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Behavioral changes following disease progression in the TNFtg mouse model of rheumatoid arthritis

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Alterations in motor activity following disease progression in the TNFtg mouse model of rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic auto-inflammatory condition that affects multiple joints in the body, causing synovial thickening, joint swelling and significant bone erosion. The inflammatory effects of the disease are known to cause significant pain, disability and depression in many patients. Mouse models of RA, including a transgenic mouse model that over-expresses the inflammatory cytokine, TNF-α (i.e. the TNFtg mouse strain), have been used to explore the pathogenesis of RA. However, the role that TNF-α over-expression plays on behavior is understood poorly. The purpose of the present study was to quantify the progression of disease in TNFtg mice using two behavioral assays: the rotorod, and locomotor activity. The rotorod has been shown to be an effective method for the analysis of motor coordination, while locomotor activity has been an effective tool in assessing spontaneous ambulatory behavior. These data were compared to another study in which mechanical sensitivity was assessed.

On both procedures, mice were tested from the age of 6 weeks to 18 weeks, in two-week increments. The data gathered showed a significant decrease in the time spent on the rotorod by the TNFtg mice compared to their wild type controls, and these effects emerged following 12 weeks of age. On locomotor activity, TNFtg mice had a significant decrease in ambulatory behavior compared to their wild type controls with significant effects also emerging after 12 weeks. The observations in this study provide further evidence that TNF-α plays a major role in RA and establishes a method of quantifying the progression of the disease.

Keywords: Rheumatoid arthritis, inflammation, TNF-α, mechanical sensitivity

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that is known to affect multiple tissues (skin, heart, blood vessels, lungs) but primarily destroys the joints and causes an inflammatory synovitis. The prevalence of this debilitating condition is approximately 1%, as it is primarily affects women more than men. Clinical manifestations of RA are known to peak in the second to fourth decades of life (1).

Many different factors contribute to the pathogenesis of RA such as environmental insults, genetic predisposition (HLA susceptibility), and contributory co-morbid conditions. However, the pathologic inflammatory process in RA is primarily initiated and mediated by CD4+ T-cells (1). Essentially, a combination of genetic susceptibility and environmental insults leads to a strong immune response to self-antigens. The immune response then causes the proliferation of fibroblasts, chondrocytes, synovial cells, which leads to the release of elastase, PGE2, collagenases, and other enzymes (1). This induces the formation of a pannus, which is an abnormal layer of fibrovascular tissue (hypertrophied synovial membrane) between the bones of a joint. The formation of the pannus between the joints and other tissues, leads to significant destruction of bones, cartilage, and can cause ankylosis (1). Although many cytokines play a role in the pathogenesis of RA, the cytokines, IFN-γ, IL-17, IL-1, TNF (Tumor necrosis factor), and RANKL are known to be contributory to the pathogenesis of the disease. However, TNF has been identified as being the most central to the pathogenesis of RA and has been studied intensively to produce disease-alleviating therapies (1).

The clinical course of RA is known to begin with fatigue, generalized musculoskeletal pain, and then proceeds to affect the joints. Symptoms typically affect the hands (metacarpophalangeal and proximal interphalangeal joints). The affected joints are typically warm, painful, stiff, and have a decreased range of motion from overnight inactivity. The end result of unresolved RA is a joint with no stability, or range of motion (1). To further explore the role of RA on human behavior, it is appropriate to initially analyze the effect of the disease on animal models.

Though several animal models have shown to manifest several limitations, the rodent model has been shown to be consistent and has led to significant advances in understanding the complicated nature of RA (6). Despite the emergence of mouse models of RA, over the years, the TNF-α transgenic mouse model of inflammatory arthritis has been employed in this study. This transgenic mouse model (developed in 1991 by Kollias and his co-workers) utilized TNF-α in mice to cause a chronic progressive nature (debilitating, erosive polyarthritis) that's similar to the human disease. In his experiment, Kollias instituted a treatment with a monoclonal antibody against TNF-α, which completely prevented the disease (6). This finding led to the formation of biologic agents that are used today to manage RA.

Though there is an abundance of literature about the pathogenesis and immunology of RA and TNF- α; there appears to be a limited amount of evidence discussing the role that TNF-α over-expression plays on behavior. The purpose of the present study is to quantify

the progression of disease in TNFtg mice using two behavioral assays: the rotorod and locomotor activity. In addition, a clinical scoring guide will be used to correlate the physical progression of disease, with the behavioral progression of the disease.

Materials and methods

This study involved working with 17 female C57BL/6 mice that were divided into 3 test groups. The first group consisted of five female wild type C57BL/6 mice. The second group consisted of five female TNF transgenic (TNFtg) C57BL/6 mice. The third group consisted of four female TNFtg mice (Table 1). Since the mice had different date of births, they were separated and identified by ear piercings (L-left ear pierced, R- right ear pierced, RR- right ear pierced twice, RL- right and left ear pierced).

TNFtg mice of the 3647 strain (carrying a single copy transgene) were kept at the hemizygous state by backcrossing to C57BL/6 mice, and identified by PCR for the human TNF transgene. Mice were group housed in standard plexiglass microventilator cages in a colony room maintained on a 12-h light–dark cycle (lights on at 7:00 AM). All mice had continuous access to food and water throughout the study. For behavioral studies, mice were habituated to the colony room environment for 2 weeks prior to any experimental manipulation. Mice were also exposed to the testing environment and handled for 2 days prior to initiation of an experiment. All testing procedures were conducted between 11:00 AM and 3:00 PM. Animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of Rowan University and all testing adhered to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, National Academy of Sciences, Washington, D.C., USA, 2011).

Cage	Mouse ID	Date of Birth	Sex	ID
Group I	C57BL/6	4/10/2015	F	L
	C57BL/6	4/10/2015	F	RL
	C57BL/6	4/10/2015	F	RR
	C57BL/6	4/12/2015	F	R
	C57BL/6	4/12/2015	F	RL
Group II	TNFtg (+)	4/15/2015	F	R
	TNFtg (+)	4/15/2015	F	L
	TNFtg (+)	4/12/2015	F	L
	TNFtg (+)	4/10/2015	F	N/A
	TNFtg (+)	4/10/2015	F	R
Group III	TNFtg (+)	6/18/2015	F	R
	TNFtg (+)	6/01/2015	F	R
	TNFtg (+)	6/01/2015	F	L
	TNFtg (+)	6/01/2015	F	RL
Group IV	TNFtg (-)	5/17/2015	F	RL
	TNFtg (-)	5/17/2015	F	RR
	TNFtg (-)	5/17/2015	F	RR_L

Table 1. Mice Group data set

Behavioral Assays

The rotorod is an apparatus that consists of a horizontal rod that rotates around its long axis. The apparatus is designed in such a way that it is able to increase its speed from 4-40 revolutions per minute (RPM). Essentially, the objective of the mice is to try to stay on the rotating apparatus, as the horizontal rod increases its speed. The rotorod behavioral assay has been shown to be an effective tool in analyzing the motor coordination of mice (2).

Locomotor activity is an assay that consists of a square chamber [27.3 cm L x 27.3 cm W x 20.3 cm H] (with perforated holes for ventilation & removable lid) and mechanical sensors that record the distance traveled by the mice (cm). Studies have showed that the locomotor activity assay has shown to be an effective method of assessing spontaneous behavior in mice and neuromuscular disorders (3,4).

Clinical Scoring system

The classical clinical disease scoring is carried out by direct observation of inflammatory swelling and joint deformities in hind and forepaws by trained handlers according to a set of defined parameters, on a scale of 0-4 for each paw (0-no evidence of inflammation; 1-minor swelling/redness of carpal/tarsal regions; 2-extensive swelling, limited digit involvement, no deformities; 3-extensive swelling, possible tarsal subluxation, multiple digits swelling, limited deformities; 4-major diffuse swelling, tarsal subluxation, multiple obvious digit deformities), with each mouse therefore scored on a 0-16 scale. The clinical disease scoring was implemented in the study to correlate the results of the behavioral assays, with the results of the physical progression of the disease in the mice.

Methods

From the age of 6 weeks to18 weeks, the mice were tested in two-week increments (6 weeks, 8 weeks, 10 weeks, etc.). Each test session involved testing the mice with the rotorod, locomotor activity, and clinical scoring assays. For the rotorod assay, the mice were put on the rotorod apparatus and the apparatus was set to increase its speed from 4-40RPM's within a 300 second period. With increasing speed, the mice must increase their gait speed to prevent falling off the rod. The time (seconds) that the mice spent on the rotorod before falling off was recorded for each mouse. For the locomotor assay, the mice were put in the square shaped container for 30 minutes. The distance traveled (centimeters) was measured for the mice. After the assays have been performed, experienced immunologists (Igor Kuzin & Andrea Bottaro) performed the clinical disease scoring on the C57BL/6 mice.

Statistics

The data gathered for the three groups of mice were separated into two main data groups (wild-type & TNFtg). Using ANOVA analysis, the locomotor activity, rotorod, and clinical scores were analyzed.

Results

The rotorod assay (Figure 1) showed that at the age of 6 weeks, both groups (WT & TNFtg) spent a similar amount of time on the rotorod (Wild type mice: 298+/- 0.95 SEM/ Transgenic mice: 273+/- 12 SEM). As the mice increased in age, the graph (Fig. 1) clearly showed an increasing separation (time spent on the rotorod) between the wild type and TNFtg groups, especially at the age of 12 weeks.

The locomotor assay study (Figure 2) showed that at the age of 6 weeks, both groups had a similar amount of distance covered in the chamber (within a 30-minute period). At the age of 6 weeks, the wild type group traveled a distance of 3125 cm (+/- 490 SEM), while the TNFtg group traveled a distance of 3359 cm (+/- 600 SEM). But as both groups of mice grew older, there was an increased difference in the distance covered by the wild type and TNFtg groups. Both groups traveled similar distances until the age of 12 weeks, in which the wild type mice traveled an average distance of 3421 cm and the transgenic mice traveled an average distance of 1996 cm. Significant differences in the distance traveled were noticed after 12 weeks of age (difference of 1425 cm between both groups), similar to the results of the rotorod assay.

The clinical disease scoring showed a complete difference between the wild type and TNFtg mice groups. The graph shows that the wild type group had a mean clinical score that was closer to zero (Figure 3). Whereas, the TNFtg group had clinical scores that rose as the mice increased in age; peaking to an average score of 13 (not sure if this is the exact value on the graph) during the age of 18 weeks.

The observational clinical scores and locomotor activity measurements were closely correlated ($-0.84<r<-0.6$ for sequential scores in individual TNFtg mice, and cumulative $r=-0.61$, $p<0.001$, for the entire dataset) independently validating the two assessment methods.

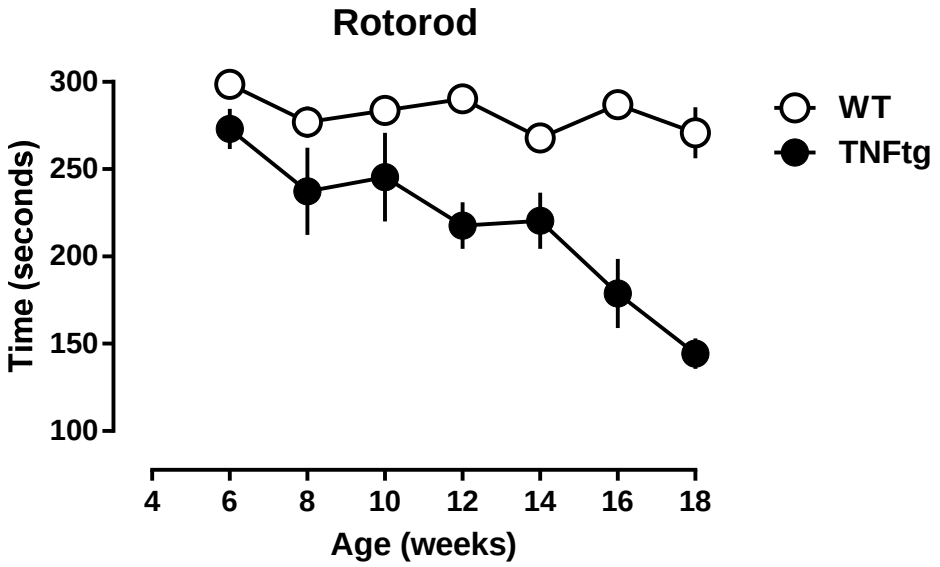

Figure 1. Comparison of TNFtg & wild-type (WT) mice rotorod activity.

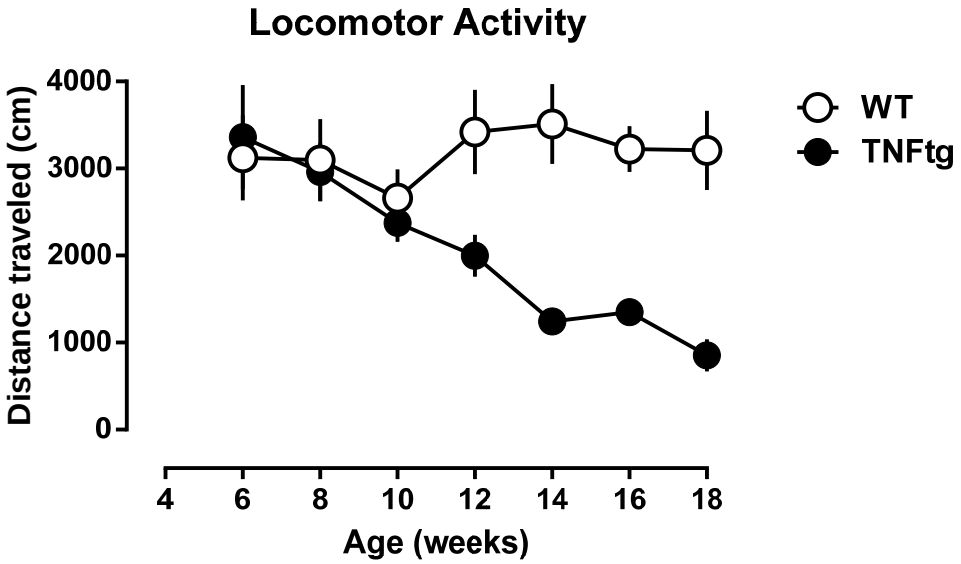

Figure 2. Comparison of TNFtg & wild-type (WT) mice locomotor activity.

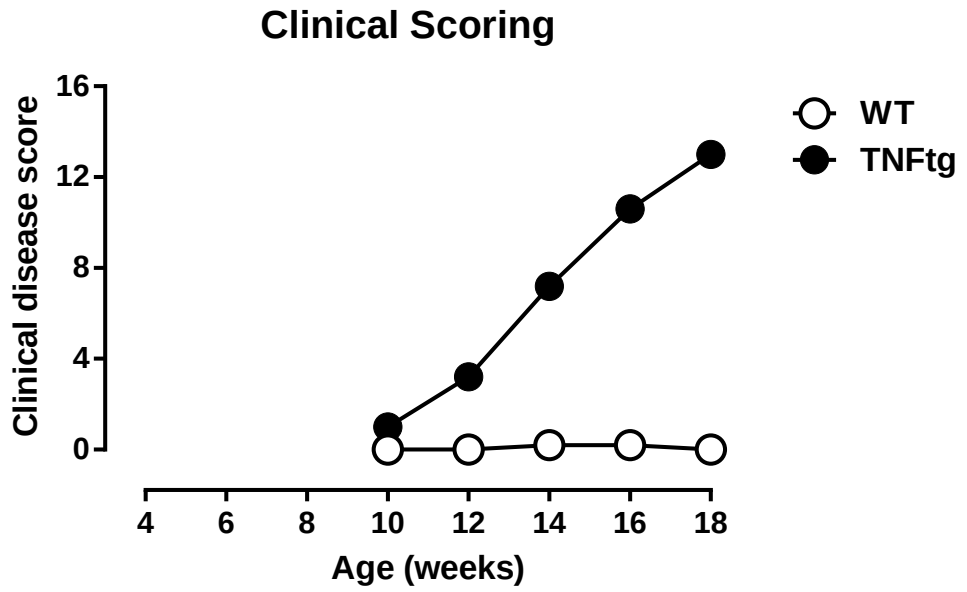

Figure 3. RA clinical scoring comparison of TNFtg & wild-type (WT) mice.

Discussion

In the present study, we aim to look at the disease progression in the TNF-α mouse model of RA, and attempt to quantify the behavioral progression of disease (in TNF-α mice), using behavioral assays. The results from the behavioral assays clearly show that TNF-α over-expression plays a role in the pathogenesis of RA. More so, the results showed that TNF-α has an effect on the behavior of mice. With a decrease in the distance traveled in TNFtg mice, as evidenced by the locomotor activity results (Fig. 2); it is suggested that there is a decrease in spontaneous behavior in the TNFtg mice, as they age. Though the rotorod data showed similar data as the locomotor activity, only the locomotor activity data was closely correlated with the clinical scoring system. As previously mentioned, significant differences in the distance traveled between the groups (wild-type & TNFtg) were noticed after 12 weeks of age (Fig. 2). Correlating those results to the clinical scoring graph (Fig. 3), there is a clear separation between the clinical scores of both groups (wild-type & TNFtg) at the age of 12 weeks. The results from the study seem to suggest that at the age of 12 weeks, signs and symptoms of RA seem to further progress and become evident in the behavioral and mechanical assays.

Though the literature has not attempted to quantify the behavioral progression of RA in mice, there is some literature exploring the relationship between behavior change and TNF-α. Results from a study by Kaster et al, determined that TNF-α produces a depressive like state in mice, and further supports the notion that inflammation may play a role in the pathophysiology of depression (8). Also, the study suggested that the

administration of a TNF-α inhibitor was able to produce an anti-depressive like response in mice performing the forced swimming test (8). Another study by Süß et al, reinforced the idea that chronic peripheral inflammatory disorders mediated by cytokines such as TNF-α, are associated with psychiatric disorders like depression and anxiety. The study showed that though severe peripheral inflammation limited locomotor activity, it didn't have an effect on eliciting depressive-like symptoms (9).

The literature has unfortunately not shown enough studies that have attempted to quantify the progression of RA in mice. Hence, the utility of this present study is quite significant. This study further supports the notion that TNF-α plays a central role in the pathophysiology of RA. This study also suggests that the progression of RA in mice has an effect on spontaneous behavior (locomotor activity). The findings in this study show that in mice over-expressing TNF-α, physical signs of RA (clinical scoring) and worsening spontaneous behavior become evident at the age of 12 weeks. The results of this study proves to be a starting point for beginning to identify the behavioral progression of RA in humans; and physical symptom worsening can be used as a guide to identify any behavioral manifestations.

Limitations

This present study accurately identified the effect of RA progression on behavior in mice. However, the lack of a similar study to compare the study results, makes it is difficult to assess the significance of the study findings. The present study did not use other behavioral assays, such as the forced swim test, which could have been useful in identifying other behavioral conditions, such as depression and anxiety. The rotorod assay in particular, was prone to error due to the variability of the mice (both groups) being able to stay on the apparatus (falling off at an earlier). A few of the mice in the study were habituated to the apparatus and did not fear falling off the rod. Also, the experimenter performing the rotorod test manually identified when the mice fell off the rotorod (time in seconds), which is deemed to be subjective. But the same experimenter performed all of the timing during the rotorod testing, so there remained a consistency in the timing of the rotorod apparatus.

Though RA is more prevalent in the female populations, the use of only female mice in this study makes it difficult for this study to be generalized to the population. Also, having an equal number of mice within each test group would have made the statistical analysis more ideal.

Conclusion

The results of this study show that TNF-α plays a role in the physical and behavioral manifestations of Rheumatoid arthritis. In the case of the C57BL/6 female mice, the progression of disease (wildtype vs. transgenic mice) appeared to have become evident after the age of 6 weeks. After the age of 6 weeks, there appeared to further delineation between the spontaneous behavior and physical activity of both groups of mice. This study will facilitate many projects examining the behavioral aspect of chronic inflammatory conditions. Literature reviews have shown the efficacy of biologics

reversing the physical manifestations of Rheumatoid arthritis. Future studies reproducing the methods of this project, in addition to the utility of biologics in mice after the age of 18 weeks, may provide further evidence for the potential efficacy of biologics in behavior.

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