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No. 26

医薬品 研究報告 調査報告書

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				2007. 9. 20	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		Wroe S, Pal S, Webb T, Alner K, Hewitt P, Brander S, Wadsworth JD, Collinge J. Prion 2007; 2007 Sep 26-28; Edinburgh.	英国

研究報告の概要

○73歳受血者における変異型クロイツフェルト・ヤコブ病の非定型症状
73歳の受血者に生前に特定された変異型クロイツフェルト・ヤコブ病(vCJD)の非典型的症状の報告である。当該患者は、1997年12月の外科手術後に輸血を受けた。血液製剤の追跡により、供血から11ヵ月後に神経病理学的に確定されたvCJDを発現した供血者由来の白血球非除去赤血球製剤一製剤が特定された。この患者は、輸血から9年後に専門家の診察を受けるためNational Prion Clinicへ紹介された。輸血から6年後、受血者は変動する疲労および集中困難を訴えた。この時点での神経学的検査及び脳MRI(T1/T2 weighted/DWI)は正常であった。この6ヵ月後に、平衡失調と認知の低下をともなう進行性症状が発現した。神経学的症状発現から2ヵ月後の検査では、認知障害、失行、視空間障害が示されたが、運動、感覚、歩行検査は正常であった。6週間後、振せん、手指の作業能力障害、四肢の失調を伴う認知障害が認められた。血清学的検査は正常であった。MRI(T1/T2 weighted/FLAIR/DWI)では、vCJDの所見と一致する、視床背側核全体の顕著な信号変化が示された。PRNP遺伝子型検査では、変異はなく、コドン129のメチオニンはホモ接合体であることが判明した。vCJDの長期潜伏期間と無症候性キャリア状態の可能性があることは、重大な公衆衛生問題を提示する。本症例は、汚染血液製剤の受血者が遭遇した重大な危険性と、専門家のモニタリングの必要性を浮き彫りにした。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
輸血によって変異型クロイツフェルト・ヤコブ病(vCJD)に感染したと疑われる73歳の受血者が生前に診断されたvCJDの非典型的症状の報告である。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

39

Epidemiology, Risk Assessment and Transmission

P04.49

Case Report of Variant Creutzfeldt-Jakob Disease in a Macaque after Blood Transfusion

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A fourth human case of probable transmission of vCJD through transfusion has now been reported but a number of features affecting transfusion-related infection remain imprecise, including infectious dose, length of incubation period and critical infectious window of blood donors.

We report here the first case of experimental transmission of vCJD in primates by blood transfusion. Experimental infection of Cynomolgus macaque has been demonstrated to be a sensitive model for the investigation of human prion diseases, inducing similar distribution of infectivity in peripheral lymphoid tissues and equivalent brain pathology. In our study, transfusion was performed with 40 ml of whole blood drawn from a vCJD-infected macaque at the terminal stage of the disease. Clinical symptoms of vCJD appeared in the recipient animal after five years of incubation. The total amount of infectivity in the transfused blood was approximately 106 fold lower than in the brain (titration still in progress). In several animals infected intravenously with brain homogenate, the presence of PrPres in serial lymph nodes biopsies and in other organs at autopsy was examined and results will be presented.

P04.50

Regional and Global Apparent Diffusion Coefficient in Inherited Prion Disease: Correlation with Disease Severity

Wroe, S[1]; Hyare, H[2]; Thornton, JS[2]; Yousry, T[2]; Siddique, D[1]; Webb, T[1]; Collinge, J[1]
[1]National Hospital for Neurology and Neurosurgery, UK; [2]National Hospital for Neurology and Neurosurgery, UK

Cerebral diffusion weighted MR imaging (DWI) has recently emerged as the most sensitive sequence for the diagnosis of prion diseases with reports of apparent diffusion coefficient (ADC) changes in specific anatomical regions. This study examined regional and global changes in cerebral ADC in a large cohort of patients with inherited prion disease, and by correlation with clinical indices investigated their potential as biomarkers of disease progression. Twenty five patients (14 female, mean age 45.2 years, range 32-69 years) with inherited prion disease underwent echo-planar DWI (b1000, TE101ms) and conventional T2W and FLAIR imaging at 1.5T. Mean region-of-interest (ROI) ADCs for the head of caudate, putamen and pulvinar nuclei were determined bilaterally and volume-normalised whole-brain ADC histograms computed following tissue segmentation. Clinical assessment included the Mini Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale (ADAS-COG), Clinician's Dementia Rating (CDR) and the Rating of Global Severity (GS). The Spearman rank correlation coefficient was calculated for each of the ROI mean ADCs, the whole-brain mean and median ADCs, and histogram peak height and peak position, versus clinical score, with $p<0.01$ considered significant. For the whole-brain measures, significant correlations were with MMSE: whole-brain mean ADC vs. MMSE: $r=-0.53$, $p=0.008$, whole-brain ADC histogram peak height vs. MMSE: $r=0.51$, $p=0.01$ and median whole-brain ADC vs. MMSE: $r=-0.52$, $p=0.009$). Of the ROIs investigated, bilateral pulvinar mean ROI ADCs alone correlated significantly with each of the clinical scores, the most significant correlations being between the pulvinar mean ADC and ADAS-COG: right: $r=0.77$, $p<0.001$ (Fig 3) and left: $r=0.56$, $p=0.009$ (Fig 4). No pathological signal changes were detected on conventional MR imaging. Both anatomically specific and whole-brain ADC metrics correlated with disease severity. Conventional MR imaging, including visual assessment of DWI, was relatively insensitive to cerebral pathology in this patient group. Despite this, we have shown that quantification of cerebral ADC provides both regional and global measures that correlate with clinical neurological status and therefore show promise as quantitative pathological biomarkers in inherited prion disease.

P04.51

Atypical Presentation of Variant Creutzfeldt-Jakob Disease in a 73 Year Old Blood Transfusion Recipient

Wroe, S[1]; Pal, S[1]; Webb, T[1]; Alner, K[2]; Hewitt, P[3]; Brander, S[4]; Wadsworth, JD[5]; Collinge, J[1]
[1]National Hospital for Neurology and Neurosurgery, National Prion Clinic, UK; [2]National Hospital for Neurology and Neurosurgery, Department of Neuropsychology, UK; [3]Health Protection Agency, UK; [4]National Hospital for Neurology and Neurosurgery, Department of Neuropathology, UK; [5]Institute of Neurology, UCL, UK

We report atypical presentation of variant Creutzfeldt-Jakob Disease (vCJD) identified ante-mortem in a 73 year-old recipient of blood products. This patient was transfused following orthopaedic surgery in December 1997. Tracing of blood products identified a single unit of non-leucodepleted red cells from an individual who developed neuropathologically confirmed vCJD eleven months after donation. Nine years post transfusion, this individual was referred to the National Prion Clinic for specialist investigation. Six years post transfusion the recipient complained of fluctuating fatigue and impaired concentration. At this time neurological examination and MRI brain (T1/T2 weighted/DWI) were normal. Progressive symptoms emerged six months later with imbalance and deteriorating cognition. Examination two months after onset of neurological symptoms demonstrated cognitive deficits, dyspraxia or visuospatial dysfunction and normal motor, sensory and gait examination. Six weeks later cognitive impairment was identified alongside tremulousness, impaired manual dexterity and limb ataxia. Serological investigations were normal. MRI (T1/T2 weighted/FLAIR/DWI) demonstrated prominent signal change throughout the dorsal thalamus, consistent with vCJD. PRNP genotyping revealed no mutations and homozygosity for methionine at codon 129. The prolonged incubation period of vCJD and possibility of asymptomatic carrier states pose major public health concerns. This case highlights the significant risk encountered by recipients of contaminated blood products and the necessity for their specialist monitoring.

P04.52

Quantification of Brain Atrophy Rates in CJD Using Volumetric MRI

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Brain atrophy has been described in all forms of human prion disease. Most reports are from visual inspection and subjective impression by radiologists who may or may not be blinded to diagnosis. This study applied quantitative measures of the rate of brain atrophy to patients with prion disease. Subjects were patients with any form of human prion disease. A control group of 12 age and sex matched normal subjects was also identified. MRIs were performed at 0, 1, 2, 4 and 6 months from trial enrolment and 3 monthly thereafter. All subjects and controls had T1 weighted volumetric scans acquired at 1.5T. Image processing was performed using MIDAS (Medical Image Display and Analysis Software) with a protocol for whole brain and cerebellar segmentation. Each scan was bias corrected to even out any intensity inhomogeneity and then underwent segmentation, registration and brain boundary shift integral (BSI) calculation from which annual rates of whole brain and cerebellar atrophy were calculated. All image processing was done in a blinded manner. 217 MRI scans were performed in 44 patients. Each patient had between 1 and 19 scans each (mean 4.50, median 3). Seventy nine scans were discarded, mainly due to movement artefact, leaving 138 scans remained in 31 patients. The mean interval between first and last scans in each patient was 1.24 years. One variant CJD, two sporadic CJD and 28 inherited prion disease patients (the majority with 6-OPRI or P102L PRNP mutations) were studied. Control patients were identified from a familial Alzheimer's trial and had two scans each, an average of 1.78 years apart (range1.05-3.15). This study showed that rates of brain atrophy and cerebellar atrophy were significantly higher in symptomatic CJD patients than in control patients. Mean whole brain atrophy rates were 1.3%/year higher than controls (whose mean rate was 0.17%/year). Whole brains did not atrophy at a significantly different rate to cerebelli. Atrophy rates were not significantly different according to presence of symptoms, sex, disease type or inherited disease type in this set of patients. No asymptomatic patients were deemed to have become symptomatic during the study. This is the first description of quantification of rates of brain atrophy in prion disease. Rate of brain atrophy may be a useful prognostic indicator or outcome measure in future clinical trials in human prion disease.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 7. 5	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Solomon A, Richey T, Murphy CL, Weiss DT, Wall JS, Westermark GT, Westermark P. Proc Natl Acad Sci U S A. 2007 Jun 26;104(26):10998-11001. Epub 2007 Jun 19.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○フォアグラのアミロイド生成の可能性

ヒトの脳及び全身性アミロイドーシスならびにプリオンに関連した海綿状脳症は、後天的、遺伝的なタンパク質折りたたみ構造の異常である。正常の場合、可溶性タンパク質やペプチドは線維性凝集体に変換される。これは核形成依存性プロセスであり、アミロイド沈着促進因子(AEF)である同種・異種アミロイド前駆体から形成される繊維の種(seed)によって始まるか、あるいは加速すると考えられ、これらの構造的に変化した成分の経口摂取又は非経口投与により疾患が伝播するという重大な病原性を持つ。感染脳組織以外に、はっきりとしたAEFの食物源は特定されていない。本稿では、二次性(アミロイドAタンパク質;AA)アミロイドーシスを誘起したトランスジェニックマウスモデルにおいて強力なAEF作用を示す血清アミロイドA関連タンパク質からなる複屈折性コンゴレッド親和性線維状物質が、市販のアヒルまたは鴨由来フォアグラに含まれることを報告する。フォアグラから抽出したアミロイドをこのマウスに投与または摂取させると、全身性異常沈着が多数発現した。これらの実験データは、アミロイド含有食品が、感受性集団においてAAアミロイドーシスを促進させるというエビデンスを示している。以上のデータから、二次性アミロイドーシスと(おそらく)その他のアミロイドーシスが伝播性であり、プリオン関連疾患の感染性と類似する可能性があると推測される。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
フォアグラから抽出したアミロイドを二次性アミロイドーシスを誘起したトランスジェニックマウスに投与または摂取させたところ、全身性異常沈着が多数発現し、二次性アミロイドーシスとその他のアミロイドーシスが伝播性であり、プリオン関連疾患の感染性と類似する可能性があると推測されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

Amyloidogenic potential of foie gras

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Communicated by D. Carleton Gajdusek, Institut de Neurobiologie Alfred Fessard, Gif-sur-Yvette, France, January 30, 2007 (received for review October 10, 2006)

The human cerebral and systemic amyloidoses and prion-associated spongiform encephalopathies are acquired or inherited protein folding disorders in which normally soluble proteins or peptides are converted into fibrillar aggregates. This is a nucleation-dependent process that can be initiated or accelerated by fibril seeds formed from homologous or heterologous amyloidogenic precursors that serve as an amyloid enhancing factor (AEF) and has pathogenic significance in that disease may be transmitted by oral ingestion or parenteral administration of these conformationally altered components. Except for infected brain tissue, specific dietary sources of AEF have not been identified. Here we report that commercially available duck- or goose-derived foie gras contains birefringent congophilic fibrillar material composed of serum amyloid A-related protein that acted as a potent AEF in a transgenic murine model of secondary (amyloid A protein) amyloidosis. When such mice were injected with or fed amyloid extracted from foie gras, the animals developed extensive systemic pathological deposits. These experimental data provide evidence that an amyloid-containing food product hastened the development of amyloid protein A amyloidosis in a susceptible population. On this basis, we posit that this and perhaps other forms of amyloidosis may be transmissible, akin to the infectious nature of prion-related illnesses.

amyloid protein A amyloidosis | amyloid-enhancing factor | protein aggregation | rheumatoid arthritis | transmissibility

Amyloid protein A amyloidosis (AA) occurs in patients with rheumatoid arthritis and other chronic inflammatory diseases and results from a sustained elevation of the apolipoprotein serum amyloid A (SAA) protein produced by hepatocytes under regulation by interleukin (IL)-1, IL-6, and tumor necrosis factor (1). This acute-phase reactant is cleaved into an ≈76-residue N-terminal fragment deposited as amyloid predominately in the kidneys, liver, and spleen. The disorder also can be induced experimentally in susceptible strains of mice by inflammatory stimuli that result in an >1,000-fold increase in SAA concentration (2). Further, the lag phase of this process is greatly decreased by injecting or feeding animals extracts of amyloid-laden spleens of affected mice (2–5).

To determine whether amyloid-containing food products exhibit amyloid enhancing factor (AEF) activity, we used a more robust *in vivo* murine model of AA amyloidosis involving mice carrying the human *IL-6* (*hIL-6*) gene under control of either the murine metallothionein-1 (MT-1) (MT-1/hIL-6) or histocompatibility H2-L^d (H2/hIL-6) promoter (6). Typically, AA amyloid develops in these animals at ≈5 mo of age and is initially located predominately in the perifollicular regions of the spleen. Over the next 2–3 mo, the deposits spread rapidly into the liver parenchyma, renal glomerular and intertubular regions, cardiac muscle, tongue, and gastrointestinal tract and lead to death at ≈8–9 mo. However, by injection into 8-wk-old transgenic mice of a single 100-μg i.v. dose of an exogenous source of AA fibrils, amyloid deposits are formed within 3 wk, and severe systemic disease (akin to that found in 8-mo-old animals) occurs within 2 mo, at which time the resultant pathology is lethal (7).

AA amyloid deposits are commonly found in waterfowl, particularly Pekin ducks, in which the liver is predominately involved (8–10). This pathological alteration is noticeably increased in birds subjected to stressful environmental conditions as well as to the forced feeding that is used to produce foie gras (8). This culinary product, derived from massively enlarged fatty livers results from gorging young ducks or geese up to three times daily over a 4-wk period with corn-based feed.

We now report the results of our studies that have shown that AA-containing fibrils extracted from duck or goose foie gras have potent AEF activity when administered by i.v. injection or gavage into our IL-6 transgenic mice.

Results and Discussion

We analyzed several commercial sources of foie gras histochemically and found amyloid to be present. Microscopic examination of hematoxylin/eosin- and Congo red-stained sections cut from formalin-fixed, paraffin-embedded specimens revealed virtual replacement of the normal hepatic parenchyma by fat; additionally, green birefringent congophilic areas in residual vasculature were noted by polarizing microscopy (Fig. 1 *a* and *b*). Further, these deposits were immunostained by a specific anti-AA antiserum (Fig. 1*c*). Similar material was found in marketed pâtés prepared from duck or goose liver (Fig. 2).

The AA composition of the hepatic amyloid deposits was confirmed chemically through analysis of material derived from acetone-defatted specimens extracted first with 0.15 M NaCl and then distilled water. The isolates were strongly congophilic, and, when examined by transmission electron microscopy, contained fibrils with the typical ultrastructural features of amyloid; namely, ≈10-μm-thick unbranched structures (Fig. 3*a*). Electrophoresis of the water-suspended product on a SDS/polyacrylamide gel in the presence of 0.1 M DTT and 8 M urea revealed, after Coomassie blue staining, a protein band with a M_r of ~6,000, comparable to that of amyloid extracted from the spleen of a mouse with AA amyloidosis (Fig. 3*b*). After transfer to a PVDF membrane, this component was subjected to automated Edman degradation with which 14 residues identical in amino acid sequence to that of the N-terminal portion of duck SAA were detected. In a similar study of tryptic digests obtained from cleavage of this molecule after reduction and alkylation, six peptides that included 45 of the first 60 residues of duck SAA were identified by MS/MS (Fig. 3*c*) (9).

Author contributions: A.S., J.S.W., and P.W. designed research; T.R. and C.L.M. performed research; J.S.W., G.T.W., and P.W. analyzed data; A.S. and D.T.W. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Abbreviations: AA, amyloid protein A amyloidosis; AEF, amyloid enhancing factor; IL, interleukin; SAA, serum amyloid A protein.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0700848104/DC1.

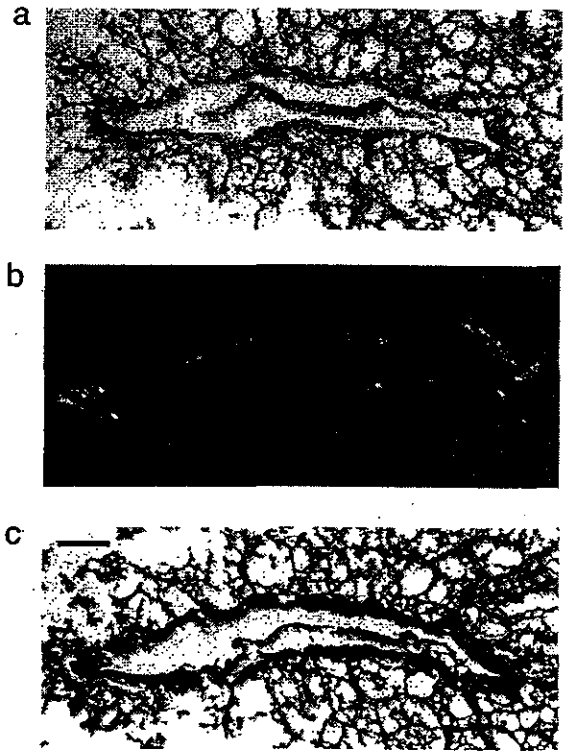

Fig. 1. AA deposition in foie gras. (a) Large venule surrounded by residual, extensively vacuolated fatty hepatic tissue (hematoxylin/eosin stain). (b) Green birefringent amyloid deposits in the blood vessel wall (Congo red stain). (c) Immunohistochemical identification of vascular AA amyloid. (Scale bar, 62 μm.)

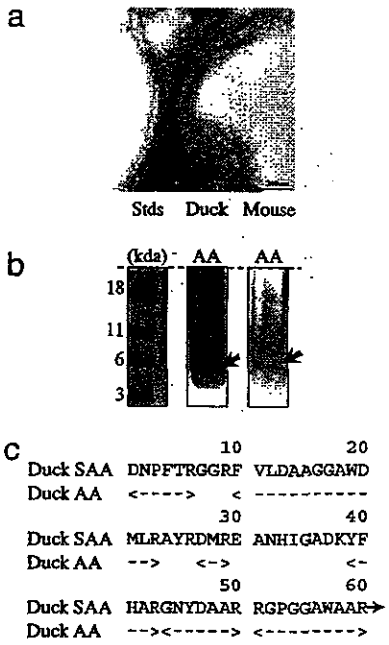

Fig. 3. Ultrastructural and chemical characterization of amyloid extracted from foie gras. (a) Fibrillar nature of proteins contained in the pellet (electron micrograph, negative uranyl acetate stain). (Scale bar, 200 nm.) (b) SDS/PAGE of congophilic components extracted from duck foie gras and the spleen of a mouse with AA amyloidosis (Coomassie blue stain). The M_r values of the standard proteins are given; arrows show the location of AA-containing protein bands. (c) Comparison of the amino acid sequence of duck foie gras AA amyloid with that of duck SAA (9). Homologous residues are indicated by dashes.

To determine whether amyloid-containing duck- or goose-derived foie gras had AEF activity, groups of up to nine MT-1/hIL-6 or H2/hIL-6 mice received tail vein injections of either 100 μg of extract suspended in 0.1 ml of PBS or the equivalent volume of PBS alone. Both sets were euthanized 8 wk later and multiple organs (liver, spleen, kidney, pancreas, heart, lung, tongue, and intestines) were obtained at necropsy for histochemical analysis. Examination by polarizing microscopy of Congo red-stained sections revealed the presence of varying amounts of amyloid deposits in one or more tissues of virtually all of the treated mice; most affected were the liver, spleen, and a lesser extent, the kidneys and pancreas (Fig. 4*a*). In contrast, control animals that received PBS had no detectable amyloid.

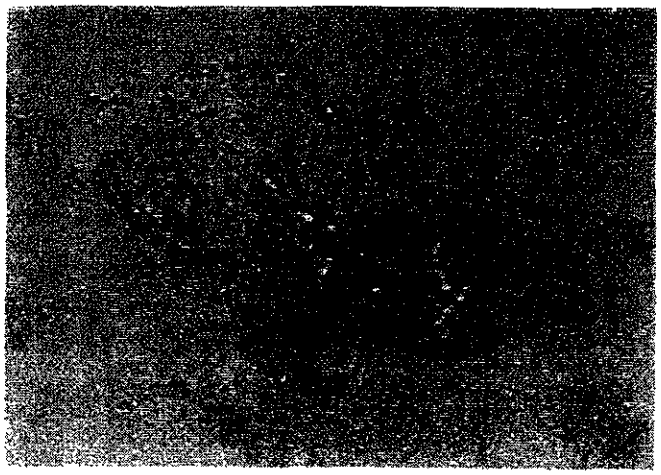

Fig. 2. Tissue fragment with amyloid in duck pâté. Congo red stain.

Similar results were obtained in the conventional murine model of AA amyloidosis in which SAA overexpression was induced by an inflammatory stimulus (4). Two groups of wild-type BALB/c mice were given two 0.5-ml s.c. injections of aqueous 1% $AgNO_3$ (days 1 and 10), and one set also were injected i.v. with 100 μg of the foie gras extract; the others (controls) received PBS only. At the time of euthanizing (day 21), 8 of 10 mice from the first group had detectable amyloid in the liver and spleen. In contrast, no amyloid was found in the control animals.

The amyloid induced by administration of fibril-containing foie gras into both the wild-type and transgenic mice was immunostained by an anti-AA antibody. Further, the deposits were AA in nature as confirmed by MS of protein extracted from the spleen of a recipient animal. MS/MS analyses of tryptic peptides generated from an HPLC-purified reduced and alkylated water pellet identified residues 19–56 of murine SAA.

AA-containing foie gras extracts also had AEF activity when administered orally to the hIL-6 transgenic animals. Five of eight mice that were gavaged for 5 consecutive days with 100 μg of material suspended in 50 μl of PBS were found 8 wk later to have amyloid deposits in virtually all organs examined, and, as in the case of animals injected i.v. with this material, this effect was most pronounced in the liver and spleen (Fig. 4*b*).

The AEF activity of foie gras was reduced, but not abolished, by cooking, as specified by the supplier. Intravenous injections into nine hIL-6 transgenic mice of 100-μg doses of extracts prepared from liver that had been heated to ≈95°C for 20 min in an oven resulted in 4+, 2+, and 1+ hepatic and/or splenic amyloid deposits in two, one, and two animals, respectively (in four cases, no amyloid was found). In contrast, when this material was dissolved in 6 M guanidine HCl, incubated at 37°C for 24 h, dialyzed against PBS, and injected into six transgenic

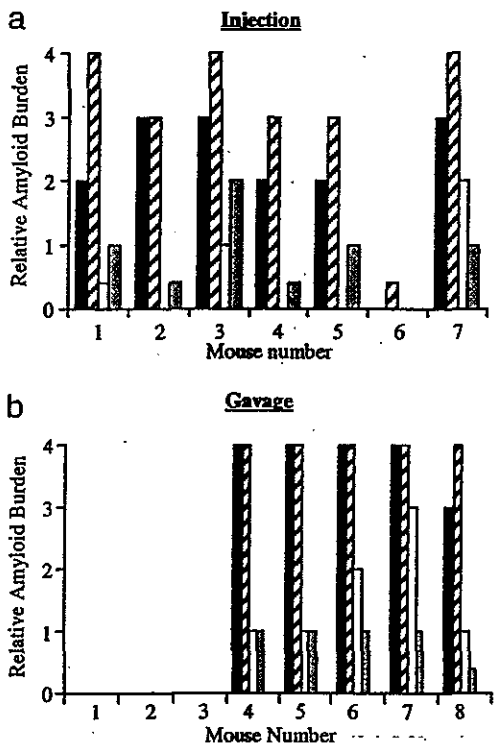

Fig. 4. AEF activity of foie gras. Hepatic and splenic amyloid deposits found in hIL-6 transgenic mice 8 wk after they were injected (*a*) or gavaged (*b*) with AA fibrils extracted from foie gras. The extent of amyloid burden in the liver (■), spleen (▨), kidney (□), and pancreas (▤) is as indicated. (Scale bars, 100 μm.)

mice, only traces of splenic amyloid were found in two mice. A summary of the results of all of the above studies is presented in Table 1 and supporting information (SI) Fig. 5.

The prevalence of AA amyloidosis in the human population is unknown. In most developed countries, sustained inflammatory processes, particularly rheumatoid and juvenile chronic arthritis (as opposed to infectious diseases), now account for the majority of such cases (11). Notably, there is a marked geographic variation in incidence among such patients, e.g., the number is relatively high in Europe compared with the United States but particularly high in parts of Papua New Guinea (12). This difference has been attributed, in part, to genetic factors, including expression of a more amyloidogenic SAA allotype (13) or other genes encoding inflammatory molecules (11). Notably, increased blood levels of SAA do not necessarily result in amyloidosis (11, 14).

Given our experimental findings, exposure to exogenous substances with AEF activity also may be an important epigenetic or environmental factor in the development of AA amyloidosis in a susceptible population. In this regard, it would seem prudent for children and adults with rheumatoid arthritis or other diseases who are at risk for this disorder to avoid foods that may be contaminated with AA fibrils (15). In addition to foie gras, meat derived from sheep (16) and seemingly healthy cattle (17) may represent other dietary sources of this material. Further, the fact that chemically heterologous fibrils can serve as AEF, as demonstrated in experimental models of AA (18–20) and AApoAII amyloidosis (21–23), suggests that it may be hazardous for individuals who are prone to develop other types of amyloid-associated disorders, e.g., Alzheimer's disease or type II diabetes, to consume such products.

Materials and Methods

Materials. Whole fresh duck and goose liver (foie gras) was purchased from three commercial venders located in the United States and France.

Mice. Mice carrying the *hIL-6* gene under control of the mouse MT-1 or histocompatibility H_2-L^d promoter were obtained from Gennaro Ciliberto and Michael Potter, respectively, and generated as described previously (6). At 4 wk the mice were weaned, and the presence of the transgene was confirmed through analysis of genomic DNA derived from tail snips. Wild-type BALB/c mice were purchased from Charles River Laboratories (Boston, MA). Animals were housed in groups of eight in a positive pressure environment with a 12-h light/dark cycle and provided filtered tap water and a standard laboratory rodent chow (Harlan Teklad, Madison, WI) ab libitum. The animals were treated in accordance with National Institutes of Health regulations under the aegis of a protocol approved by the University of Tennessee's Animal Care and Use Committee.

Histochemical and Immunohistochemical Analyses. Samples of foie gras and mouse organs obtained at necropsy were placed in 10% buffered formalin (Fisher Scientific International, Inc., Hampton, NH) and embedded in paraffin. To detect amyloid, 6-μm-thick deparaffinized sections were stained with a freshly prepared solution of alkaline Congo red and examined under polarizing microscopy. A qualitative assessment of amyloid deposition was made by an experienced microscopist (T.R.) based on the relative extent of green birefringence seen in at least 10 fields at ×20 magnification; a value of 1+, 2+, 3+, or 4+ was assigned if such material occupied, respectively, trace, minimal, moderate, or extensive portions of the sections studied, and these values were corroborated by quantitative image analyses. Electron microscopy on Epon-embedded sections stained

Table 1. Summary of the amyloidogenic potential of foie gras preparations

Group	Mice (*n*)	Treatment	Route	Positive (%)	Mean score*
1	H-2/hIL-6 (8)	Extract 1	i.v.	3 (37.5)	3+
2	H-2/hIL-6 (6)	Extract 2	i.v.	2 (33.3)	4+
3	H-2/hIL-6 (7)	Extract 3	i.v.	6 (85.7)	1.9+
4	H-2/hIL-6 (5)	PBS	i.v.	0 (0)	0
5	H-2/hIL-6 (7)	Extract 4	i.v.	7 (100)	3.1+
6	MT-1/hIL-6 (8)	Extract 4	Gavage	5 (62.5)	4+
7	MT-1/hIL-6 (7)	Extract 5	i.v.	5 (71.4)	4+
8	H-2/hIL-6 (9)	Extract 5/cooked	i.v.	5 (55.6)	1.75+
9	H-2/hIL-6 (6)	Extract 5/guanidine HCl	i.v.	2 (33.3)	0.5+
10	BALB/c (10)	Extract 5	i.v.	8 (80)	1.6+
11	BALB/c (5)	PBS	i.v.	0 (0)	0

*Mean score of amyloid-positive spleens in each group.