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Full paper

The establishment of a porcine rheumatoid arthritis model: Collagen induced arthritis minipig model

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ABSTRACT

Rheumatoid arthritis (RA) research has been largely dependent on collagen induced arthritis (CIA) rodent models, however, they may not translate well to humans due to innate differences in the size, physiology and lifespan. The present study aimed to establish a CIA porcine model with the physical, hematological, histopathological and etiological properties closer to their human equivalent in an attempt to better meet the needs of RA research.

Three month old minipigs were administered of bovine type II collagen (CII) emulsified with complete Freund's adjuvants on Day 1 and incomplete Freund's adjuvants on Day 22, via an intradermal or intra-articular route. The clinical, radiological and hematological assessments of immunized animals were made periodically until Day 43, during which period the onset and progression of arthritis was recorded and characterized. In addition, the histopathological and micro-tomographic assessments of the cartilage degradation with regard to mononuclear cell infiltration, and joint deformity indicated a higher severity in the intradermal injection group over the intra-articular group.

With confirmation of the susceptibility to heterogeneous CII for arthritis induction in minipig, the potential suitability of this test system as a large animal model for RA has been demonstrated.

1. Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease that affects about 0.5–1% of the adult population in the world (1). RA is characterized by synovial hyperplasia, inflammatory cell infiltration, cartilage degradation, bone erosion, joint destruction and an increase in the levels of pro-inflammatory cytokines. Understanding of the disease pathogenesis and etiology, however, is as-yet insufficient for the development of new therapeutic strategies (2).

To explore novel therapeutic strategy, and in a bid to understand the pathogenesis of RA, several animal models have been established in the preceding decades; these include mouse, rat, guinea pig, and non-human primate (NHP) models. Of particular note are the rodent models, which have had induced arthritis via specific antigens as well as chemical reagents. These include models based on collagen induced arthritis (CIA), streptococcal cell wall arthritis, adjuvant induced arthritis, proteoglycan-induced arthritis, and serum transfer induced arthritis. In addition to the above, genetically modified TNF-α transgenic mice and K/BxN transgenic mice were developed as animal models for spontaneous RA (3).

Particularly, the preclinical research of RA has been enormously relied on CIA rodent models (4). Since it was first described by Trentham et al. in 1977, immunization to heterologous type II collagen (CII) has been widely applied due to the high incidence of arthritis with similar pathology of human RA (5,6); the histopathological changes associated with T-cell specific cellular and humoral immunity against CII were revealed in the CIA model, with induced autoimmune arthritis characterized by an increase in CII reactive immune cells, synovial hyperplasia, bone erosion, cartilage degradation, mononuclear cell infiltration and a sensitivity to the MHC class II haplotype (7). The utilization of CIA has, in this way, contributed to an understanding of RA pathogenesis, and the development of novel therapeutic agents (8–11).

Despite the high efficiency and proven achievement of inducing RA in CIA rodent models, research applications have been limited due to constraints imposed by small size and short lifespan of the

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rodents. Similarly, the translational potential is limited by innate differences in the anatomy, physiology and genetics between rodents and humans. Therefore, large animal model has become of main importance in the preclinical and pharmacological evaluations, but a large animal CIA model has, to date, not been successfully established (8,11).

Porcine disease models, owing to a greater similarity in the anatomy and physiology with their human equivalents, have proven to be more effective in aiding the understanding of human disease pathogenesis as well as in the development of new therapeutic substances. In light of this, the present study focused on establishing a large animal RA model by way of CIA minipigs. Assessments were made of the clinical, hematological, radiological and histopathological features of minipigs post immunization to heterologous CII. Collectively, CIA porcine model is expected to have a greater translational potential than the current rodent models, which enables researchers to study a wide range of long term trials or the clinical treatments requiring human-equivalent size models.

2. Methods

2.1. Ethics

All procedures, including induction of arthritis, animal care and animal termination, were approved by the Institutional Animal Care and Use Committee (IACUC).

2.2. Induction of arthritis

Arthritis was induced according to the previous DBA/1 CIA mouse protocol with minor modifications (6,12). A total of 11 specific pathogen free (SPF) minipigs (PWG Genetics, Singapore), 3–4 month old males with approximately 7 kg body weight, was employed in this study. They were divided into 3 experimental groups; the intradermal injection group (ID group) had 5 animals assigned, the intra-articular injection group (IA group) drew 3 animals, and the final 3 animals were placed in control group. All animals were sedated with an intramuscular injection of ketamine (10 mg/kg) and xylazine (2.5 mg/kg), and anesthesia was maintained by isoflurane inhalation. The ID group was administered with a mixture of 1 mg/ml/kg bovine type II collagen (CII; Chondrex, WA, USA) emulsified with an equivalent volume of complete Freund's adjuvant (CFA; Chondrex) intradermally on Day 1. To maintain the stability of intradermal injections and to reduce the risk of possible skin ulceration, each 0.1–0.2 ml of mixture was distributed over 40 to 80 spots across the dorsal region. The second immunization was performed on Day 22 using same procedure except the use of incomplete Freund's adjuvant (IFA; Chondrex) instead of CFA. The IA group received 1 mg/ml mixture of CII emulsified with equivalent volume of CFA or IFA on Day 1 or Day 22, respectively, via direct injection into both the knee joint cavities. Control group received injections of an equivalent volume of phosphate buffered saline (PBS; Sigma, MO, USA) via both the ID and IA routes.

2.3. Gross observation and clinical evaluation

The clinical evaluations and body temperatures were recorded every three days from the second immunization day (Day 22) to Day 43 by three separate personnel. The criteria of the clinical evaluation at each limb were based on previously established standards, and were summarized in Table 1 (6). Rectal temperatures were measured using a digital centigrade thermometer. Hind paw thickness was measured in triplicate by three different evaluators with digital caliper ruler at the widest region of the tarsal joint on Day 22 and Day 43. The difference of hind paw thickness was calculated by subtracting Day 22 values from those on Day 43.

2.4. Radiological and hematological assessment

The tarsal joints of all groups were scanned for the morphological changes on Days 22 and 43 using a C-arm X-ray (Zen-2090, Genoray, Sungnam, Korea). Upon sacrifice on Day 43, the tarsal joints were harvested and subsequently scanned by micro-tomography (micro-CT; SMX-100CT, Shimadzu, Kyoto, Japan) to examine structural changes on the tarsal joints in response to arthritis development. Additionally, complete blood counts (CBC) were analyzed using an ADVIA 2120 Hematology Analyzer (Siemens, NJ, USA) on Days 1, 15, 29 and 43; special attention was paid to the total number of WBCs, neutrophils, lymphocytes and monocytes in a bid to monitor any inflammatory response.

2.5. Histopathological assessment

All groups were euthanized on Day 43 with a thiopental sodium overdose. The metatarsophalangeal joints were harvested and fixed in 10% formaldehyde (Sigma) for 3 days. Fixed samples were then trimmed in 2 cm width $\times$ 2 cm length $\times$ 2 mm thickness, decalcified with Decalcifying Solution-Lite (Sigma) for 24 h, dehydrated and embedded in paraffin. Slides of 4 μm sections were stained with Masson's trichrome (Sigma) as well as hematoxylin and eosin (H&E; Sigma) before being observed under a light microscope (Nikon, Tokyo, Japan). The thickness of randomly selected 5 non-calcified cartilage regions was measured using the NIS elements program (Nikon).

2.6. Statistical analysis

One-way ANOVA was conducted in SPSS (IL, USA) with a Games-Howell post-hoc test to discern significant differences. Data were presented as the mean $\pm$ standard error of the mean (SEM). The p value less than 0.05 was considered as significant difference.

3. Results

3.1. Gross observation

One minipig in the ID group perished shortly after the first immunization; it was speculated that the pig suffered accidental intravenous injection of CII emulsified with CFA, but no further examination was conducted. Another minipig from the same group showed no clinical changes up to Day 43 and was therefore considered an unsuccessful induction. Symptoms of swollen joint such as the disappearance of plantar creases, thickened metatarsal joints and increased distance of each digit were featured prominently in the other 3 animals in the ID group, as well as all 3 animals in the IA group. Cases representative of the aforementioned symptoms may be observed in Fig. 1.

3.2. Clinical assessment

The clinical score of the ID group was significantly higher ($p < 0.05$) than the IA group from Day 28 onwards (Fig. 2a). The maximum clinical score was reached into 1.95 $\pm$ 0.3 or 1.25 $\pm$ 0.19 points in the ID or IA group at terminal end point, respectively. Limb swelling and erythema in both immunized groups were limited to distal regions such as the metacarpal/metatarsal joints, digits and palms, as opposed to more severe clinical signs including severe

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