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Neurodegenerative diseases

ROLE OF IL-6 TRANS-SIGNALING IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Juan Hidalgo Pareja
Facultat de Biociències UAB

1. Summary of the project

1. Alzheimer's disease (AD) is one of the most common neurodegenerative diseases in older humans. The main characteristics of this disease are the extracellular presence of amyloid plaques and the intracellular presence of neurofibrillary tangles. Amyloid plaques and amyloid angiopathy result from aggregates of beta-amyloid peptides (Aβ) formed from the amyloid precursor protein (APP) in the parenchyma of the brain and the perivascular regions respectively. All this leads to a prominent neuroinflammation, which could possibly strengthen the disease. Interleukin-6 (IL-6) is one of the cytokines affected by AD, and it has been seen that it exerts many of its effects through trans-signaling.

Our hypothesis postulates that a specific inhibitor of trans-signaling, the human protein sgp 130-Fc, would have a significant impact on the progression of AD.

The objective of our project is to characterize AD progression in a widely used animal model, the Tg2576 mouse, when simultaneously coproducing the trans-signaling specific inhibitor, the sgp 130-Fc protein, specifically produced in astrocytes. This bigenic mouse (Tg2576 / GFAP-sgp130) will be compared to its littermates, Tg2576, GFAP-sgp130 and WT animals.

In addition to the objectives of the original project, and at the request of the reviewers, we included the study of another AD mouse model, triple transgenic 3xTg-AD, using a strategy similar to that of the monogenic (Tg2576).

2. Animals

Two AD mouse strains are used, Tg2576 (or APPSwe, carriers of the Swedish mutation K670N / M671L, from Taconic Europe A / S (Ry, Denmark)), and 3xTgAD, from LaFerla's laboratory (via L. Giménez-Llort, from the UAB)); 3xTgAD is a triple transgenic mouse carrier of APPSwe, PS1M146V and tauP301L. Also, GFAP-sgp130 mice were provided by Dr. S. Rose-John (Christian-Albrechts-University of Kiel, Germany). These mice will be crossed to achieve homogeneous genetic background. As it has been described that Tg2576 animals exhibit a very high mortality it will be necessary to establish groups with a high number of animals. It is important to make it clear that there will be successive crossings of crossings since they cannot be done in

parallel. After weaning (3 weeks of age) animals are separated by sex and identified by PCR by taking a small piece of tail to get the DNA. In addition, during the life of the animal weight and mortality are controlled, and blood glucose levels are measured at the time of weaning.

Behavioral analysis

The behavioral characterization of the model is performed using a battery of tests at two different ages, before and after the appearance of amyloid plaques. In the Tg2576 the plaques appear at about 12 months of age, so the tests will be performed over 5-7 months and at 13-14 months. Before performing the tests, mice are handled a few days for their acclimatization to the room. The battery of tests includes: Open-field (locomotion), Hole-Board (exploration), Elevated-plus Maze (anxiety) and Morris Water Maze (space memory). The study of 3xTgAD will be similar to that of the monogenic mouse model of AD.

Neuropathological analysis

Both AD models were intended to be euthanized at 14-15 months of age (but see below), and if possible (depending on the success of the crosses and mortality), a group of animals would also be euthanized at 5-6 months. The brains are extracted and the right hemisphere is dissected in the cortex, hippocampus and remaining brain tissue, while the left hemisphere is submerged in 4% paraformaldehyde (PFA) for 24h, then washed in PBS 1x, and conserved in 70% ethanol until its inclusion in paraffin. These techniques will be used:

- Western Blot, to measure APP, Aβ and other proteolytic fragments of the precursor protein.
- Enzyme-linked immunosorbent assay (ELISA) to determine the amount of $A\beta_{1-40}$ and $A\beta_{1-42}$.
- Immunohistochemistry to determine the amyloid plaque load, glial responses around them and Tau phosphorylation.

3. First year: obtaining the colonies of mice (Tg2576, 3xTgAD and GFAP-sgp130) and establishing the first crossings.

Second year: animal genotyping, monitoring of the different colonies (mortality and weight control etc.). First battery of behavioral tests at 5-7 months and, if all goes well, also the second battery at 13-14 months of some mice.

Third year: Last behavioral tests in the last sets of mice. Animal euthanasia was intended at approx. 14 months of age for neuropathological analysis, but we have modified the euthanasia times of the 3xTgAD strain at >20 months of age because the development of the phenotype (Aβ production) was much slower than anticipated. In the Tg2576 model, we also decided to extend euthanasia time to approx. 18 months to consolidate the phenotype.

2. Results obtained

Control of body weight, glycemia and mortality:

Both in Tg2576 and 3xTg-AD (at more advanced ages) models there is a significant weight loss. This effect is not reversed with the inhibition of the trans-signaling of IL-6 with sgp130.

Glycemia has been measured at the time of weaning (3 weeks). Tg2576 mice have a lower blood glucose level than control animals. This effect is not reversed with the inhibition of the trans-signaling of IL-6. In the 3xTg-AD model there are no significant differences in glucose levels.

As expected, mortality was prominent in the Tg2576 animals. In this case, however, the inhibition of the trans-signaling of IL-6 significantly reversed the mortality in both sexes. 3xTg-AD animals show an increase in mortality relative to controls only at advanced ages (approximately 20 months), and much lower compared to that of the Tg2576 model. The inhibition of trans-signaling of IL-6 has a sex-dependent effect, harmful in males and beneficial in females.

Behavioral analysis:

Whenever possible we carried out behavioral analyses before and after the appearance of amyloid plaques.

Before amyloid plaques

The preliminary results suggested studying animals at 5-6 and 9-11 months of age in the Tg2576 and 3xTg-AD models respectively. The following order was followed: Open-field, Hole-Board (scan), NORT (memory), Elevated-plus Maze (anxiety) and Morris Water Maze (space memory).

In the Tg2576 model the effects are sex-dependent, being more prominent in females. The Tg2576 females have more locomotive and exploratory activity than controls, and less anxiety. The inhibition of the trans-signaling of IL-6 with sgp130 reverses the effect on exploration and anxiety. In the case of males, we also see that the Tg2576 animals are less anxious than the respective controls, but we do not see an effect of blocking the trans-signaling of IL-6. There are deficits of spatial memory in Tg2576 animals, but the inhibition of the trans-signaling of IL-6 has no effect on this parameter. The results with the NORT were not conclusive.

In the 3xTg-AD model the effects on behavior are not as marked as in the Tg2576 model, probably due to the slow progression of the disease. However, we observe that 3xTg-AD animals explore more than controls, and in the case of females, they have less anxiety. No effect of the inhibition of IL-6 trans-signaling was observed in these parameters, but there is a small effect on males, decreasing anxiety regardless of the presence of AD transgenes. Regarding spatial memory, we observe that 3xTg-AD animals have a higher swimming speed than the respective controls. On the other hand, in females, the inhibition of the trans-signaling of IL-6 increases the latency for arriving to the platform; thus suggesting a detriment to spatial learning. The results with the NORT were not conclusive.

After amyloid plaques

The previous results suggested studying animals at 15-16 months of age in the Tg2576 model, and discarding further behavioral studies in the 3xTg-AD model given the older ages to be considered, the small phenotype in heterozygous animals, and the time available.

The Tg2576 mice have more locomotive activity than controls. This effect is reversed in males with inhibition of the trans-signaling of IL-6, but not in females, where in fact

there is a small increase independently of the Tg2576 genotype. They also tend to show more exploratory activity, although this effect is significant only in females, although in this case we do not observe the effect of the inhibition of the trans-signaling of IL-6. Anxiety is reduced in Tg2576 animals compared to controls, and there is no effect of the inhibition of trans-signaling. Finally, the Tg2576 animals have deficits in memory and learning that are not affected by the inhibition of the trans-signaling of IL-6.

Neuropathological analysis:

These are carried out using cortex and hippocampus samples of euthanized mice at different ages in order to monitor the progression of the disease. The amyloid cascade (APP, Aβ and other fragments from the proteolysis of the precursor protein) is analyzed using Western Blot, the amount of $A\beta_{40}$ and $A\beta_{42}$ using ELISA, as well as neuroinflammation and amyloid plaque load by immunohistochemistry.

Before amyloid plaques

In the Tg2576 model at 10-11 months of age we detect the presence of precursor APP by WB, and $A\beta_{40}$ using ELISA. No clear effect was found with inhibition of IL-6 trans-signaling. In the 3xTg-AD model at 5-6 and 10-11 months of age animals present a precursor APP protein, but the Aβ levels are too low to be detected with the assays employed. We observed an increase in precursor APP in animals aged 10-11 months compared to mice at 5-6 months. We do not observe an effect of the inhibition of trans-signaling of IL-6.

After amyloid plaques

In the Tg2576 model at 16-17 months of age, ELISA results indicate that there is no effect of the inhibition of trans-signaling of IL-6 in the amount of $A\beta_{40}$ and $A\beta_{42}$. Plaque measurement by immunohistochemistry shows a decrease in amyloid plaques load by the inhibition of trans-signaling of IL-6. The Tg2576 females also have a higher glial response measured by immunohistochemistry, but in this case we do not observe the effect of the inhibition of trans-signaling of IL-6. We are in the process of transcriptome analysis (by microarrays) in the hippocampus.

In the 3xTg-AD model at 20-28 months of age, we observe that the pathology progresses more slowly than we expected. The amount of $A\beta_{40}$ and $A\beta_{42}$ measured by

ELISA is very low compared to the Tg2576 model. Additionally, amyloid plaques are not observed for immunohistochemistry, or inflammation associated with them. The inhibition of the trans-signaling of IL-6 has no effect on these parameters. The results for tau immunohistochemistry revealed very faint staining that precludes meaningful results.

Effect of a high-fat diet-induced obesity in the 3xTg-AD mouse model

As the 3xTg-AD animals had a delayed phenotype, it was decided to look at the effects of obesity induced in this model. A group of animals of both sexes were exposed to a diet rich in fat for 10 weeks; several physiological variable parameters were measured before and after the diet. The variables analyzed were:

- Body weight and food intake: 3xTg-AD animals present a diminished weight compared to controls. In addition, they present an increased food intake, suggesting a hypermetabolic phenotype. The inhibition of trans-signaling of IL-6 accentuates this phenotype in females.

- Response to fasting: After a fast, 3xTg-AD animals lose weight faster than the controls. Once the 3xTg-AD mice feed again they present hyperphagia, leading to a faster recovery of the previously lost weight. The blockage of the trans-signaling of IL-6 inhibits the recovery of the weight, especially in males.

- Temperature: 3xTg-AD animals have a higher body temperature than their respective controls. This effect is more marked in males than in females, and is not affected by the blockage of the trans-signaling of IL-6.

- Sensitivity to insulin and glucose tolerance (by means of an ITT and OGTT): 3xTg-AD animals present an insulin hypersensitivity, which is only reversed in males due to the inhibition of IL-6 trans-signaling after being fed with a fat-rich diet. Before the fat diet we observe that 3xTg-AD animals have more tolerance to glucose, decreased in females GFAP-sgp130. Once exposed fed with a fat-rich diet, we observe that the 3xTg-AD females diminish the tolerance observed previously.

- Hormones and metabolites in serum: 3xTg-AD animals showed a decrease in insulin, leptin and cholesterol blood levels. In females the inhibition of the trans-signaling of IL-6 diminishes leptin levels even more.

- Hypothalamic neuropeptides: We see an increased expression of IL-6 in 3xTg-AD males, an effect that is rescued by the inhibition of the trans-signaling of IL-6.

3. Relevance and future possible implications

Collectively, the results of this project provide evidence that the trans-signaling of IL-6 plays a role in the mouse model brain of Alzheimer's disease (AD). The administration of the soluble form of the co-receptor gp130 (sgp130), which is already used in other inflammatory pathologies, could be a therapeutic strategy in humans with AD, and women would potentially be the most sensitive group. The results also suggest that treatment should take place in the early stages of the disease.

4. Publications

Manso Y, Comes G, López-Ramos JC, Belfiore M, Molinero A, Giralt M, Carrasco J, Adlard PA, Bush AI, Delgado-García JM, Hidalgo J. Overexpression of metallothionein-1 modulates the phenotype of the Tg2576 mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* **51** (2016) 81-95.

Comes G, Manso Y, Escrig A, Fernandez-Gayol O, Sanchis P, Molinero A, Giralt M, Carrasco J, Hidalgo J. Influence of transgenic metallothionein-1 on gliosis, CA1 neuronal loss and brain metal levels of the Tg2576 mouse model of Alzheimer disease. Int J Mol Sci. 18 (2017) 251.

Fernández-Gayol O, Sanchis P, Aguilar K, Navarro A, Comes G, Molinero A, Giralt M, Hidalgo J. Different responses to a high-fat diet in IL-6 conditional knock-out mice driven by constitutive GFAP-Cre and Synapsin 1-Cre expression. Neuroendocrinology (2019) doi: 10.1159/000496845

Publications under review:

Escrig A, Molinero A, Méndez B, Sanchis P, Fernández-Gayol O, Montilla A, Comes G, Giralt M, Giménez-Llort L, Becker-Pauly C, Rose-John S, Hidalgo J. IL-6 trans-signaling in the brain influences the behavioral and physio-pathological phenotype of the 3xTgAD mouse model of Alzheimer's disease. Brain Behav. Immun.

Publications in preparation:

Escrig et al., IL-6 trans-signaling in the brain influences the behavioral and physio-pathological phenotype of the Tg2576 mouse model of Alzheimer's disease.