Activation of invariant NKT cells confers protection against Chlamydia trachomatis-induced arthritis

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Abstract

The role of invariant NKT (iNKT) cells in reactive arthritis is unknown. We explored the functional role of NKT cells in reactive arthritis using an established murine model of Chlamydia trachomatis-induced arthritis (CtIA). CtIA in wild-type and CD1d knockout (KO) mice was induced by intra-articular injection of C. trachomatis. The effect of α-galactosylceramide (α-GalCer) activation of iNKT cells was investigated by intra-peritoneal administration of α-GalCer. Histopathological and phenotypic changes, chlamydial clearance and cytokine and chemokine production in synovial tissue of the knee joint were investigated after onset of the arthritis. The severity of CtIA was significantly increased in CD1d KO mice, which was associated with decrease in bactericidal cytokine IFN-γ, regulatory cytokines IL-4 and IL-10 and increase in pro-inflammatory chemokines macrophage inflammatory protein-2 (MIP-2) and IFN-γ-inducible protein-10 (IP-10). Local clearance of the pathogen from the joint was also decreased. Prior treatment of mice with α-GalCer, a potent activator of iNKT cells, significantly reduced the severity of CtIA in mice. The amelioration of CtIA was associated with decrease in chlamydial load and induction of cytokines IFN-γ, IL-4 and IL-10 and significant suppression of MIP-2 and IP-10. Treatment of established CtIA with α-GalCer also demonstrated modulation of CtIA and decrease in chlamydial load. These results suggest that iNKT cells are protective against CtIA and α-GalCer-activated iNKT cells have an immunoregulatory role not only in preventing the induction of reactive arthritis but also in modulating established disease.

Introduction

Invariant NKT (iNKT) cells are a unique subset of T lymphocytes which express intermediate levels of a semi-invariant Vα14Jα281 TCR in mice or an invariant Vα24Jα15 TCR in humans (1). NKT cells recognize glycolipid antigens, such as the exogenous synthetic glycolipid, α-galactosylceramide (α-GalCer) and the endogenous isoglobotrihexosylceramide in association with the MHC class I-like molecule CD1d (2, 3). α-GalCer, originally isolated from marine sponge, specifically binds to CD1d and activates NKT cells.

The triggering of joint inflammation by infectious agents such as obligate intracellular pathogen Chlamydia trachomatis has been well established in the case of reactive arthritis (4–6). We previously established an animal model of C. trachomatis-induced arthritis (CtIA) that closely mirrors clinical reactive arthritis (7). It has been demonstrated that innate immunity is important in the initial phase of chlamydial infection. In our CtIA model, host resistance was characterized by enhanced synovial expression of IFN-γ, tumor necrosis factor (TNF)-α and IL-4 (8). iNKT cells, upon activation, produce both T1,1 and T1,2 cytokines which play critical roles in the regulation of innate and adaptive immune responses by iNKT cells (9, 10). Recently, the presence of iNKT cells within an inflamed joint has been demonstrated in both clinical and experimental rheumatoid arthritis (11, 12). These cells demonstrated a protective role in collagen-induced arthritis (13). However, in another study, these cells also acted as effector cells (14). Although iNKT cells have also been shown to play an important role in certain infectious diseases, the role that iNKT cells play in reactive arthritis has not been studied (15). Since these cells figure critically in innate host responses, we hypothesized that iNKT cells might play an important role in the early phase of reactive arthritis. To test this hypothesis, we examined CtIA in CD1d knockout (KO) mice and subsequently the effect of α-GalCer-activated iNKT cells on murine CtIA.

Materials and methods

Mice

Eight- to 10-week old male BALB/c mice and CD1d KO mice on a BALB/c background (Jackson Laboratories, Bar Harbor, Maine, USA) were used. Mice were housed in the Animal Resource Centre of the University of Toronto which is a fully accredited facility by the Canadian Council on Animal Care. The animal care and handling procedures were reviewed and approved by the University of Toronto Animal Care Committee in accordance with the guidelines established by the Canadian Council on Animal Care. Mice were maintained on a 12 h light cycle with free access to food and water. All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Toronto Animal Care Committee.
Harbor, ME, USA) were used in all experiments. CD1d KO mice were bred and maintained in pathogen-free animal care facility of the institute. All procedures were carried out in accordance with the Guide for the Humane Use and Care of Laboratory Animals and were approved by the University Health Network Animal Care Committee.

Organisms
The culture and preparation of C. trachomatis serotype L2 was performed as described (16). Briefly, C. trachomatis was cultured in L cells in cell culture medium [MEM containing 10% fetal bovine serum (FBS), 2 mM L-glutamine] at 37°C in 5% CO₂ for 48 h. The infected cell monolayer was harvested and then sonicated in a harvest cocktail [7 mg DNase, 5 mg heparin and 1 tablet of Complete Mini protease inhibitor (Roche) in 10 ml MEM]. The partially purified organisms were sedimented by centrifugation at 40 000 g for 30 min. Chlamydia elementary bodies were purified by centrifugation through a discontinuous 20/50% Renografin (Squibb) gradient. These were re-suspended in sucrose–phosphate–glutamic acid (SPG) buffer (10 mM sodium phosphate, 220 mM sucrose, 0.5 mM L-glutamic acid) and stored at −80°C until used.

α-GalCer treatment and C. trachomatis injection of mice
Mice were given an intra-articular (i.a.) injection with 4 × 10⁶ inclusion forming units (IFU) of C. trachomatis in 10 µl of SPG buffer. For activation of iNKT cells in vivo, 2 h before chlamydia injection, mice received a single intra-peritoneal (i.p.) administration of 4 µg α-GalCer (Pharmaceutical Research Laboratories, Kirin Brewery, Gunma, Japan). Control mice were injected i.p. with 200 µl PBS and i.a. with 10 µl SPG buffer. For reproducible and accurate injections of the mouse joint, a 30-gauge needle connected to a microinjection pump (CMA-400 Syringe pump, Carnegie Medicin, Stockholm, Sweden) was used.

Histopathology and immunohistochemistry
The knee joints were fixed in 10% formalin, decalcified with formic acid and sections of 4-μm thickness were stained with hematoxylin and eosin. Each joint was assigned a histopathological score according to the Yang–Hamilton grading system, which assesses inflammatory cell infiltrates, joint space exudates, synovial hyperplasia, pannus formation and cartilage/bone damage (17). Each category was evaluated with hematoxylin and eosin. Each joint was assigned a histopathological score according to the Yang–Hamilton grading system, which assesses inflammatory cell infiltrates, joint space exudates, synovial hyperplasia, pannus formation and cartilage/bone damage (17). Each category was assigned a score of 0–4, where 0 = normal and 4 = extreme pathologic change, for a total maximal score of 20.

For neutrophil staining, the sections of formalin-fixed, EDTA-decalcified and paraffin-embedded joint tissues were stained with mAb specific for murine neutrophils (CL8993B; Cedarlane, Hornby, Ontario, Canada) (18).

Enumeration of IFU in synovial tissue
Synovial tissue was homogenized. L cells grown to confluent monolayer in 96-well flat-bottom plate were inoculated with serially diluted synovial tissue homogenate and incubated in cell culture medium at 37°C in 5% CO₂ for 48 h. Chlamydia was identified with FITC-conjugated anti-chlamydia mAb (Pathfinder Chlamydia Culture Conformation System; Bio-Rad). The number of inclusions was counted using a fluorescence microscope. The microbial load in the tissue was determined based upon dilution titers of the original inoculum.

Cell isolation
Single-cell suspensions from spleen and popliteal lymph nodes (PLN) were prepared using a cell strainer followed by RBC lysis. Synovial cells were isolated with minor modifications of methods described previously (19). In brief, the synovial tissue was carefully removed from the knee and treated twice with collagenase (1 mg ml⁻¹), polymyxin B (33 µg ml⁻¹) and DNase (150 µg ml⁻¹) for 45 min at 37°C on an orbital shaker. The synovial cells were purified by centrifugation on a 30% Percoll gradient at 600 × g for 20 min. Fibroblast-like synoviocytes (FLS) from Balb/c mice were isolated and cultured as described (7).

Flow cytometry
Cells (0.5 × 10⁶) pre-incubated with anti-CD16/32 mAb (clone 2.4G2, BD Biosciences) to block Fcγ receptors were incubated with the allophycocyanin-conjugated-CD3 mAb C3 (clone 145-2C11, BD Biosciences) and PE-conjugated CD1d tetramer (PE-mCD1d-PBS57, an α-GalCer analog-loaded tetramer from NIAID MHC Tetramer Core Facility, Atlanta, GA, USA) for 45 min on ice. In some cases, the cells were incubated with PE-conjugated CD4 (clone GK1.5, BD Biosciences) and PerCP-conjugated CD8 (clone 53-6.7, BD Biosciences) mAbs for 30 min. After washing, the cells were acquired on FACSCalibur flow cytometer (Becton Dickinson). Data were analyzed using WinMDI version 2.8.

Intracellular cytokine staining
Cells isolated from synovial cavity or spleen were stimulated with phorbol myristate acetate (50 ng ml⁻¹) and ionomycin (1 µg ml⁻¹) and incubated for 6 h in RPMI 1640 medium containing 10% FBS and 2 mM L-glutamine at 37°C. For the last 4 h of incubation, brefeldin (Sigma) was added to accumulate cytokines intracellularly. Cultured cells were washed twice and surface stained as described above. The cells were then fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) as per the manufacturer’s instructions and stained intracellularly with anti-IFN-α, -γ (clone XMG 1.2, BD Biosciences), anti-IL-4 (clone 11B11, BD Biosciences) and anti-IL-10 (clone JESS-16E3, BD Biosciences) mAbs or with corresponding isotope control antibodies for 30 min in permeabilization buffer (BD Biosciences). After washing twice in permeabilization buffer, the cells were re-suspended in PBS and acquired on FACSCalibur flow cytometer (Becton Dickinson). Data were analyzed using WinMDI version 2.8.

RNA extraction and real-time PCR
Total RNA was extracted from synovial tissue and FLS by TRIzol (Invitrogen Life Technologies). cDNA was synthesized with oligo (dT) 12–18 primer and superscript II reverse transcriptase (Invitrogen Life Technologies) after DNase treatment.
The following real-time PCR primer sets were used: IFN-γ, CCAAGCGGCTGACTGAACT forward, TGGCCGGAGTGTA-AGACAT reverse; TNF-α, ACAAGGCTGCCCGACTAC forward, TGGAAGACTCCTCCCAGGTATATG reverse; IL-10, TCTTCAAACAAAGGACCAGCTG forward, GGCAACCCAA- GTAACCCTAAA reverse; IFN-inducible protein-10 (IP-10), CCAGCGTGGTCACATCAG forward, ACCTCCACATAGC- TACG reverse and β-actin, GGCTATGCTCTCCCTCAG

Fig. 1. CD1d KO mice develop more severe CIA and increase chlamydial load. Mice injected i.a. with Chlamydia trachomatis (4 × 10^6 IFU) were sacrificed on days 5 and 14 of infection. Histological examination (original magnification ×10) (A) and histology score (B) of knee joints. (C) Chlamydial load in synovial cavity. (D) Histology score of knee joints of CD1d-blocked mice. Anti-CD1d (clone 1B1) or rat IgG2b isotype control antibody (eBiosciences) (200 micrograms per mouse) were injected i.p. on days −1, 0, 2, 4, 6, 8 and 10 of i.a. injection of C. trachomatis (4 × 10^6 IFU). Each symbol in (B, C and D) represents an individual joint and horizontal bars represent the mean of eight joints in each group (some of the symbols overlap due to same score). Results represent one of the three independent experiments with similar results. *P < 0.05 compared with chlamydia-infected WT or isotype control antibody-treated mice.
forward, CGCTCGGTCAGGATCTTCAT reverse. IL-4 primers were purchased from Biosource International, Camarillo, CA, USA. Vα14Jα281 primers to detect iNKT cells and macrophage inflammatory protein-2 (MIP-2) primers have been described previously (12, 20). Quantitative real-time PCR was performed using the IQ-SYBR Green Supermix (Bio-Rad) in a Bio-Rad iQ5 cycler. After an initial denaturation step at 95°C for 3 min, 40 cycles of two-step PCR with 10 s of denaturation at 95°C and 1 min of annealing/extension at 60°C were used for all reactions. The concentrations were normalized to β-actin in each sample and the data were presented as normalized fold expression.

Statistical analysis
Data were expressed as mean ± SD and analyzed for statistical significance using Student's t test.

Results
CD1d KO mice demonstrate enhanced CtlA
To assess the intrinsic role of iNKT cells in CtlA, C. trachomatis-infected wild-type (WT) and CD1d KO mice were compared. The severity of CtlA was increased in CD1d KO mice (Fig. 1A and B), with histology scores significantly increased in these mice compared with WT mice. This was associated with significant increase in chlamydial load in...
the synovial tissue of CD1d KO mice (Fig. 1C). The blocking experiment in which the Balb/c mice were treated with anti-CD1d antibody to block CD1d was also performed. The histopathology results showed a trend toward increased histology score in anti-CD1d antibody-treated mice compared with isotype control antibody-treated mice (Fig. 1D). To examine the effect of activation of iNKT cells on cytokine and chemokine responses during the course of CtIA, mRNA levels for Th1 (IFN-γ and TNF-α) and Th2 (IL-4 and IL-10) cytokines and pro-inflammatory chemokines (MIP-2 and IP-10) were measured with real-time PCR in synovial tissue of these mice. In the CD1d KO mice, IFN-γ, IL-4 and IL-10 levels were significantly decreased and MIP-2 and IP-10 chemokine levels were significantly increased compared with C. trachomatis-infected WT mice (Fig. 2A). A direct cellular level analysis on cytokine patterns was also performed. Intracellular cytokine staining analysis of synovial cells in CD1d KO mice for IFN-γ, IL-4 and IL-10 showed decreased proportion of IFN-γ-, IL-4- and IL-10-producing cells compared with C. trachomatis-infected WT mice (Fig. 2B).

α-GalCer treatment decreases the severity of CtIA
Since our results demonstrated that iNKT cells modulate CtIA, the influence of α-GalCer treatment on the course of CtIA was investigated. α-GalCer treatment attenuated the CtIA. Histopathology results showed a decrease in cellular infiltration such as neutrophils in the synovial cavity and an overall decrease in histology score of α-GalCer-treated mice (Fig. 3). α-GalCer treatment also enhanced clearance of the organism, with reduced chlamydial load in the synovial cavity.

Effect of α-GalCer treatment on cytokine and chemokine production in joint
CtIA was associated with a significant increase in the levels of the cytokines and chemokines in the synovial tissue compared with uninfected control mice (Fig. 4A). In comparison

**Fig. 3.** Pre-treatment with α-GalCer decreases CtIA and the chlamydial load. Two hours prior to an i.a. injection of *Chlamydia trachomatis* (4 × 10^6 IFU), the mice were administrated i.p. with α-GalCer (4 micrograms per mouse). Mice were sacrificed after 5 and 14 days after infection. (A) Histological examination (original magnification ×10), (B) immunohistochemistry showing neutrophil staining (original modification ×20) and (C) histology score of knee joints. (D) Chlamydial load in synovial cavity. Each symbol in (C and D) represents an individual joint and horizontal bars represent mean of eight joints in each group (some of the symbols overlap due to same score). Results represent one of the three independent experiments with similar results. *P < 0.05 compared with chlamydia-infected mice.
to CtIA alone, α-GalCer treatment significantly increased the levels of IFN-γ, IL-4 and IL-10 and decreased the levels of chemokines MIP-2 and IP-10 in synovial tissue of C. trachomatis-infected mice. α-GalCer injection of control mice did not induce local alteration in the basal levels of any of the cytokines or chemokines compared with control mice (IFN-γ, 1 ± 0.2 versus 2.59 ± 0.96; TNF-α, 1 ± 0.2 versus 2 ± 0.8; IL-4, 1 ± 1 versus 3.6 ± 0.74; IL-10, 1 ± 0.8 versus 2.2 ± 1; MIP-2, 1 ± 0.8 versus 0.3 ± 0.1 and IP-10, 1 ± 0.5 versus 0.5 ± 0.4 in control and α-GalCer-treated mice, respectively). A direct cellular level analysis on cytokine patterns was also performed. Intracellular cytokine staining analysis of synovial cells for IFN-γ, IL-4 and IL-10 showed that α-GalCer treatment increased the proportion of IFN-γ-, IL-4- and IL-10-producing cells in C. trachomatis-infected mice (Fig. 4B).

α-GalCer treatment increases the proportion of iNKT cells in the CtIA joints
To address the mechanisms whereby α-GalCer treatment decreased the severity of CtIA, FACS analysis of synovial and PLN cells was performed. Real-time PCR was also performed to evaluate the synovial tissue level of Vα14Jα281 TCR mRNA as a measure of infiltration of iNKT cells in synovial cavity. In control mice, both real-time PCR and FACS

![Fig. 4](https://academic.oup.com/intimm/article-abstract/21/7/859/821197/1197)
analyses did not demonstrate iNKT cells in the synovial tissue (Fig. 5). Real-time PCR results demonstrated that α-GalCer treatment significantly increases the level of Vα14Jα281 TCR mRNA in C. trachomatis-infected mice. FACS analysis also showed local iNKT cell population (CD3+ CD1d Tet+) both in the synovial cavity and PLN of C. trachomatis-infected mice, the proportion of which increased with α-GalCer treatment. iNKT cell proportion in spleen of C. trachomatis-infected mice was also increased with α-GalCer treatment. However, on day 14 of infection, there was no significant difference of iNKT cell proportion in PLN and spleen. The absolute numbers of iNKT cells in these regions of C. trachomatis-infected mice increased because of significant increase in cell infiltration (Table 1). However, there was no significant difference between α-GalCer-treated and untreated C. trachomatis-infected mice except in spleen where the iNKT cell population was significantly increased with α-GalCer treatment.

α-GalCer treatment induces iNKT cytokine response in CtIA
Since α-GalCer treatment showed cytokine induction in CtIA, we next investigated cytokine profile of iNKT cells in α-GalCer-treated CtIA. Intracellular cytokine staining analysis of synovial cells isolated after 5 days of infection showed that α-GalCer treatment increased the proportion of IFN-γ, IL-4- and IL-10-producing iNKT cells in C. trachomatis-infected mice with maximum increase in IL-4 (Fig. 6). Intracellular cytokine staining analysis was also performed of synovial cells isolated after 14 days of infection. There was no significant difference in the proportion of IFN-γ-producing iNKT cells between α-GalCer-treated C. trachomatis-infected mice and C. trachomatis-infected mice (13.4 ± 3.1 versus 15.8 ± 3% in C. trachomatis and α-GalCer + C. trachomatis, respectively). However, there was a significant increase in the proportion of IL-4-producing iNKT cells in α-GalCer-treated C. trachomatis-infected mice compared with C. trachomatis-infected mice (11 ± 3 versus 33.5 ± 6.2%).

Activation of iNKT cells induces adaptive immune responses
To further evaluate whether activation of iNKT cells influences T cells in the adaptive immune phase of infection, intracellular cytokine staining analysis of spleen cells of α-GalCer-treated C. trachomatis-infected mice was performed after 14 days of infection. Both CD4+ and CD8+ cells were analyzed for IFN-γ and IL-4. α-GalCer treatment increased the proportion of IFN-γ-producing both CD4+ and CD8+ cells in C. trachomatis-infected mice (Fig. 7). Results did not show any significant change in the proportion of IL-4-producing T cells. α-GalCer treatment also increased the absolute number of CD4 and CD8 cells (8.1 ± 0.74 × 10^6 versus 13.5 ± 0.84 × 10^6 and 3.9 ± 0.7 × 10^6 versus 6.1 ± 1.4 × 10^6 in chlamydia and α-GalCer + chlamydia, respectively, P < 0.05) along with B cells (10.5 ± 1.5 × 10^6 versus 30.3 ± 5.5 × 10^6 in chlamydia and α-GalCer + chlamydia, respectively, P < 0.05) in the spleen of C. trachomatis-infected mice. These results suggested that iNKT cells could activate cells of adaptive immune responses. Increase in the IFN-γ-producing CD4+ and CD8+ T cells supports previously published work showing the importance of these cell responses as a major protective mechanism against chlamydia (21).

IL-4 and IL-10 cytokines down-regulate MIP-2 and IP-10 expression in FLS
To investigate the effect of cytokines on MIP-2 and IP-10 production, an in vitro experiment with the FLS was performed. The C. trachomatis-infected FLS were treated with combination of IFN-γ (10 ng ml^-1) and TNF-α (5 ng ml^-1) in the absence or presence of IL-4 (10 ng ml^-1) and IL-10 (10 ng ml^-1). Combination of IFN-γ and TNF-α up-regulated mRNA levels of MIP-2 and IP-10 in C. trachomatis-infected FLS (Fig. 8). Combination of IL-4 and IL-10 down-regulated mRNA levels of MIP-2 and IP-10 in IFN-γ + TNF-α-treated C. trachomatis-infected FLS.

α-GalCer treatment ameliorates established CtIA
To investigate the effect of α-GalCer on established CtIA, 7 days after i.a. C. trachomatis injection, mice received a single i.p. injection of α-GalCer. Single injection of α-GalCer decreased the severity of the histopathological changes in established CtIA (Fig. 9). The histology score was significantly decreased. The chlamydial load in synovial tissue was also significantly decreased with α-GalCer treatment.

**Discussion**
This is the first study to address the role of iNKT cells in reactive arthritis. Here we demonstrate that iNKT cells have an intrinsic role in amelioration of joint inflammation induced by
C. trachomatis since (i) there was an enhancement in severity and in the local chlamydial load in CtIA occurring in CD1d-deficient mice and (ii) α-GalCer, an activator of iNKT cells, treatment enhanced the protective effect in CtIA. Previous studies have also shown their protective role against several infectious diseases such as tuberculosis and Pseudomonas aeruginosa pneumonia (15, 22).

Fig. 5. α-GalCer treatment increases the iNKT cell population. (A) Real-time PCR of synovial tissue (n = 8 joints per experiment) showing expression of Vα14Jα281 mRNA levels on day 2 of infection. (B, C and D) CD1d tetramer staining showing iNKT cell (CD3+ CD1d Tet+) population in synovial cavity, PLN and spleen, respectively, on days 5 and 14 of infection. Results represent one of the three independent experiments with similar results. #P < 0.05 compared with control mice and *P < 0.05 compared with chlamydia-infected mice. Light gray bar, control; open bar, chlamydia and filled bar, α-GalCer + chlamydia.

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The cytokine analyses in the present study have shed light on the mechanisms by which iNKT cells might influence the host response to chlamydia infection. It is known that IFN-γ
and TNF-α are indispensable for an effective defense against intracellular pathogens. In reactive arthritis, failure of effective bacterial elimination during the initiating phase of the disease may be attributable to a relative lack of Th1 cytokine production. Several studies have demonstrated a relative decrease in Th1 cytokines during reactive arthritis (23, 24). IFN-γR−/− bone marrow-derived macrophages showed increased Chlamydia pneumoniae load compared with WT controls (25). Our recent study showed that rats resistant to CtIA have more IFN-γ production than susceptible rats (10). It seems that with the induction of both T,1 and T,2 cytokine arms of the immune response, iNKT cell activation may play an important role in the modulation of CtIA.

Studies have shown that MIP-2 and IP-10 induce pro-inflammatory responses. MIP-2 produced by synoviocytes attracts neutrophils to the site of inflammation, which interact with macrophages and synoviocytes to induce IP-10 (26, 27). Since IP-10 recruits NK and T,1 T cells into synovial
cavity and up-regulates T_{H}1-type cytokine synthesis in T cells (27, 28), the ability of neutrophils, macrophages and synoviocytes to produce this chemokine might contribute to the initiation and progression of the inflammatory response in the joint. Recently, induction of IP-10 production was observed in septic and rheumatoid arthritis (29–31). A recent study has shown that neutrophils enhance the replication of C. pneumoniae and therefore increase the chlamydial load...
and inflammation in the lung (32). In light of these findings, we speculated that modulation of CtIA by activated iNKT cells might be because of suppression of cell infiltration in the synovial cavity by the regulatory effects of T<sub>1</sub>2 cytokine on MIP-2 and IP-10. Consistent with this notion, our results showed that iNKT cell activation suppressed the expression of both MIP-2 and IP-10 and decreased the infiltration of cells in the synovial cavity. The enhanced T<sub>1</sub>2 cytokine response might ameliorate severity of arthritis by suppressing MIP-2 and IP-10 expression. In a previous study, the increase in arthritis susceptibility in IL-4<sup>-/-</sup> mice was correlated with elevated MIP-2 transcripts (33). In <i>in vitro</i> studies demonstrated suppression of IP-10 production in human neutrophils and macrophages with IL-4 and IL-10 treatment (34, 35). Present <i>in vivo</i> study also demonstrated similar results in FLS. Therefore, the effect of iNKT cell activation in CtIA might be mediated primarily through the up-regulation of both T<sub>1</sub> and T<sub>2</sub>-type cytokines. IFN-γ contributes to clearance of the chlamydia from the tissue and IL-4 and IL-10 might restrict the cell infiltration in the synovial cavity by down-regulating the expression of MIP-2 and IP-10. A recent study also demonstrated α-GalCer-induced protection against collagen-induced arthritis which occurred by an IL-10-dependent pathway (36).

In <i>P. aeruginosa</i>-infected mice, iNKT cells induced MIP-2 level and infiltration of neutrophils in the bronchoalveolar lavage fluid (37). In a recent study of <i>C. trachomatis</i>-induced pneumonia, in which the mice were infected intra-nasally, iNKT cells promoted the chlamydia infection and induced the inflammation in the lung (38). In contrast to these studies, our study demonstrated that the iNKT cells in joint have different functional characteristics than other target organs. iNKT cells induced fundamentally different immune responses in the joint, the site of chlamydia challenge. Two recent studies, one in rheumatoid arthritis and one in murine antibody-induced arthritis demonstrated distinct functional characteristics of iNKT cells in synovial cavity as compared with iNKT cells from peripheral blood (11, 12). Therefore, these studies taken together demonstrate different functional characteristics of iNKT cells at different sites. Interestingly, α-GalCer treatment not only protects in the early phase of CtIA but also ameliorates established CtIA. Although decreasing chlamydial load might be responsible for the amelioration of established CtIA, further studies need to be done to understand the mechanism of protection.

At the present time, there is no established treatment for reactive arthritis. Our findings indicate that iNKT cells activated with α-GalCer can act as potent immunoregulatory cells and ameliorate both incipient and established CtIA in mice.

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Abbreviations
α-GalCer α-galactosylceramide
CtIA Chlamydia trachomatis-induced arthritis
FBS fetal bovine serum
FLS fibroblast-like synoviocytes
i.p. intra-peritoneal
IFU inclusion forming units
iNKT invariant NKT
KO knockout
MIP-2 macrophage inflammatory protein-2
PLN popliteal lymph node
SPG sucrose–phosphate–glutamic acid
TNF tumor necrosis factor
WT wild type

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