

## Progression of an antigen-induced arthritis model in rat assessed by MRI

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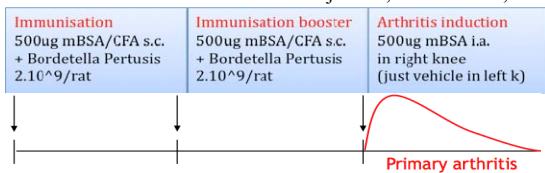
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### Introduction

Diagnosis of early signs of arthritis is important for the prompt administration and resulting efficacy of treatment. An antigen-induced arthritis model was developed in rat and assessed over its development by MRI imaging. One of the early manifestations of the disease that changes rapidly over the time scale studied is the development of extra and intra-articular oedema, closely followed by bone erosion.

### Methods

Female Lewis rats (Janvier, France), weighing 150-175g/2 months were used throughout this study. 24 rats were scanned at the first time point, with n reduced by 3 at each following stage for histology. The protocol for Antigen Induced Arthritis (AIA) was as follows: At day -21, animals were sensitized by an intradermal injection at four sites (two on the back and one on each forepaw) with 100 $\mu$ L antigen solution (500 ug methylated bovine serum albumin (mBSA, Sigma-Aldrich)/50  $\mu$ L saline plus 50  $\mu$ L complete Freund's adjuvant (Becton Dickinson)). At the same time, animals received an intraperitoneal injection of 2x10<sup>9</sup> heat-inactivated Bordetella pertussis (Institut Pasteur - Paris). The sensitization procedure was repeated after 7 days (D-14) by an intradermal injection at four sites on the back. Mono-arthritis was induced 14 days later (D0) in the sensitized animals. The right knee joint was challenged by an intra-articular (i.a.) injection of 500 $\mu$ g mBSA in 50 $\mu$ L saline, while the left knee joint received 50  $\mu$ L of saline. Injections into each joint cavity were achieved by inserting the needle point just below the patella after incision of the skin to reveal the joint. Both for sensitization and i.a. mBSA injection, and for MRI, rats were anaesthetized with isoflurane (Rothacher-medical) 1.5-3% in an air/oxygen mixture.



Bordetella pertussis is given as systemic adjuvant, whereas the antigen in the model is methylated BSA (mBSA). mBSA is a cationic protein that is first used for immunization and boosting, and later on for intra-articular injection to give arthritis onset. Bordetella pertussis triggers toll-like receptors, and thereby enhances the T and B cell immunity during immunization.

Scanning was carried out on a Siemens MAGNETOM Trio, a Tim system, 3T clinical scanner (Siemens AG, Erlangen, Germany) using the system 4cm loop coil. Time points were a pre scan before the model was started (day -21), a baseline scan after immunization, but before induction (day 0) followed by simultaneous scanning of the diseased knee and the contra lateral control joint at days 3, 6, 10, 13 and 17. The protocol parameters included T2 STIR 2D for oedema, TR/TE 3700/20ms, Flip angle 150°, Resolution 0.156mm, FOV 60mm, Slice thickness 1mm and T1 3D gradient echo for bone erosion, TR/TE 14.3/5.9ms, Flip angle 12°, Resolution 0.31mm FOV 100mm. Anaesthetized animals were monitored with a respiratory pad, and ethical committee approval was obtained for the complete protocol. Histological staining (HE) was carried out on animals sacrificed at each time point.

Statistical analysis of data included exponential fits of the development of each component of the disease and comparison of the derivative coefficients by Friedman non-parametric comparison and by Wilcoxon for the placement of the time of maximum score.

### Results

Images showing the disease progression are shown in figure 1. Scoring shows the development and diminution of oedema over the timescale of the imaging.

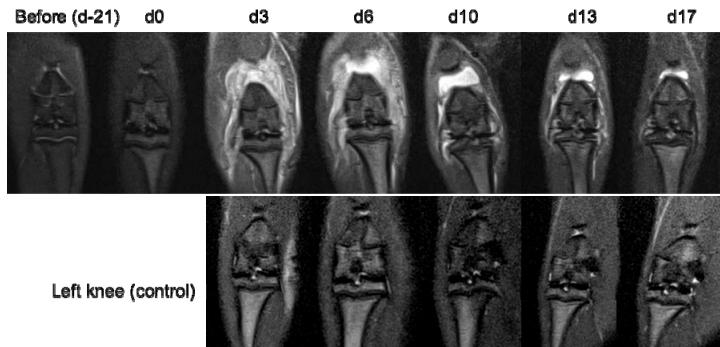


Figure 1 T2 STIR images of AIA progression in the same rat

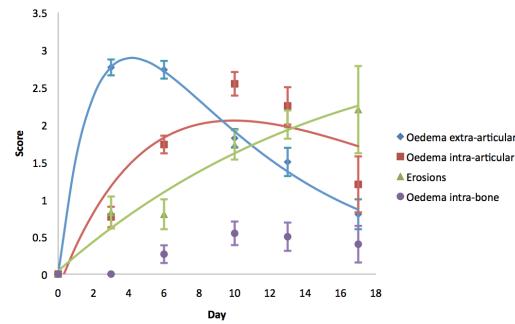


Figure 2 Scoring of oedema types and bone erosion mean  $\pm$  SEM with double exponential fit of rise and decay (d0 n=24, n=3 at each time, fit  $R^2 > 0.8$ )



Figure 3 Corresponding histology slices at days 3 and 17 from different animals showing oedema swelling and bone loss.

All left control knees scored zero for oedema and bone erosion, unlike diseased right knees that showed the rapid development of a synovitis with periarticular oedema, followed by bone erosion and joint effusion. Slight intra-bone oedema appears at day 6 and remains constant for the time period studied. Figure 2 shows fitting of mean scores with time and SEM shows consistency between animals.  $R^2$  also shows good fits for the data from the individual animals (0.60 to 0.94). Comparing the position of the maximum (tmax) of a double exponential fit of the data for individual animals with images at all time points, the Wilcoxon analysis shows a significant difference ( $p=0.043$ ) for the time to peak of extra-articular oedema and intra-articular oedema. Fits for erosion did not produce maxima and continued to grow outside the timescale studied, however, the exponential coefficients from the fit produce a  $p=0.007$  when compared by Friedman test between erosion and oedema. Correlation is seen between the scores on MR images and the corresponding histology (Figure 3).

### Discussion and Conclusions

A robust and predictable AIA model in rat has been developed that is easily characterized by T2 STIR imaging. The progression of the different disease components can be tracked on MR imaging over time. The left knee that does not have the induced disease acts as an internal control throughout the serial study. Further serial treatment and progression studies can now be based on this well-defined model.