White Paper

Cartilage Oligomeric Matrix Protein (COMP) Induced Arthritis in the C57BL/6 Mouse - The Methodology

Keywords: rheumatoid arthritis, cartilage oligomeric matrix protein, C57BL/6 mice

1. Introduction

Genetically modified mouse strains have most often been developed on the C57BL/6 genetic background, which has restricted their use for rheumatology research due to the lack of reliable model for rheumatoid arthritis (RA) for this strain. The C57BL/6 mice possess the MHC class II haplotype H-2b, which does not allow induction of collagen induced arthritis (CIA), the most widely used animal model of rheumatoid arthritis (1, 2).

Recently, a novel mouse model for arthritis for C57BL/6 mouse, the cartilage oligomeric matrix protein (COMP) induced arthritis, was developed, and characterized (3). It induces severe arthritis with high incidence, accompanied by a strong auto-antibody response in C57BL/6NJ mice.

This white paper describes the methodology for COMP induced arthritis for C57BL/6 mice, summarizes the characteristics and discusses the pros and cons of the model.

2. Methodology

Animals for arthritis experiments

COMP induced arthritis experiments can be performed in both male and female mice. If female mice are used, they should be kept together for some time to synchronize their estrous cycle. If males are used it is of particular importance to group them before sexual maturity to avoid intermale aggressiveness.

In experiments all mice must be age- and sex-matched, randomized to experimental groups, and experimental groups mixed in cages. Experiments are carried out when mice are at least 9 weeks old. Experimental investigators must be blinded to the experimental groups until the end of experiments.

Mice are housed in standardized conditions, preferably in specific pathogen-free facility having a climate-controlled environment with a 12-h light-dark cycle, in cages with standard chow and water given ad libitum. However, experiments can be done in conventional facilities but all variable conditions (infections, ventilation, light cycles, feeding, enrichment) need to be carefully described since they could influence the results. The experimental protocols must be approved by local animal welfare authorities.

Induction of experimental arthritis

Arthritis is induced by immunizing the mice with native COMP. Native COMP (100 µg/mouse) is emulsified 1:1 in Freund's complete adjuvant (CFA, #263810, Difco, BD) or in Freund's incomplete adjuvant (IFA, #263910, Difco, BD) containing *Mycobacterium Tuberculosis* H37Ra (M.T., #231141, Difco, 0.5 mg/ml), to prepare an emulsion with 100 µg native COMP per 100 µl of emulsion. Each mouse is immunized with 100 µg of COMP by administering 100 µl of emulsion intradermally (i.d.) at the base of the tail on day 0.

On day 35 a booster immunization is given with native COMP (50 µg/mouse) emulsified in IFA in a total volume of 50 µl/mouse, administered intradermally (i.d.) at the base of the tail.

The choice of the adjuvant (CFA or IFA) for the first immunization is likely to affect disease severity, CFA resulting in a more severe disease than IFA containing *Mycobacterium Tuberculosis* H37Ra (3). Timing of the disease is expected to be the same irrespective of the adjuvant in the first immunization, and a more prominent disease is expected to develop after the booster immunization (3), see Figure 1. Environmental conditions such as the microbiota of the experimental mice may affect disease severity, which can be controlled by selecting the adjuvant best suited for the environment and for the purpose of the experiment.

Visual scoring to follow development of COMP induced arthritis

Mice are investigated at least three times per week for the peripheral joint inflammation, starting 2 weeks after the first immunization. Signs of arthritis in the paws are followed up macroscopically with blind scoring of each red and swollen joint following a standardized protocol (4). To define clinical arthritis, two criteria, namely swelling and redness must be fulfilled. Thus, e.g. paws which remain swollen after an active arthritis but erythema is not observed any longer are not scored or defined as arthritis.

Scoring for clinical arthritis results in 0 to 60 points for each mouse: 1 point is given for each inflamed toe or knuckle, and 1–5 points are given to an inflamed wrist or ankle according to the severity of disease in that joint, resulting in a scale of 0 to 15 for each paw, which then sums up to maximum of 60 points per mouse. Typical arthritis course in C57BL/6 mice after immunization with native COMP in CFA or IFA with *Mycobacterium Tuberculosis* H37Ra is shown in Figure 1.

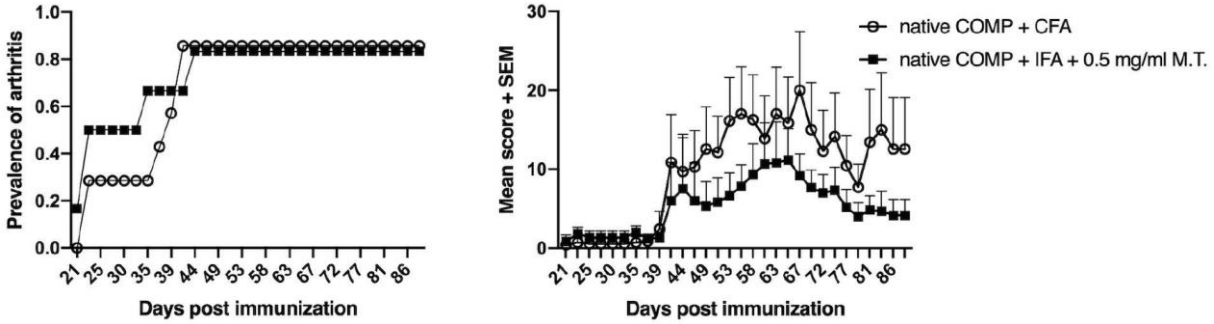

Figure 1. Native COMP induces arthritis in C57BL/6NJ mice with high incidence and severe arthritis. Mice were immunized with native COMP emulsified in CFA (n=7) or IFA + 0.5 mg/ml Mycobacterium Tuberculosis H37Ra (M.T.; n=6), and booster immunization given on day 35. Arthritis was followed by visual scoring to investigate prevalence (left panel) and severity (right panel) (3)).

Characteristics of COMP induced arthritis

Similar to human RA, COMP induced arthritis results in prominent synovial hyperplasia, inflammatory cells infiltration and cartilage and bone destruction in the arthritic joints (3).

COMP induced arthritis results in a prominent IgG, IgG1 and IgG2b anti-COMP autoantibody response irrespective of the adjuvant used for first immunization (3). In C57BL/6NJ mouse, significant anti-Col2 (type II collagen) antibody responses were not observed, pointing to COMP-specific B cell responses. COMP response seems to be peptide specific, as few peptides were identified as major autoantibody epitopes. Antibodies to COMP are pathogenic and can be used for passive transfer of arthritis (5).

COMP induces severe clinical arthritis only in native conformation. Immunization with denatured COMP resulted in very mild arthritis with low incidence. However, both conformations induce anti-COMP antibody response, but stronger IgG, IgG1, and IgG2b anti-COMP responses were observed when native COMP was used for immunization (3). It was concluded that conclude that antibodies to conformational epitopes are pathogenic and need for the induction of arthritis and that these antibodies are likely triggered by help from COMP specific T cell (3).

The major T cell epitope and the critical amino acids within the immunodominant COMP T cell epitope have been reported in (3) .

Methodology to follow arthritis development *ex vivo* and *in vitro*

Arthritis development may be followed by standard histological analyses of the joints after hematoxylin and eosin staining (for details, see (3)).

Serum antibodies against COMP can be determined by enzyme-linked immunosorbent assay (ELISA) using recombinant human COMP as the antigen in the assay. The protocol, the secondary antibodies for detecting IgG, IgG1 and IgG2b class antibodies, and typical results in C57BL/6NJ mice have been described in detail (3).

Peptide specific T cell activation may be measured by stimulation of splenocytes ex vivo and quantifying IFN-γ secretion by ELISpot assay, as described in (3).

3. Benefits

COMP induced arthritis in C57BL/6 mice has many similarities with CIA, the classical mouse model for RA, and induces a severe polyarthritis in diarthrodial joints. It is MHC class II associated, and the COMP-derived peptide recognized by T cells has been characterized. Like in CIA, the induction of arthritis is facilitated by a T cell response to non-self protein antigen because the immunodominant peptide does not bind well to the MHC class II molecule and thus fails to induce tolerance (6, 7). COMP immunization induces a strong antibody response, like type II collagen does in CIA.

The major advantage of COMP induced arthritis is that it can induce severe arthritis in C57BL/6 mice carrying the H-2^b haplotype, which limits their use in CIA requiring MHC class II molecule A^q for proper induction. C57BL/6 mice are regarded as a standard background in immunology research and most often the genetically modified mouse strains are established on this background. With COMP induced arthritis genetically modified C57BL/6 mice can be used for arthritis experiments without extensive backcrossing.

COMP induces arthritis also in mice expressing the murine A^q, A^p on the C3H background and the human DR*0401 MHC class II molecules (8). Thus, the COMP induced arthritis is useful in many mouse strains to study arthritis and the immune response is directly comparable with human RA. COMP induced arthritis in mice is inducible in both females and males.

In addition, COMP also induces arthritis in rats (9). It is associated with the RT1u MHC haplotype with LEW, E3 or DA backgrounds.

4. Limitations

COMP induced arthritis develops into severe clinical disease after the booster immunization, and it thus takes several weeks to perform an experiment. On the other hand, that feature is considered as an advantage, as the disease model involves the same immunological, T and B cell dependent mechanisms characteristic to human RA.

5. Conclusive remarks

The COMP induced arthritis is useful for studies on disease mechanisms and pathogenesis of arthritis involved in immunological priming, effector phase and the clinical phase of arthritis, even in the C57BL/6J mice. The models may be useful for screening and validating pharmacological agents such as candidate drugs targeting any of these phases of arthritis development.

Abbreviations: CFA, Freund's complete adjuvant; CIA, cartilage oligomeric matrix protein; Col2, type II collagen; COMP, cartilage oligomeric matrix protein; ELISA, enzyme-linked immunosorbent assay; IFA, Freund's incomplete adjuvant; MHC, major histocompatibility complex; RA, rheumatoid arthritis.

6. References

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