

Animals. Sg hamsters (*Mesocricetus auratus*) were purchased from Harlan Sprague Dawley, Inc. Armenian (*Cricetulus migratorious*), Chinese (*Cricetulus griseus*), Djungarian (*Phodopus campbelli*), and Siberian (*Phodopus sungorus*) hamsters and RML Swiss mice and Tg (haPrP) mice ("Sg hamsterized;" also called Tg7-haPrP/moPrP$^{-/-}$ mice) (18) were bred at NIAID/Rocky Mountain Laboratories (RML). C57BL10 mice were purchased from Jackson Laboratory. Protocols for using animals in these studies were reviewed and approved by the NIAID/RML Animal Care and Use Committee and complied with relevant NIH guidelines. Animals were housed at NIAID/RML facilities accredited by AAALAC International.

CWD primary inocula. New supplies and aseptic technique were used for the following preparations to minimize the potential for contamination from any other TSE source. In addition, inoculations for these experiments were done separately from any other TSE work. For each of the individual brain inocula used (see Fig. 3A), the brainstems of a CWD-affected elk, mule deer, and white-tailed deer were removed. The brainstems were confirmed to be CWD positive by histology and immunoblot analysis of PrP^{CWD}, and homogenates were inoculated into Sg hamsters and RML and Tg (haPrP) mice. Each of the CWD brain pools used (see Fig. 3B) has been described previously and contains heterogeneous genotypes (20). Brain pool-derived homogenates were inoculated into Sg, Djungarian, Chinese, and Armenian hamsters and C57BL10 mice. For each of the inocula, 10% brain homogenates in Dulbecco's phosphate-buffered saline (DPBS; Invitrogen) were made using a separate, new Dounce (Wheaton) homogenizer. Suspensions were sonicated for 5 min at maximum power (Heat Systems-Ultrasonics), diluted to 1% in DPBS, and inoculated intracerebrally (i.c.; 0.05 ml per animal).

Second- and third-passage inocula. For the second passage, each brain from rodents inoculated with the individual animal primary inocula and suspected of being TSE positive by progressive signs of neurological disease was excised using new tools and divided sagittally. Half of each brain was used for immunohistochemical analysis (described below). A 20% homogenate was made from the other half in DPBS, using new disposable plastic pestles and microtubes (Kontes). A portion of this homogenate was used for PrP-res detection on immunoblots (see below), and another portion was diluted to 1%, with 0.05 ml inoculated i.c. into each animal as outlined in Fig. 3. The CWD brain pools from elk, mule deer, and white-tailed deer were passaged only once.

Immunoblot analysis of PrP-res from brain tissue. Aliquots (0.1 ml) of 2% Triton X-100, 2% sodium deoxycholate, 0.2 M Tris-HCl, pH 8.3 (at 22°C), 0.3 M NaCl, and 0.01 M EDTA were added to 0.1-ml aliquots of the 20% brain homogenates described above. After incubation for 60 min at 22°C, the samples were placed on ice and sonicated in a cup horn at maximum power for 1 min. After centrifugation at 2,500 × *g* for 10 min at 4°C, the tubes were inverted three times to resuspend the softer portions of the pelleted material. The resulting supernatant suspensions, including all but the hardest parts of the pellets, were removed, and aliquots were saved at −20°C. A 15-μl aliquot of each suspension was digested by adding PK to 50 μg/ml and was incubated at 37°C for 60 min. One microliter of 0.1 M Pefabloc (Roche) was added, and the sample was held on ice for 5 min, followed by the addition of 25 μl of 2× PAGE loading buffer with 50 mM dithiothreitol and boiling for 5 min. Ten microliters of 0.25 M iodoacetamide (Sigma) was then added and incubated at 37°C for 10 min. A 10-μl aliquot of each sample was subjected to electrophoresis on 10% NuPAGE bis-Tris gels, using morpholineethanesulfonic acid (MES) running buffer (Invitrogen). Proteins were transferred to Immobilon-P (Millipore) membranes by semidry electroblotting. Membranes were immunostained using the following primary Abs (as described in references 20 and 22): monoclonal Ab 3F4 (11), rabbit antiserum 505 (against sheep peptide residues 100 to 111 [20]; generously provided by J. Langeveld, CIDC-Lelystad, The Netherlands), and rabbit antiserum R30 (against Sg hamster PrP residues 89 to 103) (9). The immunoblot was incubated with either alkaline phosphatase-conjugated anti-mouse or anti-rabbit immunoglobulin G (IgG; Zymed) secondary Ab, developed using AttoPhos (Promega) substrate, and scanned using a Storm fluorescence detection instrument (GE Healthcare).

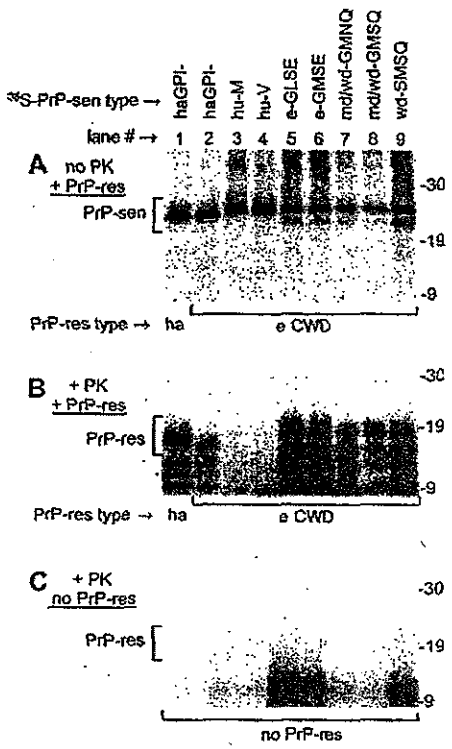

FIG. 1. CFC reactions of various species' PrP-sen molecules induced by PrP-res. PrP-res isolated from either Sg hamsters (ha; lane 1 of each panel) or CWD-affected elk (e CWD; lanes 2 to 9) was incubated with various immunopurified ^{35}S–PrP-sen molecules, identified at the tops of the lanes. (A) Aliquots (1/11) of each reaction mix that were not PK treated. The PrP-sen bands (bracketed; 22 to 26 kDa) were not glycosylated, as explained in Results. (B) Remaining portion of each reaction mix, treated with PK, resulting in the radiolabeled PrP-sen molecules being converted to PK-resistant PrP bands (PrP-res bracket; 16 to 18 kDa). (C) Parallel set of PK-digested reaction mixes incubated without PrP-res. The following PrP-sen molecules (descriptions are given in reference 20) were used in the conversion reactions: cervid PrP polymorphic types present in elk (e-GLSE and e-GMSE; lanes 5 and 6), PrP types present in mule deer and white-tailed deer (md/wd-GMNQ and md/wd-GMSQ; lanes 7 and 8) or white-tailed deer only (wd-SMSQ; lane 9), human Met/Met (hu-M; lane 3) and human Val/Val (hu-V; lane 4) PrP (amino acid residue 129 is given), and Sg hamster PrP lacking the GPI anchor (lanes 1 and 2; haGPI−). The md/wd GMNQ (lane 7) has recently been shown to be only the predicted translation product of an unexpressed pseudogene in deer (6). All data except those using hamster PrP molecules were previously published (20) and are shown here only to compare interspecies conversion efficiencies. The hamster conversion reactions were done at the same time as the other conversion reactions, using identical PrP-res isolates. The migration of molecular mass standards, in kilodaltons, is shown to the right of each panel.

Immunohistochemical analyses. Brains were excised from PBS-perfused animals and divided sagittally. One-half was fixed in 3.7% phosphate-buffered formaldehyde for 3 to 5 days prior to routine dehydration and paraffin embedded. Sections were cut 4 to 6 μm thick and placed onto charged microscope slides. The remaining half was not fixed and was used for the biochemical analyses and serial passages described above. A Ventana NesES automated stainer was used for immunohistochemical staining of the sections. For PrP analysis, slides were deparaffinized, rehydrated with 0.1 mM citrate buffer, pH 6.0, and pretreated for 20 min at 120°C, using a decloaking chamber (Biocare). Using standard avidin-biotin technique (12), 3F4 was diluted 1:50, followed by biotinylated horse