Peripheral immune system and neuroimmune communication impairment in a mouse model of Alzheimer´s Disease

Lydia Giménez-Llort[1], Ianire Maté[2] Rashed Manassra[2], Carmen Vida[2] and Mónica De la Fuente[2]

[1]Dept. of Psychiatry and Forensic Medicine, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[2]Dept. of Physiology (Animal Physiology II), Faculty of Biology, Complutense University of Madrid, 28040 Madrid, Spain

Corresponding author: Mónica De la Fuente. Department of Physiology (Animal Physiology II), Faculty of Biology, Complutense University of Madrid, 28040 Madrid, Spain. Phone: 34913944989. Fax: 34913944935
E-mail: mondelaf@bio.ucm.es

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Abstract

Neurodegenerative diseases such as Alzheimer´s disease (AD) can be understood in the context of the aging of neuroimmune communication. Although the contribution to AD of the immune cells present in the brain is accepted, the role of the peripheral immune system is less well known. The present review examines the behavior, and the function and redox state of peripheral immune cells in a triple-transgenic mouse model (3×Tg-AD). These animals develop both beta-amyloid plaques and neurofibrillary tangles with a temporal- and regional-specific profile that closely mimics their development in the human AD brain. We have observed age and gender-related changes in several aspects of behavior and immune cell functions, which demonstrate premature aging. Lifestyle strategies such as physical exercise and environmental enrichment can improve these

aspects. We propose to analyze the function and redox state of peripheral immune cells as a useful tool for measuring the progression of AD.

Neuroimmunomodulation in aging

Currently it is recognized that the three regulatory systems, namely the nervous, endocrine and immune systems are intimately linked and interdependent. Thus, it is accepted that a "neuro-immune-endocrine" system allows the preservation of homeostasis and therefore of health[1,2] (Figure 1). The communication between these regulatory systems is mediated by cytokines, hormones and neurotransmitters through the presence of their receptors on the cells of the three systems. Moreover, mediators of the three systems coexist in lymphoid, neural and endocrine tissues. These facts show the complexity of the regulation not only at general levels, but also at local levels. This neuro-endocrine-immune communication allows the understanding of why depression, anxiety and emotional stress are accompanied by a greater vulnerability to infections, cancers and autoimmune diseases.[3-5]

In addition, the aging process, which may be defined as a progressive and general impairment of the functions of an organism that leads to a lower ability to adaptively react to changes and preserve homeostasis, affects all the physiological systems but especially the regulatory systems and the communication between them (Figure 1). This difficulty in preserving the homeostasis is the basis of the increase of age-related morbidity and mortality. [2,6,7] With aging the nervous system suffers a progressive loss of its function, which can be shown, for example, in aspects such as sensation, cognition, memory and motor activity. The hippocampus shows an age-related decrease of neurogenesis, which explains the learning and cognitive impairment in aged subjects. [8] In the endocrine system there are also several changes, which accompany healthy aging. These include, for example, the decrease of several hormones such as the growth hormone and sexual hormones. [9] Moreover, the age-related disturbances of the hypothalamic-pituitary-adrenal (HPA) axis are responsible for decreasing the stress adaptability in old subjects, this being, at least in part, the cause of their health impairment.[10] With respect to the immune system, it is presently accepted that its components undergo striking age-associated re-structuring, leading to changes that may include enhanced as well as diminished functions. This is

termed immunosenescence,[2,11,12] appearing with age in both the periphery and in the central nervous system (CNS).

The age-related changes in the communication among the homeostatic systems were proposed as the main cause for physiological senescence.[13] Although more than 300 theories have been proposed to explain the aging process,[14] the free-radical theory proposed by Harman[15] is now probably the most widely accepted to explain how this process occurs. This theory proposes that aging is the consequence of the accumulation of damage by deleterious oxidation in biomolecules caused by the high reactivity of the free radicals produced in cells as a result of the necessary use of oxygen. Thus, the age-related changes in the organism are linked to a chronic oxidative and inflammatory stress (a progressive imbalance between endogenous antioxidant/anti-inflammatory and oxidant/pro-inflammatory compounds, with higher levels of the first) affecting all cells and especially those of the regulatory systems, which explains their impaired function.[2,6,7,12] Moreover, oxidation and inflammation are the basis of immunosenescence. In fact, although immune cells need to produce oxidant and inflammatory compounds in order to perform their defensive functions, if these are produced in relatively high concentrations they cause damage to these cells.[2,16] Since the function capacity of the immune system has been considered the best marker of health,[17] the preservation of this functional and redox state is relevant to the maintenance of a healthy condition and successful aging.[2] Moreover, we have proposed the theory of oxidation-inflammation in aging, with an "oxi-inflamm-aging" situation, in which the age-related impairment of the immune system could affect the functions of the other regulatory systems through increased oxidative and inflammatory stress, resulting in the alteration of homeostasis and an increase in morbidity and mortality.[2,6,7,11]

In addition, aging is a very heterogeneous process and thus there are different rates of age-related physiological changes in each system or tissue of the organism and in the diverse members of a population of the same chronological age. This fact justified the introduction of the concept of "biological age", which determines the rate of aging experienced by each individual having a better predictive value for longevity than chronological age.[2,18] The biological age is related to the mean longevity (the mean of the age to which the members of a population that have been born on the same date live), the subjects of a population with a higher rate of aging showing an older biological age and a shorter lifespan. If the maximum longevity (the maximum time that a subject belonging to

a determined species can live) is fixed in each species, the mean lifespan of individual organisms shows marked variability and can be improved by environmental factors. In order to determine the "biological age" the parameters that change with age and show the tendency to a premature death should be determined.[19] Since a positive relation has been shown between a good function of immune cells and longevity,[2,11,17,20] we have proposed several immune function parameters as adequate markers of "biological age" and therefore as predictors of longevity.[2] Moreover, the redox situation and inflammatory state of the immune cells are related to their functional capacity and the lifespan of a subject. Thus, the immune system state allows us to know the rate of aging of the organism and can modify this rate.[2]

Neurodegenerative diseases in the context of the age-related changes in nervous and immune communication

We can understand age-related neurodegenerative diseases in the context of the nervous and immune system communication. Since the mediators of immune cells can affect the nervous system at peripheral and central levels, it is necessary to consider both influences (Figure 1). It is currently accepted that the immune cells present in the CNS have neuroprotective mechanisms, but also, if they show uncontrolled responses, which is frequent in aging, they can be the cause of neurological disorders[21,22] increasing an oxidation and inflammation situation.[8,15] Thus, the age-related progressive accumulation of immune cells in brain areas such as the hippocampus, which are from systemic sources, might have a great impact on the progressive cognitive impairment that occurs with aging[26] and they were proposed as contributing to neuropathologies.[24] Also, age-related peripheral chronic inflammation can influence cells in the CNS, leading to neuroinflammation and neuro-oxidation, which play a key role in the behavioral and cognitive deficits associated with aging and age-related diseases such as Alzheimer´s disease (AD).[25] Thus, immunosenescence is important because the inflammatory signals that are initiated in the periphery can be propagated through CNS immune cells.[26] Interestingly, both humans and mice that achieve longevity in healthy conditions have been shown to preserve the functions and a low peripheral chronic inflammatory and oxidative status of the immune cells.[2,11,27]

Characteristics of Alzheimer ´s Disease (AD)

AD, the most common neurodegenerative disorder and cause of senile dementia, is a progressive age-related disease whose prevalence in the elderly is dramatically increasing in parallel to that of life expectancy and social aging. AD is characterized by deficits in memory, spatial vision, language and executive function. While cognitive deficits have traditionally been emphasized in defining AD, there are a variety of neurobehavioral symptoms that are also commonly associated with the disease, including increased apathy, agitation, anxiety, and other psychiatric symptoms, such as delusions or hallucinations.[28] The main histopathological hallmarks of AD are extracellular amyloid plaques and neurofibrillary tangles (NFTs). The AD brain is further characterized by massive neuronal cell density and synapse number loss that appear to precede overt neuronal degeneration.[29] The most severe neuropathological changes occur in the hippocampus, followed by cortical and subcortical structures, including the amygdala.[30]

The component of the plaques is a 40-42 amino acid polypeptide beta-amyloid (Aβ, $A\beta_{40}$ and $A\beta_{42}$) that is derived from the larger amyloid precursor protein (APP) by proteolytic cleavage[31] accomplished through the action of β-secretase and γ-secretase. This γ-secretase activity depends on a proteolytic complex[32,33] and dictates the length of Aβ, $A\beta_{42}$ being the more neurotoxic because of its propensity to readily aggregate into oligomers and fibrils.[34] The second histopathological hallmark of AD is the appearance of intraneuronal aggregates composed of highly phosphorylated protein tau.[35] Under physiological conditions, tau stabilizes the microtubules, but under pathological conditions, its basal phosphorilated ratio increases,[36] and tau dissociates from microtubules forming intraneuronal aggregates, which lead to neuronal dysfunction and synapses loss.[37] Although the amyloid hypothesis of AD has focused majority of studies in this research area, it has been proposed that APP intracellular domain (AICD) levels, which are elevated in brain from AD patients and cause hyperphosphorylation and aggregation of tau protein, can contribute to this pathology independently of Aβ.[38]

Besides these hallmark lesions, other reactive processes occur such as inflammation[39] and oxidative stress.[40] A large body of evidence supports the hypothesis of a direct contribution of the inflammatory response to amyloid plaque progression as well as hyperphosphorylation of tau protein and, thus, to the neurodegeneration associated with AD. Moreover, Aβ itself has been shown to act as a pro-inflammatory agent[41] and many inflammatory mediators, such us cytokines, which are up-regulated by Aβ, can serve to increase tau pathology.[42] Reactive oxygen species (ROS) are also produced as a result of

this inflammatory response,[43] which damage the cells and may further exacerbate this inflammation state.

It is currently accepted there is an associated immunological response in AD, but it is still unclear whether this is beneficial or harmful. In fact, several authors have proposed that in AD the key pathogenic phenomena consist in the long-term maladaptive activation of innate immunity.[44,45] Many components of this immunity, which are expressed throughout the brain, are disregulated in AD and they may act as a double-edged sword, with either beneficial or detrimental effects. Thus, the disturbed balance between complement activators and complement regulatory proteins seems to mediate neuronal lysis in AD.[46] Monocytes recruited into brain shown ineffective Aβ phagocytosis in AD patients.[47] With respect to microglial cells (brain macrophages), which are found upregulated in the AD brain, although they attempt to phagocytose Aβ plaques and secret anti-inflammatory cytokines, they fail at restricting Aβ plaque formation, and their over-activation results in the secretion of proinflammatory cytokines, chemokines and ROS, as well as hyperphosphorylations of tau protein, all of which exacerbate the pathology.[40,44,48] In addition, it has been suggested Aβ is an antimicrobial peptide that may normally function in the innate immune system.[49] Moreover, the presence in amyloid plaques and tangles of many immune-related proteins in addition to viral proteins has suggested plaques and tangles represent cemeteries for a battle between virus and the host´s defence network.[50]

Murine models of Alzheimer´s Disease

During the last two decades it has been a challenge to model behavioral and neuronal symptoms of AD.[51] Advances in gene transfer techniques and the identification of the genes implicated in the autosomal dominant familiar AD, have made possible the development of many experimental models, particularly in mice. These target the major aspects of the neuropathological characteristics of AD, such as Aβ plaques and NFTs. In this regard, several mutations in the genes of human APP, presenilin-1 (PS1), presenilin-2 (PS2) and protein tau have been reported to produce AD-like pathology. Nevertheless, a valid animal model for AD should exhibit progressive AD-like neuropathology and cognitive deficits that closely mimic human disease progression, and should be verified in different laboratories.[52] Although several transgenic animal models of AD based on the expression of mutant familiar AD transgenes have been developed,[34] the triple-transgenic

mouse model (3×Tg-AD, harbouring APP_{Swe} and tau_{P301L} transgenes on a mutant $PS1_{M146V}$ knock-in background) represents an unique model that develops both Aβ plaques and NFTs with a temporal- and regional-specific profile that closely mimics their development in the human AD brain.[53,54] Thus, since LaFerla´s laboratory created the triple-transgenic 3xTg-AD mice in 2003[53] this animal model has been the object of an important number of publications based on the singularity mentioned above. Moreover, this model manifests other hallmarks of the disease such as the characteristic reactive gliosis inflammatory profile, cholinergic deficits, synaptic dysfunction, deficits in learning and memory and the behavioral and psychological symptoms of dementia (BPSD) in an age-dependent manner.[51,53,55-57] This mouse model has been used extensively to dissect pathogenic mechanisms and for therapeutic gene approaches,[42] and is continually evolving, which should lead to a better transfer of mouse discovered therapies into the clinic.[34]

Age-related changes of the behavioral parameters in 3 xTg AD mice

At the behavioral level, the 3xTg-AD model provided evidence that Aβ peptide caused the onset of early Alzheimer's disease-related cognitive deficits in learning and memory.[58] Thus, the earliest cognitive impairment manifests itself in these animals at 4 months as a deficit in long-term retention correlated with the accumulation of Aβ in the hippocampus and amygdala, which worsens with aging and the advancement of the neuropathological stages.[51] In addition to these limbic-dependent spatial learning and memory deficits observed in the Morris Water Maze, the 3xTg-AD has also shown deficiencies in working memory,[59] deficits in emotional learning using passive avoidance[60] and we have also provided evidence of the influence of emotional behavior in fear conditioning learning tasks.[61] Moreover, the 3xTg-AD mice can also be considered a valuable animal model for AD because of its gender- and age-dependent course of severity of non-cognitive disturbances resembling BPSD or neuropsychiatric symptoms.[62] These BPSD-like symptoms such as increased anxiety-related behavior with reduction of exploratory activity in 3xTg-AD mice appear prior to cognitive deficits, as early as 2.5 months of age and are indicative of increased responsiveness to stressful situations.[51,62] Progressively, in correlation with the appearance of intraneuronal Aβ immunoreactivity and further Aβ and tau pathologies, the 3xTg-AD mice exhibit increased neophobia, freezing behavior, reduced exploratory efficiency and other behavioral variables indicative of reduced coping with stress strategies.[51,60,62-65] In addition, there is a lack of regulation of behavior (i.e.,

impulsivity and desinhibition) and deficits in sensorimotor gating (in startle response and prepulse inhibition) which progressively worsens with the neuropathological affects of limbic areas[61] (Table I).

The immune system in 3xTg-AD mice. An early marker of the disease

The impairment in the neuroimmunendocrine network that occurs with aging is accelerated and more pronounced in the 3xTg-AD mice.[65,66] With respect to the peripheral immune system the organometrics of immune organs are relevant to the understanding of the physical variation and changes due to disease. Thus, the weight of thymus has been established as an indirect indicator of immunological functional state. In 3xTg-AD mice we have reported that both total weight and relative weight of peripheral immunoendocrine organs such as thymus, spleen and adrenal glands correlate with the gender-dependent impairment of the neuroimmunoendocrine network described at both initial[67] and advanced[65] stages of the AD neuropathology.

In relation to the function parameters in the immune cells we have analyzed several proposed as biological age markers, such as chemotaxis, proliferation in response to mitogens (ConA and LPS), antitumoral NK activity and IL-2 secretion.[2,6,7,12] We have observed an age-related decrease in these functions and that subjects with lower values of these parameters show an older biological age and earlier death than those with the same chronological age that have higher values.[2,7,12] Moreover, individuals reaching a high longevity (human centenarians and long-lived mice) show these functions with values similar to those in healthy adults.[2,11,27] In 3xTg-AD we have studied these parameters in immune cells from peritoneum, spleen and thymus, and the changes in comparison to NTg animals. These changes were similar in cells from all these locations (Table IIA).[65,68] As can be seen in 3xTg-AD mice there is a decrease in these functions with respect to the NTg animals in adult-young and adult mice (4 and 9 months of age, respectively). In young mice (2.5 months of age) the differences have not appeared yet, and in old animals (15 months of age) the 3xTg-AD mice show an increase in chemotaxis and IL-2 secretion.[65,68,69,70] With respect to the oxidative stress parameters analyzed (Table IIB) both adult-young and adult transgenic mice show a decrease in spleen antioxidant defenses such as total glutathione (TG) levels and activity of GPx and GR antioxidant enzymes as well as an increase in xantin oxidase (XO) activity (an enzyme that produces oxidant compounds) with respect to those in NTg animals.[71,72] These changes in antioxidants and oxidant compounds are characteristics of prematurely and chronologically aged subjects.[2,12,16,73]

Thus, there is a premature immunosenescence in the 3xTg-AD mice, which has as a base an oxidative stress situation. These facts confirm the premature aging of transgenic mice and explain their early mortality. Moreover, the analysis of the peripheral immune functions seems to be a good marker of the progression of AD. All this confirms the idea of the early involvement of the immunity in the pathogenesis of AD.[45]

Strategies to improve the behavior and the immune system in 3xTg-AD mice

We have proposed several lifestyle strategies such as the performance of physical exercise and environmental enrichment to improve the redox state, immune function and behavioral response in aging mice and in several animal models proposed as premature aging models.[2,7,12] These strategies retard the aging process, improving homeostasis and increasing the longevity of the individuals.[7,12] Thus, these strategies could also exert beneficial effects on 3xTg-AD mice, which show high oxidative stress, impaired peripheral immune cell functions and shorter longevity.[64-66]

Physical exercise in 3xTg-AD mice

It is well known that physical exercise is an effective mean of preventing or delaying chronic diseases. Together with the muscle and cardiovascular systems, physical activity strongly modulates the regulatory systems, showing beneficial effects in the behavioral response and the immune functions. Moreover, physical exercise represents a physiological stress model in which highlight the relevance of interactions between the regulatory systems.[12,74]

In 3xTg-AD mice we have studied the effects of forced and voluntary exercise in both male and female 3xTg-AD mice and as compared to non-transgenic mice. Thus, an exhaustive treadmill exercise administered at a moderate stage of AD neurodegeneration (7 months of age) partially protected 3xTg-AD mice both in brain and peripheral organ functions, whereas voluntary physical exercise in a freely available running wheel ameliorated many of the 3xTg-AD pathological behaviors, brain oxidative stress changes, as well as the immune functions.[64,67] Thus, the exercise exerted its benefits in 3xTg-AD mice in addition to the improvement of physical condition *per se,* already observed in non-transgenic healthy animals studied in parallel.[64]

Environmental enrichment

Environmental enrichment (EE) is a good experimental model for the maintenance of a life, which is socially, mentally and physically active. The most common EE protocol in

rodents is grouped housing using large cages with a variety of objects, which are changed frequently. This more complex and stimulating habitat, as opposed to the regular monotonous housing, induces sensory, cognitive, motor and social stimulation.[75] The EE reverses many of the adverse effects of the aging process on behavior, immune function and oxidative stress, extending the life-span of animals.[76,77] It is also known that EE reduces brain pathology and improves cognition and behavioral responses in a variety of murine models for age-related neurodegenerative diseases such as AD.[78] We have shown that EE can benefit several immune functions in old male 3xTg-AD mice. [66]

Gender differences in behavior and the immune system in 3xTg-AD mice and in the response of these animals to physical exercise and environmental enrichment

There are gender differences in the changes of the neuroimmunoendocrine network with aging and it is known that females live longer than males in a great range of animal species.[79] In general, females show stronger immune responses than males.[80,81] Although the prevalence of AD is higher in females, the higher vulnerability of the neuroimmunoendocrine network in males could result in a higher susceptibility to the deleterious effects of aging and be responsible for the increased morbidity and mortality observed in 3xTg-AD male mice.[65,66,67] This is possibly a consequence of the regulatory role of sex hormones promoting AD pathogenesis.[59] There is a gender-dependent behavioral phenotype, which is exacerbated at early and moderate stages of the disease but losses relevance with the advancement of the neuropathology.[51,53,55,60,62,65] In general, at moderate pathological stages of the disease male 3xTgAD mice show more homeostasis redox derangement that females, while females show greater brain AD pathology compared to males.[64] There are gender-related differences in functions and redox state of immune cells from 3xTg-AD mice, which are in some cases different to those found in NTg mice and they depend on the age of animals (Table III). The 3xTg-AD male mice show worse immune functions and antioxidant levels than females, especially adult and old animals. [65,70] Thus, transgenic males mice show a premature immunosenescence and aging with respect to females, starting at adult age.

There were gender-differences in the effects of the above mentioned strategies, both exercise[64,67] and EE,[66] on the behavior and immune functions of 3xTg-AD, with males showing better responses than females.

Conclusions and proposals

Our results in 3xTg-AD mice show the premature peripheral immunosenescence of these animals and seem to confirm the involvement of systemic immunity and inflammation in behavioral and cognitive deficits,[26] such as those in AD,[82] as well as in the acceleration of the progression of the disease.[83] For this, we propose the study of the peripheral immune system as a marker of the course of AD as well as of the effects of preventive and/or therapeutic interventions. Moreover, interaction effects between age, gender, genotype, and treatment should be always taken into consideration when assessing the outcome of those interventions.

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Table 1. Temporal course of behavioral changes observed in males and females of the Spanish colony of 3xTg-AD versus NTg mice.

	Stages of neurodegeneration			
Behavior parameters	*Onset (2.5 m)*	*Early stages (4 m)*	*Moderate stages (6 m)*	*Advanced stages (12 m or more)*
Increased sensorimotor function	n.s.	n.s.	+	++
BPSD-like symptoms				
Emotionality	+	+	++	+++
Neofobia	n.s.	+.	n.s.	+++
Reduced exploration in ansiogenic places	+	++	++	+++
Anxiety-like behaviors	+	++	++	+++
Hyperactivity	n.s.	+	+	++
Desinhibition	n.s.	n.a.	++	n.s.
Impulsivity	n.s.	n.a.	+	+++
Reduced novelty seeking	n.s.	n.a	n.s.	n.a.
Dysfunction of startle response	n.a.	n.a.	+	n.a.
Dysfunction of prepulse inhibition	n.a.	n.a.	+	n.a.
Cognition				
Spatial Working memory deficits	n.s.	n.a.	+	+++
Spatial Short-term memory deficits	n.s.	n.a.	n.s.	+++
Spatial Long-term memory deficits	n.s.	+	++	+++
Instrumental conditioning deficits	n.a.	n.a.	++	n.a.
Alteration Circadian rhythms	n.a.	n.a.	+	+++

Genotype effects: n.s. non-significant differences. n.a. not assessed in our colony of mice. +, ++, +++ : higher levels in 3xTg-AD than NTg mice ($p<0,05$, $p<0,01$ and $p<0,001$, respectively). The appearance of AD pathology at different ages: 2.5 months (onset of AD; no neuropathological manifestation); 4 months (early stages; intracellular Aβ immunoreactivity); 6 months (moderate stages; extracellular Aβ deposits but still no tau alterations); 15 months (advanced stages; Aβ deposits in many cortical regions and tau hyperphosphorilation). References: 31, 61, 64, 65, 67.

Table IIA. Several function parameters in immune cells from young to old 3xTg-AD versus NTg female mice.

Parameters (function)	3xTg-AD vs. NTg			
	Age			
	2.5 months	*4 months*	*9 months*	*15 months*
Chemotaxis	=	↓↓↓	↓↓↓	↑↑↑
Proliferation	=	↓↓	↓↓	=
NK	=	↓	=(↓)	=
IL-2 secretion	n.a.	↓	=(↓)	↑

The appearance of AD pathology at different ages: 2.5 months (onset of AD; no neuropathological manifestation); 4 months (early stages; intracellular Aβ immunoreactivity); 9 months (moderate stages; extracellular Aβ deposits but still no tau alterations); 15 months (advanced stages; Aβ deposits in many cortical regions and tau hyperphosphorilation).

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Table IIB. Oxidative stress parameters (antioxidants and oxidants) in spleen of young and adult 3xTg-AD versus NTg mice.

Parameters	3xTg-AD vs. NTg
Antioxidant	
TG	↓↓↓
GPx	↓↓
GR	↓
Oxidant	
XO	↑↑

TG: Total Glutathione; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; and XO: Xantine Oxidase.

↓,↓↓ and ↓↓↓: lower levels and activities in 3xTg-AD than NTg mice ($p<0,05$, $p<0,01$ and $p<0,001$, respectively); ↑, ↑↑ and ↑↑↑: higher levels in 3xTg-AD than NTg mice ($p<0,05$, $p<0,01$ and $p<0,001$, respectively); "=", similar levels and activities in 3xTg-AD and NTg mice; "=(↓)", decreased tendency without significant differences in 3xTg-AD with respect to NTg mice; "n.a." not assessed in our colony of mice. References: 65, 66, 68, 69, 70, 71, 72.

Table III. Gender differences in several parameters of function and redox state in immune cells from young, adult and old, NTg and 3xTg-AD mice.

	Female vs. Male					
	4 months		*9 months*		*15 months*	
Parameters	NTg	3xTg-AD	NTg	3xTg-AD	NTg	3xTg-AD
Function						
Chemotaxis	$<^{*}$	$>^{***}$	$>^{***}$	$>^{*}$	>	$>^{***}$
Proliferation						
Basal	$<^{***}$	=	$>^{*}$	$>^{**}$	=	=
ConA	$<^{***}$	$<^{***}$	$<^{***}$	=	=	>
LPS	$<^{***}$	$<^{***}$	$<^{***}$	$>^{*}$	=	$>^{**}$
NK	$<^{**}$	$<^{**}$	$>^{**}$	$>^{**}$	=	=
Antioxidant						
TG	$>^{**}$	=	$>^{*}$	$>^{**}$	$>^{*}$	$>^{*}$
GPx	=	=	=	=	n.a.	n.a.
GR	=	=	=	=	n.a.	n.a.
Oxidant						
XO	=	=	=	<	n.a.	n.a.

TG: Total Glutathione; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; and XO: Xantine Oxidase. "<, $<^{*}$, $<^{**}$ and $<^{***}$", lower levels and activities in female versus male NTg and 3xTg-AD mice (no significance (n.s.), p<0,05, p<0,01 and p<0,001, respectively); ">, $>^{*}$, $>^{**}$ and $>^{***}$", higher levels and activities in female versus male NTg and 3xTg-AD mice (n.s., p<0,05, p<0,01 and p<0,001 respectively); "=", similar levels and activities in female and male NTg and 3xTg-AD; "n.a." not assessed in our colony of mice. References: 65, 66, 69.

LEGEND

Figure 1. With age, all regulatory systems (the nervous and immune system) involved in homeostasis and therefore of health, as well as the communication between them, show an impairment due to an increase of inflammatory and oxidative stress. This age-related loss of homeostasis is more exacerbated in Alzheimer´s Disease (AD), and supports the hypothesis of a premature immunosenscence as a relevant factor of AD. Moreover, the analysis of immune function could be a marker of the progression of AD. Several strategies, such as physical exercise and environmental enrichment, seem to reestablish

the homeostatic systems. Aβ plaques: Beta amyloid plaques; NFTs: Neurofibrillary tangles; (+) positive effect; (-) negative effect.