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Research report

Decreased behavioral impairments in an Alzheimer mice model by interfering with TNF-alpha metabolism

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ABSTRACT

The performance of mice expressing PDAPP (+/+ or +/−) was studied in the Morris place navigation task. Different lines of questions were investigated using PDAPP+/− mice in which the activity of the cytokine Tumor Necrosing Factor α (TNFα) was attenuated by chronic treatment with anti-TNF or deleting TNFα (TNF−/−). Two different categories of behavior were analyzed in adult (6 months) and middle aged (15 months) subjects. Classically, the cognitive performance was assessed from the escape efficacy and quantitative bias toward the training position in a Morris water maze. Second, stereotyped circling was quantified, along with more qualitative behavioral impairments such as self-mutilation or increased reactivity.

Our results can be summarized as follows. (1) All of the PDAPP mice expressed reduced cognitive performance in the Morris task, but only those with a clear-cut amyloid burden in the hippocampus showed behavioral abnormalities such as stereotyped circling. (2) Chronic treatment with anti-TNF prevented the development of pathological circling in the 6-month-old mice but not in the 15-month-old mice and had no significant effect on amyloid burden. (3) The absence of TNFα prevented the development of stereotyped circling in 6- and 15-month-old mice but increased amyloid burden after 15 months. These data indicate that PDAPP mice express cognitive impairments disregarding absence of TNF. The pathological behavioral anomalies related to the PDAPP mutation seem reduced by treatments interfering with TNFα.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly population, affecting nearly 10% of people over 65 years of age. AD is clinically characterized by a progressive intellectual decline and morphologically by specific neuropathological changes, including the presence of both neurofibrillary tangles (NFT) and β-amyloid deposits in diffuse and organized senile plaques (SP), together with clear signs of chronic inflammation. The well known amyloid hypothesis came from the overall presence of β-amyloid in AD brains as well as from the discovery that mutations on the amyloid precursor protein (APP on chromosome 21) and on the presenilin's genes (PS1 and 2 on chromosomes 14 and 1) led to an overproduction of β-amyloid peptide (Aβ) in early-onset AD families. For review see [20,28,29].

Within this framework, numerous transgenic (TG) mice models were produced by manipulating either APP or presenilin genes or both. In particular, the TG mice PDAPP were largely studied [6,8,14,16]. However, few of these studies take into account the multiple biological and behavioral nature of the pathological signs, i.e., cognitive, behavioral and histological.

Furthermore, prominent inflammatory processes have been observed in the brains of AD patients, although it is not clear if inflammatory pathways would drive or precede the pathological signs, without excluding that inflammation could also induce a beneficial immune response. Evidence supporting a blood–brain barrier dysfunction in AD exists [4] and peripheral cytokines are known to be transported across the blood–brain barrier [19], suggesting a monocyte origin. Among these agents, Tumor Necrosing Factor α (TNFα) is a multipotent cytokine, closely associated with the pathogenesis of infectious, inflammatory and autoimmune diseases. For review, see [26,32].

The possible effects of inflammation on AD lesions were for the most part studied through the genetic manipulation of immune and inflammatory pathways in mouse models of AD (for review, see [33,34]). Behavioral characterization of TNFα models is a way, con-

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sidering behavior in multiple tasks [3,5,13,17,18,21,27]. Therefore, a comprehensive behavioral characterization of transgenic lines manipulating TNFα represents an important avenue for research that will help not only to understand some pathogenic traits on an AD model, but eventually lead to the development of therapeutic agents.

Indeed, due to the central role of brain amyloid processing in the pathogenesis of AD, a variety of Aβ-targeted therapeutic strategies is currently being tested, including anti-inflammatory strategies. Anti-TNF treatment has been recently and successfully used, improving cognitive functions in a small number of Alzheimer patients [12,31].

This line of questioning requires correlative data of behavioral parameters and β-amyloid deposits in TG mice with pharmacological or genetic manipulation of TNF. The present study's aim was to look on behavioral and cognitive impairments in different groups of transgenic mice with an overproduction of β-amyloid peptide (PDAPP+/+ or +/−), into the effects of TNFα modulation (chronic treatment with anti TNFα serum) or of TNFα deletion in combined PDAPP+/− TNFα deficient mice. Place discrimination in a Morris water maze was taken as the reference assessment of cognitive learning abilities. The description of several behavioral abnormalities included a quantification of stereotyped locomotion in the cage or during swimming in the water maze. The behavioral assessment was related to β-amyloid deposits quantified from post mortem histological analyses.

2. Materials and methods

2.1. Subjects

A total of 136 mice (90 males and 46 females) sourced from isogenic groups were used in the study. Table 1 indicates the number and age of different groups tested in the water maze and perfused post mortem for histological analyses. Twenty-three wild mice were used for some comparison.

2.1.1. PDAPP+/+ (n = 26)

Adult *PDAPP+/+* mice were sent directly to our laboratory from the Institute Eli Lilly & Co. Ltd., Indianapolis [10,15]. These mice carry a double copy of the *APP V717L* gene which results in overproduction of Aβ and produces brain's Aβ deposits.

2.1.2. PDAPP+/− (n = 60)

All of the *PDAPP+/−* (line 1683) mice came primarily from the Institute Eli Lilly & Co. Ltd., Indianapolis. They carry the mutated APP V717L gene that results in overproduction of Aβ and produces brain's Aβ deposits. They were provided to the Hellenic Pasteur Institute, Greece [15] and further bred to receive anti-TNF treatment. From birth onwards and for the next 12 or 30 weeks, all of the mice received weekly intraperitoneal injections of a chimeric hamster/mouse monoclonal antibody to murine TNFα bearing the variable regions of the hamster TN3.19.12 antibody (30 μg/g of body weight in saline; provided by Roly Foulkes, Celltech, Berkshire, U.K. to the Hellenic Pasteur Institute). Half of the littermates received an equivalent concentration of PBS.

2.1.3. PDAPP+/−TNF−/− (n = 27)

PDAPP+/− mice from Eli Lilly (see above) were crossed with *TNF−/−* mice knockout for the cytokine TNFα in the Hellenic Pasteur Institute, Greece [25].

2.1.4. Reference control mice (n = 23)

DBAxC57xSwiss Webster mice were maintained in similar conditions and trained in the same design to provide a control baseline.

Table 1
Number and age of different groups tested.

	Total		Water maze		Histology	
Age in months	**6**	**15**	**6**	**15**	**9–12**	**16–20**
PDAPP$^{+/+}$	**26**	**–**	26	–	26	–
PDAPP+/− PBS	**18**	**16**	18	16	12	14
PDAPP+/− anti-TNF	**15**	**11**	15	11	13	11
PDAPP+/−TNF−/−	**16**	**11**	16	11	–	10
Wild	**23**	**–**	23	–	7	–
Total	**98**	**38**	98	38	58	35

2.2. Apparatus

A circular pool (diameter 120 cm and height 30 cm) made of grey PVC was used in a dimly illuminated room. The pool was filled to a depth of 15 cm with water (27 °C) opacified with milk (0.5 l). Four orthogonal starting positions were situated around the perimeter of the pool, dividing its surface into four quadrants. A platform of transparent Plexiglas cylinder (15 cm tall and 8 cm diameter) covered with a white aluminum perforated plate (14 cm diameter) was placed in the centre of one quadrant, approximately 0.5 cm under the water level and served as an escape platform. The pool was located in a room containing numerous extra-maze cues. A camera was fixed on the ceiling, above the water maze.

2.3. Procedures and analyses

The mice' behavior was analyzed in detail to detect behavioral abnormalities and non-cognitive deficits. The cognitive performance was assessed in a classical place learning task (Morris navigation task).

2.3.1. Water maze procedure

The subjects received four daily trials over five days. The hidden platform remained at a fixed spatial location for the entire acquisition period and each subject was assigned a different escape sector. Each trial started with the mice being placed into the water, at one of four starting points: N, E, S, W. Four different starting positions were pseudo-randomly used in each training block. A trial ended when the mouse reached the hidden platform and managed to remain there for 20 s. If a successful escape did not take place within 60 s, the mouse was guided to the platform and the trial was recorded as an escape failure with an arbitrary latency of 60 s. The tested mouse was left for a 30 s inter-trial interval in a dry container. At the end of the 20 training trials, each mouse received a probe trial. For this trial, the platform was removed from the pool and the mouse was allowed to swim for 60 s. During training trials, the time to reach and escape on the platform was measured (escape latency in seconds). For the probe trial, the time spent in the different quadrants was measured with help of a Noldus 2.1 videotracker and the time spent in the training sector was computed as percent of the total time.

2.3.2. Recording and statistical analyses

The video camera suspended directly above the apparatus was connected to the computer and used to record the behavior of the mice. Experiments took place during the light phase between 7:00 a.m. and 12:00 a.m. In general, analyses of variance (ANOVA, StatView 4.5) were performed on the raw data with the genotype as the between-subject factor and repeated measures such as day, session, area, as within-subject factors. For the ANOVAs, we used the method of "missing data" proposed by Snedecor and Cochran [30] for the imputation of missing values. The results of ANOVAs were confirmed by unpaired *t*-test and other paired comparisons (post hoc test, Fisher PLSD, $p < .05$) whenever necessary. Spearman's rank correlations were also computed.

2.3.3. Abnormal behavior analyses

Stereotypic behavior is defined as repeated and invariant movements without any obvious goal or function [22,23]. Stereotyped circling was observed in the home cage (*stereotyped walking*) and also during training and probe trials in the water maze (*stereotyped swimming*). The stereotyped circling was assessed from the number of consecutive loops. Once initiated, circling was pursued in the same direction, but a given mouse was able to start circling in one sense or the other. A criterion for pathological stereotypy was reached when a sequence of more than 4 complete loops was observed, thus the scores were necessarily above 4. From this stage onward, all additional loops were noted during a 60-s period (time duration of the probe trial). These loops appeared very characteristic of PDAPP mice and were not observed in normal subjects; they were observed during walking or during swimming, in the absence of noticeable motor dysfunction.

Behavioral disorders are also an important part of the clinical symptoms of Alzheimer disease beside cognitive deficits. The signs observed in PDAPP mice were assessed together with cognitive impairments, in order to better replicate clinical assessment in human pathology. It was thus be possible to evaluate to what extent they were influenced by pharmacological or genetic treatments.

At different stages of what can be qualified as an illness in the genetically engineered mice, the following disorders were observed and noted:

- Inability to feed, sometimes leading to death.
- Inability to remain still (even in their cage). Their movement was characterized by running in incessant circles.
- Repetitive aggressiveness towards other mice, to the extent that it might cause their death, meaning they had to be kept in separate cages.
- Hyper-reactivity in response to manipulation, manifested by sudden accelerations and impressive jumps. The hyper-reactivity turns the mice into real "batteries". Nothing in the general context could justify such sudden and violent reactions. This hyper-reactivity may be linked to difficulties in regulating and expressing emotions. Finally the emotional lability observed during behavioral tests corresponded with what has been described in humans.