A Review on Complete Freund’s Adjuvant-Induced Arthritic Rat Model: Factors Leading to its Success

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ABSTRACT

Arthritis-induced adjuvant (AIA) is an established animal model reflecting several clinical manifestations of human arthritis. It provides more understanding of pathogenesis and pathways involved in arthritic development and for testing various treatment modalities. Complete Freund’s adjuvant (CFA) is one of the most known algogenic agents used to develop AIA rodent model. Its wide application increases understanding of CFA effects locally and systemically following adjuvant-containing mycobacterium exposure in-vivo. This study aims to review possible factors involved in producing a successful CFA-induced arthritic rat model. We conducted a review of previous studies to determine critical factors to be emphasized. Since arthritis can be classified as gout, osteoarthritis, and rheumatoid arthritis, among others, factors that should be assessed include different dosage and volume, injection site, remission, arthritic and animal gender, and strain selections to successfully develop an arthritic rat model.

INTRODUCTION

Adjuvants have been applied to boost the immune responses of the animal host to an antigen since more than 70 years ago.\textsuperscript{1} The adjuvants include collagen type I or II, lipopolysaccharides, carrageenan, complete or incomplete Freund’s adjuvant, formalin, pristine, squalene, and 6-sulfanilamidoindazole\textsuperscript{2-6} generally produce arthritogenic signals depending on types of adjuvants used and immunisation procedures using specific antigens to cause a self-tolerance breakdown.\textsuperscript{7} Adjuvant-induced arthritis (AIA) model has been extensively developed to mimic certain conditions of human diseases, especially arthritis. In the past few decades, experimental models were developed to explore arthritic etiopathogenesis and discover the cure for inflammation, arthritic features, and pain. AIA model develops mechanical allodynia, thermal hyperalgesia, flare pain following joint movement (joint hyperalgesia), reduced mobility, and increased swelling indicating oedema formation and joint stiffness.\textsuperscript{2,4,8,9}

Amongst AIA models, CFA-induced arthritis seems the most reliable\textsuperscript{5,7} and justifiable in research.\textsuperscript{11} CFA is extensively used to develop experimental models of autoimmune diseases including rheumatoid arthritis (RA), gout, osteoarthritis, encephalomyelitis, neuritis, uveitis, thyroiditis, and orchitis either in acute or chronic types.\textsuperscript{11} CFA-induced arthritis is more severe and systemic\textsuperscript{7,11} compared to antigen-free adjuvants such as incomplete Freund’s adjuvant (IFA) and pristine.\textsuperscript{11} Its efficiency in stimulating antibody production is reported to be greater than other types of adjuvant.\textsuperscript{1,12} It also produces nociceptive responses that may assist researchers to discover new potential therapeutic drugs to combat arthritic pain. In this review, we will focus on CFA including the arthritic mechanisms involved, clinical features and symptoms produced as well as factors leading to its success in developing this rat model.

Mechanisms of arthritis induced by CFA

Arthritis development in an animal by CFA was initiated by Stoeck et al\textsuperscript{13} when attempting to produce immunity to spleen extracts emulsified in the adjuvant. The developed
arthritogenic effect. It induces haematopoiesis by increasing monocyte-macrophage colony-stimulating activity in serum, granulocyte-macrophage progenitors in the spleen, and multipotent stem cell multiplication in the bone marrow. The CFA may lengthen the antigen presentation at the injected site followed by its uptake to phagocyte-rich organs such as the lymphatic system and lungs where the adjuvant promotes immune cell assembly. These antigens bind proteoglycan to hyaluronic acid in the joint cartilage matrix which further enforces mononuclear phagocytic cells (MPCs) activation and dendritic cells (DCs) maturation. A sufficient amount of mycobacterial injection promotes the maturation of dendritic cells (DCs) but a higher dose may produce more severe implications. Another component of mycobacterium, lipoarabinomannan (LAM) is trafficked back to the cell surface in relation to CD1 and presented as a major histocompatibility complex (MHC)-independent T-cell epitope. LAM induces interleukin-1 (IL-1), IL-6, IL-8, IL-10, tumor necrosis factor-a (TNF-a), granulocyte-macrophage colony-stimulating factor (GM-CSF) and monocyte chemoattractant protein-3 (MCP-3) release. It also promotes innate immunity by orchestrating antigen-specific T- and B-lymphocytes development. The excessive polyclonal activation and T-lymphocytes proliferation infiltrate tissues and cross the blood-brain barrier. Helper T-cell type-1 (Th1) will be produced and antibody response is abnormally exaggerated (i.e. autoimmune reaction). Since heat-killed mycobacterium persists for weeks to months at the injected site and in phagocyte-rich organs, the autoimmune reaction may be prolonged and chronic.

Clinical signs and symptoms

The most common arthritic sign is body weight loss with a progressive decrease in mobilization due to joint hyperalgesia. CFA dosage determines joint oedema developmental trend which peaks on day 5 post-CFA inoculation and resolves on day 30 post-injection. CFA inoculation at the footpad also causes oedema due to inflammatory reaction. Hyperalgesia including thermal allogynia develops two weeks later. Tactile allodynia and hypersensitivity are reported although the arthritic duration is variable.

Factors for a successful arthritic rat model development

Mycobacterium strains

The mycobacterium cell wall is made of peptidoglycan layer, mycolic acid, and arabinogalactan which are responsible for bacterial virulence. In CFA, mycobacterium species either M. butyricum or M. tuberculosis (i.e. H37Ra, CDC 1551DT, and PN) are heat-killed to halt its virulence and to provide antigens for the development of animal model mimicking human disease.

In the CFA-induced arthritic model, intra-articular injection of M. butyricum rather than M. tuberculosis developed severe arthritis as evidenced by a significant joint diameter increase. Moreover, female rats
administered with *M. butyricum* at tibiotarsal and tibiofemoral joints produced prolonged monoarthritis while male rats injected with *M. tuberculosis* had less severe arthritis.\(^9\) It is postulated that the marked gender differences in the severity of arthritis are contributed by the hormones which are particularly involved in the hypothalamic-pituitary-gonadal and –adrenal axes (HPG and HPA) as oestrogen stimulates higher corticosteroid responses in female rats.\(^{24,28}\) Meanwhile, CFA injection containing heat-killed *M. butyricum* produced earlier signs of arthritis in Lewis rats which are more severe and consistent. Hyperalgesia and oedema are developed in rats injected with CFA containing *M. butyricum*. However, *M. butyricum* and *M. tuberculosis* could exert similar effects for a similar duration as *M. tuberculosis* produced a prolonged and persistent increase in inflammation for 25 days.\(^9\) Furthermore, the alldynic condition was persistent in rats injected with CFA containing *M. tuberculosis* for 25 days of experimentation.\(^{23}\) Moreover, acute inflammation with the appearance of primary arthritic lesions was observed as early as 30 minutes post-CFA injection,\(^9\) thereby possibly indicating that both mycobacterial strains produce similar signs and symptoms and duration of inflammation and arthritis.

**Types of arthritic model**

The site of injection may determine the types of arthritic model to be developed either monoarthritis or polyarthritis. Intra-articular CFA injection at a single joint or subcutaneous CFA injection at a lower dose produces monoarthritis.\(^{29}\) This CFA dose manipulation is sternly unilateral with the only minor effect of arthritis detected at the contralateral joint. Alternatively, it can be achieved by a single, lower CFA injection dose at the plantar surface of the rat’s hind paw which results in unilateral polyarthritis. However, the monoarthritis model is frequently applied as an acute or chronic inflammatory model to study pain pathways\(^9,29\) since it is easier to assess pain behaviour and pain-related markers. Meanwhile, the critical systemic changes during the polyarthritic phase are believed to make it difficult to attribute data related to peripheral and central modifications in nociceptive transmission. Polyarthritis can be achieved by injecting CFA at a higher dose intradermally at tail base\(^{29,30}\) (i.e. 50-100µL of 5mg/mL, or 25µL of 135µL of CFA)\(^{9,24,31}\) or hind paw (i.e. 5-10 mg/ml)\(^{9,26,32}\) which results in systemic modifications with light to severe arthritic symptoms.\(^14\) The initial phase resembles a monoarthritis condition which further changes into a polyarthritic state due to inflammatory mediators’ migration\(^9\) to other organs and joints producing secondary arthritis.

**Dosage and volume of CFA**

Wauben et al.\(^{25}\) proposed that the optimal dose of CFA to induce severe arthritis is 100 µL (10mg/mL) whilst 50-100µL (1.0 to 5.0mg/mL) has opted for less severe cases. Table 1 shows detailed CFA dosages, administration route, remission, and post-injection effects with the mycobacterial strain used. Wilson et al.\(^{22}\) asserted that the severity of CFA injection relies on the volume administered. A low CFA volume (100 µL, *M. tuberculosis*) administered to the rat’s knee joint produced hypersensitivity which further subsided after day 4 with 50% lower hypersensitivity. Higher CFA volume (i.e. 150 L) produced a higher degree of hypersensitivity which was then stable for more than 90 days. CFA injection may produce acute and chronic inflammatory phases. Local inflammatory responses occur soon after CFA injection indicating acute inflammatory reactions. Subsequently, it gives rise to primary arthritic lesions (i.e. oedema on the ipsilateral side) followed by secondary arthritic lesions (i.e. inflammatory oedema on the contralateral side) at day 15 post-injection indicating a chronic inflammatory phase.\(^9\)

**Route and site of injection**

Gomes et al.\(^{27}\) suggested that the best effective method to produce an arthritic model is by standardizing the interval of inoculation of 21 days with two different routes and sites of inoculation (i.e. one intradermal CFA injection followed by an intra-articular site of injection). However, some studies applied one single high CFA dose that also develops the same effect.\(^9,20,26\) CFA dose and the injection site may affect the trend and phase of arthritis produced. For example, a lower CFA dose at the tail base requires remission whilst a single higher CFA dose injection at the footpad is sufficient to develop a good chronic arthritis model.
Table 1. Previous methodologies in developing CFA-induced arthritic rat models.

<table>
<thead>
<tr>
<th>Arthritis type</th>
<th>Injection site and route</th>
<th>Remission</th>
<th>CFA dosage and duration</th>
<th>Pathogen type</th>
<th>Peak of the paw or joint swelling (days after CFA injection)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic monoaarthrosis</td>
<td>Right footpad</td>
<td>No</td>
<td>20µL (1:1 emulsion)</td>
<td>M. tuberculosis</td>
<td>15</td>
<td>Laste et al^{10}</td>
</tr>
<tr>
<td></td>
<td>Right footpad</td>
<td>No</td>
<td>30µL</td>
<td>M. butyricum</td>
<td>1</td>
<td>Yu et al^{11}</td>
</tr>
<tr>
<td></td>
<td>Right knee joint (i.a.)</td>
<td>No</td>
<td>25µL (125µL)</td>
<td>M. butyricum</td>
<td>14</td>
<td>Nagakura et al^{12}</td>
</tr>
<tr>
<td></td>
<td>Foot pad (i.p.)</td>
<td>No</td>
<td>5µg/mL (50µL)</td>
<td>M. tuberculosis</td>
<td>5</td>
<td>Wilson et al^{13}</td>
</tr>
<tr>
<td></td>
<td>Left knee joint (i.a.)</td>
<td>No</td>
<td>1µg/mL (100µL)</td>
<td>M. butyricum</td>
<td></td>
<td>Gomes et al^{14}</td>
</tr>
<tr>
<td></td>
<td>Tibio-femoral joint (i.a)</td>
<td>Yes (day 0 and 8)</td>
<td>1µg/mL (50µL)</td>
<td>M. butyricum</td>
<td>2^{nd} day after the first inoculation, and the 14th day after the second inoculation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Base of the tail (i.d.) and tibial joint (i.a.)</td>
<td>Yes (i.d. on day 0, and i.a. on day 21)</td>
<td>1µg/mL (50µL)</td>
<td>M. butyricum</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Footpad of left hind paw (i.d.)</td>
<td>No</td>
<td>0.05% w/v (100µL)</td>
<td>M. butyricum</td>
<td>28</td>
<td>Suke et al^{15}</td>
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<td></td>
<td>Base of the tail (i.d.) and tibial joint (i.a.)</td>
<td>Yes (i.d. on day 0, i.a. on day 21)</td>
<td>1µg/mL (50µL) for both i.d. and i.a. injection after 25 days of inoculation</td>
<td>M. butyricum</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Chronic polyarthrosis</td>
<td>Right hind paw</td>
<td>No</td>
<td>10µg/mL (100µL)</td>
<td>M. tuberculosis</td>
<td>15</td>
<td>Luo et al^{16}</td>
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<tr>
<td></td>
<td>Right hind paw</td>
<td>No</td>
<td>5µg/mL (100µL)</td>
<td>M. tuberculosis</td>
<td>10</td>
<td>Chen et al^{17}</td>
</tr>
<tr>
<td></td>
<td>Right footpad and base of the tail</td>
<td>No</td>
<td>10µg/mL, at the footpad and 10µg/mL, twice in the tail base</td>
<td>M. tuberculosis</td>
<td>14</td>
<td>Laste et al^{18}</td>
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<tr>
<td></td>
<td>Left hind paw (subplantar)</td>
<td>No</td>
<td>10µg/mL (100µL)</td>
<td>M. tuberculosis</td>
<td>3</td>
<td>Mahdi et al^{19}</td>
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<td></td>
<td>Right hind foot pad (at metatarsal region; i.d.)</td>
<td>No</td>
<td>10µg/mL (100µL)</td>
<td>M. tuberculosis</td>
<td>Day 20 for the palmarial paw and day 25 for the contralateral paw</td>
<td>Nasani et al^{20}</td>
</tr>
</tbody>
</table>

**Abbreviations:** i.d.-intradermal, i.a.-intra-articular, i.p.-intraplantar and s.c.-subcutaneous

CFA inoculation at the tail base usually produces a polyarthritic condition that progresses in a dual phase. The first phase begins in several hours, resolves after 3-5 days, and is visible as an acute local inflammatory response; while the second phase appears after 14 days post-injection as the chronic systemic response.\(^{30}\) Polyarthritis normally implicates several organs resulting in distal limb joints inflammation, and vertebrae with genitourinary tract, gastrointestinal tract, eyes, ears, nose, and skin lesions and marked weight loss. Laste et al\(^{13}\) revealed that simultaneous CFA injections at the tail base and footpad produce joint damage, revascularization and synovial proliferation, bone erosion, cartilage erosion, and nodular inflammatory response in rat’s external ears and nose. Costa et al\(^{13}\) demonstrated that administration of CFA (5 mg/mL) at the tail base produced prolonged arthritic symptoms for over one month with several phases: pre-clinical (1-10 days), acute (15-30 days), post-acute (30-50 days) and a late stage (more than 50 days). Tibio-tarsal joint circumference was increased after day 15 post-inoculation and peaked on day 18 before subsiding after day 25 post-inoculation. No clinical sign was observed during the pre-clinical stage whilst the highest inflammatory was reported during the acute stage. The degree of arthritic signs was minimized during the post-acute stage due to diminished inflammation and improved mobility. During the late stage, paw diameter reaches baseline value indicating recovery.

The CFA injection at the rat’s footpad is the most effective method to produce a potent immune response and obvious painful sensation in animals.\(^{9}\) Mahdi et al\(^{24}\) revealed that CFA injection (i.e. 10 mg/mL at footpad) produced maximal oedema formation on day 3 post-arthritic induction. This leads to tactile allodynia development persisting for 4 weeks,\(^{8}\) acute inflammation during the early phase that further results in secondary arthritic lesion at non-injected hind paw on day-15 post-CFA injection.\(^{9}\) However, CFA injection at the footpad is infection-prone and could produce serious ulcerations.\(^{34}\) Another preferable route is the tibiotarsal joint to develop a monoarthritis model which may result in oedema and necrosis at the adjacent paw.\(^{27}\) Monoarthritic model is preferable for behavioural and neurochemical studies involving several pain treatment methods. This model produces minimal systemic disruption as it is anatomically limited, pronounced with a prolonged and stable arthritic condition.

**Gender and strains selection**

Nociceptive stimulus modality, arthritis severity, and CFA injection site may differ significantly between gender.\(^{10}\) Arthritic male rats have better tolerance with thermal stimuli compared to female rats\(^{10}\) whilst female rats.
demonstrated longer monoarthritis duration. Osteoarthritic female rats exhibited higher inducible nitric oxide synthase (iNOS), pro-inflammatory IL-1β, and MCP-1 expressions in temporomandibular joints synovial membrane. Hormones may implicate differential arthritic signs and symptoms. Oestrogen and testosterone produce different disease incidences and severity. Oestrogen may enhance cellular memory development through epigenetic processes that possibly contribute to the sexual dimorphism of synoviocytes between genders. It possibly potentiates NF-κB's DNA-binding activity in the synovial membrane of CFA-induced temporomandibular joint inflammation in the female rat. Regarding strain selection, certain strain is highly susceptible whilst others are resistant to algogenic agents’ effects. It is affected by genetic factors contributed by certain genes, specific quantitative trait loci, T-cell proliferation, inflammatory cytokines, antibodies, heat-shock proteins, endocrine factors, and housing environment. Lewis strain is highly susceptible to arthritic symptoms whilst Long Evans, Wistar, Dark Agouti and Germfree F344 strains produce good arthritic susceptibility. Sprague-Dawley is comparable to Lewis strain for AIA susceptibility clinically, histologically, and radiologically. Since SD strain is less costly, more heterogenic, and mostly available, it is mostly preferred. Meanwhile, Buffalo and Fischer strains are reported to be less susceptible to arthritic induction. Remission requires a lower CFA dosage to produce significant arthritic development. Gomes et al suggested that single CFA administration is incapable of inducing prolonged arthritis. The initial phase of CFA injection at the rat's tail results in no clear sign of pain and oedema. It is also agreed that remission is crucial to sustaining arthritic development. CFA injection at the footpad and tail base produces polyarthritic conditions rather than a single CFA injection. Remission results in prolonged hypersensitivity and oedema in rats and it enhances the effectiveness of the first CFA inoculation. However, remission depends on the purpose of the research since either single or more inoculations may eventually produce arthritic signs and symptoms although the intensity and severity may vary.

**Effect of CFA inoculation**

CFA-induced arthritic rat displayed −7% body weight loss starting at day 14 post-CFA injection with increased spontaneous vocalization indicating distress or discomfort. In the severe stage, the rat developed coarse, ruffled fur and generally appears ill. The AIA rat exhibits four different stages of adjuvant arthritis; incubation, onset, summit, and recovery periods. Regardless of the injection site, mineral oil in CFA ensures three particular mechanisms of action: (1) establishing antigen depot with slow antigen release, (2) offering a vehicle for antigen transport throughout the lymphatic systems to immune effector cells, and (3) communicating with antigen-presenting cells including phagocytes, macrophages, and DCs. The CFA effects could be seen as the paw appeared swollen and red within 3 to 4 days indicating acute inflammation. During early inflammatory events, the erythrocyte sedimentation rate (ESR), blood leukocyte, and neutrophil counts start to increase in number starting on day 4 post-arthritic induction. It is followed by swelling in the ankle and dorsal area of tarsus on day 11 due to the neutrophil infiltration and proliferation of synovial lining. The level of neutrophils in the arthritic rat is reported to be two times higher in the blood than in normal rats. During arthritis-induced inflammation, neutrophils become persistently activated and resistant to apoptosis. Acting like macrophage or dendritic cells, the activated neutrophils secrete proteases, and various types of TNF family cytokines including TNF, B cell-activating factor, and receptor activator of nuclear factor kappa B (RANKL) in the affected joints. The expression of chemokines and their receptors on the surface of activated neutrophils facilitates its migration to the affected joints. Within several days of the CFA injection, strong and prolonged inflammatory reactions were reported at the injected site and adjacent joints. Macrophages and
endothelial cells infiltrating synovial fluid present antigenic TNF-α and IL-1β on their surfaces to cause further pro-inflammatory cytokines production found to be elevated in rat's sera and joints.¹⁹,³⁷,⁴³ TNF-α could be the culprit during early perpetuation and maintenance of synovitis and it works synergistically with IL-1β to induce intracellular adhesion and migration, production of acute-phase proteins, and proteolytic enzymes, angiogenesis¹⁹,²⁰ and other cytokines productions.¹¹,¹³,³⁰ The orchestration of these mediators leads to joint swelling in the rat.¹⁸ IL-6 also leads to the positive feedback loop of inflammation as it stimulates more MCP-1 productions¹¹ leading to chronic arthritis. It is claimed that the effects exerted by IL-1β are locally whilst TNF-α are systemically in CFA-induced arthritis.¹⁸,⁴⁵ The release of TNF-α and IL-1β may bind to their specific receptors to initiate the activation of the nuclear factor kappa B (NF-κB) pathway,⁴⁶ which plays a pivotal role during the initiation and perpetuation stages of chronic inflammation in RA.⁴⁷ Meanwhile, the activation of TNF receptor-1 (TNFR1) mediates the PI3 kinase and PKC-δ activation which results in the assembly of the TNFR-1-TRADD-RIP-TRAF2 complex for activating the anti-apoptotic signaling.⁴⁸

Furthermore, IL-12 may trigger natural cell s (NK cells) activation linking to stress-activated pathways.⁴⁵,⁴⁹ In turn, NK cells produce interferon-γ (IFN-γ) orchestrating with IL-12 and IL-6 to promote T-cells differentiation favouring Th1 responsiveness.¹¹ At the end stage, IL-6 and MCP-1 may play more significant roles. Although IL-6 is considered a pro-inflammatory mediator, it plays a disparate role during chronic inflammatory reactions to produce anti-inflammatory effects.⁵⁰ IL-6 is possibly involved both in local and systemic events as it contributes more effect at the later stage promoting recovery (Figure 1).

Systemic actions of CFA may affect organs within the lymphoid system. Hyperplasia and architectural alterations are detected in regional and distant lymph nodes. Remission of CFA induces tubercle-like lesions in lymph nodes and non-lymphoid tissues followed by the presence of local and distant granulomas containing mononuclear phagocytes (MPCs). The phagocytes may appear as epitheloid structures containing mycobacterial acid-fast rods. The presence of typical tubercles with Langerhans giant cells is also reported.¹⁵ Besides that, tissue necrosis is also detected at the injected site.¹,²² In the early 1950s, Pearson¹⁴ revealed dermatitis occurrence in AIA rats, which were possibly identified as necrosis in later works of literature.¹ They also reported the occurrence of iridocyclitis in their polyarthritic model.

The arthritic symptoms can be severe relying on injected site and duration, degree, and extent of peripheral involvement. The intradermal or subcutaneous CFA injection at 1.0mg/mL may produce arthritic lesion between the 11th to 16th days and the severity peaked on the 18th to 25th-day post-injection.⁹,¹⁴ Moreover, CFA at 5-10mg/mL produces prominent tactile allodynia initially on the inoculated hind paw followed by both hind paws started on day 15 until 25 of experimentation.⁸,⁹ The pain and distress experienced by animals may be due to local inflammatory lesions associated with IL-12 and NK cells' actions.¹¹ The intradermal injection of low CFA dose (i.e 1.0mg/mL) results in thermal hypoalgesia during polyarthritic state (day-18 post-adjuvant injection).¹⁰ Although it is unclear, it is possible that this effect is due to progressive cartilage and bone destruction. Consequently, this occurrence may directly affect the nociceptive processing of affected joints’ deep somatic tissue.

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**Figure 1.** Summary of CFA mechanisms of action. During the early phase of complete Freund’s adjuvant (CFA) injection, neutrophils migrate and infiltrate the injected site followed by MCPs and DCs migration. Micro-bacterial antigens binding leads to the production of tumor necrosis factor-a (TNF-a), interleukin-1β (IL-1β), and interleukin-12 (IL-12). TNF-a and IL-1β lead to interleukin-6 (IL-6), macrophage-chemoattractant protein-1 (MCP-1), macrophage-inflammatory protein-1α (MIP-1α), interleukin-12 (IL-12), and epithelial-neutrophil activating peptide-78 (ENA-78) release and further stimulate intracellular adhesion and migration, acute-phase proteins, and proteolytic enzymes productions as well as angiogenesis. These effects indirectly contribute to hypersensitivity and pain (i.e. allodynia and hyperalgesia). Systemic modifications lead to clinical symptoms (i.e. joint swelling and redness and body weight loss). Meanwhile, IL-12 stimulates natural killer cells (NK cells) via natural killer cell receptor (NKGD2) activation leading to stress-induced membrane ligands activation. The IL-6, interferon-γ (IFN-γ) (produced by activated NK cells), and IL-12 further drive T-cell differentiation to assume a T-helper 1 (Th1) profile. Adapted from Billiau and Mathys.¹¹
Nociceptive mechanisms following CFA administration

Since the presence of antigens from CFA initiates aberrant inflammatory reactions, these mediators may directly activate certain receptors on the joint nociceptors to cause the action potentials (APs) to fire. This mechanism leads to the development of peripheral sensitisation in which the nociceptive threshold is progressively reduced and further unmasks the previously ‘silent’ nociceptors through the action of nerve growth factor (NGF). From the joint nociceptors in the periphery, the APs are transmitted to the spinal cord dorsal horn to synapse with interneurons and projection neurons leading to the molecular changes at the CNS level. The persistent nociceptive signals enhanced by the peripheral sensitisation may cause hyperexcitability in the CNS, progressively leading to central sensitisation.

During the central sensitisation, glutamatergic neurotransmission may mediate the summation of subthreshold excitatory post-synaptic currents from the acute pain to the firing of APs in the higher-order neurons (i.e. third- and/or fourth-order neurons in the brain). The central modifications also result in the loss of tonic inhibitory controls due to the γ-aminobutyric acid (GABA) receptors and glycinegic pathway disinhibition. The changes in the glia-mediated inflammatory mechanisms also lead to increased pro-inflammatory insults such as IL-1β in the cerebrospinal fluid of RA patients, and the activation of CX3CR1 receptors in the spinal microglia to contribute to the generation of mechanical hypersensitivity in the rat model of RA. Therefore, the marked changes in the peripheral and central mechanisms during the pathogenesis of arthritis in the rat model contribute to the generation of allostynia and hyperalgesia as demonstrated in the behavioural analyses in the rat model of chronic arthritis.

Behavioural testing for nociception and inflammation in the arthritic rat model

The successful development of the arthritic model may produce similar signs and symptoms as in RA patients including the presence of joint oedema, flare pain indicated as allostynia and hyperalgesia, and reduced mobility due to joint swelling and pain. Since arthritic rats are unable to describe verbally the symptoms they experienced, several behavioural testing is commonly conducted to confirm the presence of arthritic symptoms in regards to pain and inflammation in the rat model. In terms of pain assessment, several tests can be performed to identify the specific development of pain and the pathways involved. For example, tail-flick tests and hot-plate tests can be performed to assess hyperalgesia although the pain pathways involved are different. Von-Frey and Randall-Selitto tests can be conducted to evaluate tactile and mechanical allodynia in the rat. Depending on the concentration of formalin prepared, the formalin test induces tonic chemical stimulation of pain in the animal model. In this test, the results of the pain activity could represent the changes either in the periphery or centrally in regards to the nociceptive and inflammatory mediators. The writhing test is a chemical-induced pain test that can be applied to evaluate visceral pain in the laboratory rat.

In regards to an inflammatory evaluation in the arthritic rat model, the measurement of diameter and circumference in the affected joints can be conveyed as a screening method for the development of joint swelling and oedema. Meanwhile, the more accurate procedure to confirm the development and disease progression of the arthritic rat model can be done through blood and urine tests, tissue biopsies (e.g. the affected joints), aspiration of joint fluid, or x-rays. The level of white blood cells and C-reactive protein in the blood plasma or serum may indicate the progression of arthritis in the rat. The histology conducted on specific joint tissue may reveal more extensive joint degradation resulting from the inflammatory reaction following the CFA injection.

CONCLUSION

Although the animal model does not perfectly recapitulate whole arthritic characteristics in humans, it offers an armamentarium to understand the pathophysiology involved, leading to a revolution of biological therapeutics to slow down the disease progression. In order to produce a good model for the CFA-induced arthritic rat model,
researchers should consider certain criteria to achieve a successful arthritic model. The arthritic types, animal gender and strains, mycobacterial strains, dosage and volume, injection site and route, and remission should be focused on to produce a good AIA rat.

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