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Supplementary Data

Pathogen Free Conditions Slow the Onset of Neurodegeneration in a Mouse Model of Nerve Growth Factor Deprivation

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MATERIALS AND METHODS

Transgenic mice

AD11 transgenic mice and control transgenic mice expressing the recombinant antibody against the 4-Hydroxy-3-iodo-5-nitrophenylacetyl hapten were produced as described before [1]. AD11 mice to be housed in murine pathogen free (MPF) conditions were derived from cryopreserved embryos obtained after rederivation, by embryo transfer in a clean foster mother, at Taconic Europe (Lille Skensved, Denmark). The genotype of the rederived MPF mice was confirmed by PCR as described [2].

Mice were kept under a 12 hours dark to light cycle, constant room temperature ($22 \pm 2^{\circ}$C) with food and water *ad libitum*. Experiments were performed according to the national and international laws for laboratory animal welfare and experimentation (EEC council directive 86/609, OJ L 358, 12 December 1987).

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Housing conditions

AD11 and NiP mice were kept in plastic cages under the conventional facility in the European Brain Research Institute (EBRI, Rome, Italy). For MPF conditions, we used the facility at Taconic Farms Inc. (Germantown, NY), where AD11 mice were kept under controlled 12-h light-dark cycle and room temperature, with food and water *at libitum* as for the CV facility. Health reports, performed according to FELASA standards, from both conventional and MPF conditions are summarized in supplementary Table 1.

PCR and detection of recombinant anti-NGF antibody

Identification of transgenic mice was performed by PCR, (to detect the antibody transgenes) and ELISA (to measure the levels of anti-NGF antibodies in serum) according to protocols previously described [2].

Immunohistochemical analysis

Mice were anesthetized with an excess of 2,2,2-tribromethanol (400 mg/kg) and intracardially perfused with a 4% solution of paraformaldehyde in PBS. Brains were processed for immunohistochemical analysis as described before [3, 4]. The following

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Supplementary Table 1
Conventional (CV) and murine pathogen free (MPF) conditions in AD11 mice

	CV	MPF
Viruses		
Ectromelia virus	0/5	0/5
K virus	0/5	0/5
Hantaan virus (Korean Hemorrhagic Fever)	0/5	0/10
Lactic dehydrogenase elevating virus (LDHV)	0/5	0/5
Lymphocytic choriomeningitis virus (LCMV)	0/5	0/7
Minute virus of mice (MVM)	0/5	0/5
Mouse Adenovirus type 1 (FL) (MAV1)	0/5	0/10
Mouse Adenovirus type 2 (K87) (MAV2)	0/5	0/10
Mouse Cytomegalovirus (MCMV)	0/5	0/5
Mouse hepatitis virus (MHV)	0/5	0/5
Mouse parvovirus (MPV)	0/5	0/5
Mouse rotavirus (EDIM)	0/5	0/5
Mouse Thymic virus	0/5	0/5
Murine Norovirus (MNV)	**5/5**	0/7
Pneumonia virus of mice (PVM)	0/5	0/8
Polyoma virus	0/5	0/5
Reovirus type 3 (REO3)	0/5	0/10
Sialodacryoadenitis virus (SDAV)	ND	0/1
Sendai virus	0/5	0/8
Theiler's encephalomyelitis virus (GD7)	0/5	0/8
Toolan's H-1 Parvovirus (TH1)	ND	0/1
Bacteria and Mycoplasm		
Bordetellabronchiseptica	0/5	0/8
Cilia-associated respiratory bacillus (CARB)	0/5	0/8
Citrobacterrodentium	0/5	0/7
Clostridium piliforme (Tyzzer's disease)	0/5	0/8
Corynebacteriumkutscheri	0/5	0/8
Helicobacter spp.	**5/5**	0/9
Klebsiellapneumoniae	0/5	0/8
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Leptospira spp.	0/5	ND
Mycoplasma spp.	0/5	0/8
Pasteurella spp.	0/5	0/8
Pneumocystis carinii	0/5	0/5
Pneumocystis murina	ND	0/5
Proteus spp.	0/5	ND
Pseudomonas aeruginosa	0/5	0/8
Salmonella spp.	0/5	0/8
Staphylococcus aureus	0/5	0/8
Streptobacillusmoniliformis	0/5	0/8
β-hemolytic Streptococci	0/5	0/8
Streptococcus pneumonie	0/5	0/8
Parasites and protozoa		
Aspiculuris sp.	ND	0/8
Eimeria sp.	ND	0/8
Trichomonasmuris	5/5	ND
Entamoebamuris	0/5	0/8
Giardia muris	ND	0/8
Hymenolepis sp.	ND	0/8
Liponyssus sp.	ND	0/8
Myobia sp.	ND	0/8
Myocoptes sp.	ND	0/8
Notoedres sp.	ND	0/8
Polyplax sp.	ND	0/8
Psorergates sp.	ND	0/8
Radfordia sp.	ND	0/8

Supplementary Table 1
(Continued)

	CV	MPF
Rodentolepis sp.	ND	0/8
Spironucleus sp.	ND	0/8
Syphaciamuris	0/5	0/8
Encephalitozooncuniculi	0/5	0/10
Trichomonads	ND	0/8
Trichosomoidescrassicauda	ND	0/1
Klossiellamuris	ND	0/9

primary antibodies were used: goat anti-ChAT (1 : 500, Millipore, Billerica, MA); goat anti-NH2 terminus of Aβ (1 : 100; Santa Cruz, Santa Cruz, CA) and mouse anti-human phospho-tau recognizing Ser199 (1 : 10 clone AT8; Pierce Endogen, Rockford, IL); goat anti-CD45 (1 : 100; Santa Cruz, Santa Cruz, CA).

ELISA assay

ELISA assay against IL-6 and TNF-α was performed according to the manufacturer's instructions (Peprotech, Rocky Hill, NJ).

Statistical analysis

Statistical analyses were performed using the Sigmastat v. 3.11 program (Systat Software, San Jose, CA). The alpha was set at 0.05 and a normality and equal variance test were first performed. Paired *T*-Test or One-way ANOVA was used to compare mice with different genotype and housing conditions. Post-hoc comparisons were carried out using Bonferroni test. Differences were considered significant at $p < 0.05$.

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