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Aberrant IL-1 signaling does not increase susceptibility of mice to NOD2-dependent uveitis

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Abstract

Background—*NOD2* is the genetic cause of Blau syndrome, an autoinflammatory disease that manifests as coincident uveitis and arthritis. Since dysregulation of IL-1 signaling is considered a pathogenic mechanism in a number of related autoinflammatory conditions, we examined the extent to which unimpeded IL-1 signaling influences *NOD2*-dependent inflammation of the eye versus the joint.

Methods—Mice deficient for IL-1R antagonist (IL-1Ra) were administered the *NOD2* agonist muramyl dipeptide (MDP) by systemic (intraperitoneal) or local (intraocular and/or intra-articular) injections. *NOD2*-deficient mice received an intraocular injection of recombinant IL-1 β . Uveitis was evaluated by intravital videomicroscopy and histopathology and arthritis was assessed by near-infrared imaging and histopathology. Ocular levels of IL-1 α , IL-1 β , and IL-1Ra were quantified by ELISA.

Results—IL-1Ra deficiency did not render mice more responsive to systemic exposure of MDP. Despite the increased production of IL-1R agonists IL-1 α and IL-1 β in response to intraocular injection of MDP, deficiency in IL-1Ra did not predispose mice to MDP-triggered uveitis, albeit intravascular cell rolling and adherence were exacerbated. *NOD2* expression was dispensable for the potential of IL-1 to elicit uveitis. However, we find that IL-1Ra does play an important protective role in arthritis induced locally by MDP injection in the joint.

Conclusions—Our findings highlight the complexity of *NOD2* activation and IL-1 signaling effects that can be compounded by local environmental factors of the target organ. These observations may impact how we understand the molecular mechanisms by which *NOD2* influences inflammation of the eye versus joint, and consequently, treatment options for uveitis versus arthritis.

Keywords

NOD2; IL-1 β ; IL-1Ra; anakinra; uveitis; arthritis

INTRODUCTION

Uveitis, or intraocular inflammatory disease, is one of the leading causes of visual loss and presents a considerable medical challenge due to an incomplete understanding of its pathogenesis. Uveitis is often classified in association with a number of systemic inflammatory diseases, many of which involve arthritis and as such, the involvement of common inflammatory pathways amongst the eyes and joints has been presumed. The innate immune receptor, NOD2, may provide important insight into the genetics involving uveitis and arthritis. Mutations in *NOD2* are responsible for development of an autoinflammatory disease called Blau syndrome (1), which was initially described as an inherited form of progressive and severe panuveitis that coincides with arthritis and dermatitis (2, 3). NOD2 is one of the better characterized members of the NOD-like or NOD-leucine-rich repeat (NLR) family of intracellular innate immune receptors in terms of its microbial recognition and downstream signaling responses. NOD2 is critical for host defense against bacterial infection and senses the bacterial cell wall constituent peptidoglycan (PGN), of which the minimal moiety responsible for NOD2 activation is muramyl dipeptide (MDP) (4, 5). Thus, in addition to its previously described linkage with susceptibility to Crohn's disease (6), the importance of NOD2 in immune homeostasis of the eyes and joints is underscored by the fact that a single base pair change in the *NOD2* gene invariably causes inflammatory disease in Blau syndrome. Despite the clinical observations and recent genetic work performed by international research laboratories on *NOD2* and Blau syndrome (7, 8), very little is understood of its biological function in regulation of inflammation within target organs such as the eye.

Dysregulation of IL-1 signaling is thought to be involved in the pathogenesis of a variety of autoinflammatory disorders, as best exemplified in the cryopyrinopathies (abbreviated CAPS), which collectively represent a spectrum of overlapping disease of three previously identified syndromes caused by mutations in the NLR family member *NLRP3* (also called *CIAS1* or *NALP3*) (9). Disease ensues from hyperactivation of the caspase-1-NLRP3-inflammasome complex and overproduction of IL-1 β . In turn, treatment with anakinra, a recombinant form of the endogenous inhibitor of IL-1 activity, IL-1 receptor (IL-1R) antagonist (IL-1Ra), alleviates symptoms and improve outcome in CAPS patients, thereby emphasizing the importance of IL-1 in this rare disease. Since the discovery of how IL-1 β production is regulated by the caspase-1-inflammasome complex, its potential as a pathogenic factor has been considered for a multitude of more common rheumatic diseases (9, 10). With encouraging reports of the efficacy of Anakinra (a soluble antagonist of IL-1R, IL-1Ra), its use is now under consideration as a therapy for ocular inflammatory disorders (11, 12).

Proper regulation of IL-1 signaling is an especially critical aspect of homeostatic protective mechanisms for the joints, but what about the eye? Deficiency in IL-1Ra results in

development of spontaneous and chronic inflammatory arthritis in mice on the genetically predisposed BALB/c strain (13, 14), and the arthritis phenotype can be reproduced by exposure to microbial triggers such as LPS early in life (15, 16). In contrast, very little is known how inflammatory effects of IL-1 are regulated within the eye. Given the link between NOD2 and immune homeostasis of the eyes and joint, and the importance of IL-1Ra in protective mechanisms of the joint (i.e. controlling the joint's susceptibility to inflammatory disease), it stands to reason that proper regulation of IL-1 signaling is important for resistance to NOD2-dependent uveitis. We hypothesized that IL-1Ra deficiency and the ensuing unchecked IL-1 signaling response would render mice more susceptible to NOD2 activation by its agonist MDP thereby leading to disease involving coincident arthritis and uveitis. In contrast to our proposed hypothesis, the data presented here do not support a predisposing role for IL-1Ra-deficiency in NOD2-triggered uveitis. However, IL-1Ra does play an important regulatory role within the joint when NOD2 is activated locally by MDP. Collectively, these observations demonstrate differential roles for IL-1Ra in NOD2-mediated joint versus ocular inflammation and could therefore impact how we target eyes versus joint for therapeutics.

METHODS

Reagents

Muramyl dipeptide (MDP) and mouse recombinant IL-1 β were purchased commercially (Bachem, Torrance, CA and R&D Systems, Minneapolis, MN, respectively). IL-1Ra, IL-1 α , and IL-1 β protein levels were measured in whole eye homogenates using commercially available ELISA kits (R&D Systems) as per manufacturer's instructions and previously reported (16).

Mice

Female IL-1Ra KO, which were gifted by Dr. Martin J. Nicklin (University of Sheffield, Sheffield, UK), or the NOD2 KO mice on the BALB/c background have been described (17, 18). Animal experiments were conducted according to guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International, OHSU, and the Portland VA Medical Center. Mice were used at 7–16 weeks of age for experiments. One hundred micrograms of MDP were injected via different routes: intraperitoneal (i.p.), intravitreal (i.v.t.) into the eye as described (19), and intra-articular (i.a.) into the knee joint as described (20). For examination of intra-ocular responsiveness to locally administered IL-1, 80 ng of IL-1 β was i.v.t. injected into mice. For i.v.t. or i.a. injections, mice were anesthetized (1.7% isoflurane in oxygen) and a 30 1/2 G needle under guidance of a stereomicroscope was used to inject 2 or 10 μ l volumes into the eye versus joint, respectively.

Intravital videomicroscopy

The *in vivo* cellular trafficking response within the iris vasculature and interstitial tissue was assessed using intravital videomicroscopy as previously described (19, 21). To visualize leukocytes, mice were i.p. injected with rhodamine 6G (35 mg/kg, Sigma-Aldrich) at the time of microscopy. Digital videos (10 sec each) were acquired in 3 independent regions of

the iris using a monochrome camera (Kappa, Gleichen, Germany). Measurements of vessel diameter and length and iris tissue area, and quantification of rolling, adherent, and infiltrating leukocytes were performed off-line by masked observers using ImageJ software.

Near-infrared (NIR) fluorescence imaging of joint inflammation

At the time of sacrifice, near- infrared (NIR) imaging was performed using previously described methodology (16, 20). Quantification of cathepsin activity within the joint was performed by i.v. infusion of NIR-fluorescent probe ProSense 680 (Perkin Elmer, Waltham, Massachusetts, USA) (2 nmol/150 μ l) 24 h prior to assessment. Images of ankles or joints were analyzed with Odyssey software (LI-COR Biosciences, Lincoln, Nebraska) and mean differences in fluorescence intensity were normalized to saline-injected BALB/c congenic controls.

Histopathology

Mice were euthanized and eyes, ankles and knees were dissected, fixed in 10% neutral-buffered formalin and prepared for paraffin embedding and sectioning as previously described (16). Hematoxylin & eosin stained joint tissues were evaluated for severity of arthritis and eye tissue sections were evaluated for uveitis severity by quantifying in masked fashion the number of infiltrated leukocytes in the aqueous humor of the anterior segment and vitreous body of the posterior segment.

Statistical analysis

Results are presented as the mean $\pm$ SEM. Statistical analysis of differences amongst experimental groups was by analysis of variance followed by Student's t test (Prism, GraphPad Software, La Jolla, CA). Statistical significance was considered when $p < 0.05$.

RESULTS

IL-1Ra plays a non-essential role in protecting the eye or joint from systemic MDP exposure

Based on work demonstrating that deficiency in IL-1Ra renders mice susceptible to systemic exposure to microbial triggers such as lipopolysaccharide (LPS) with resultant development of a progressive form of arthritis (15), we tested whether IL-1Ra KO mice develop disease upon direct activation of NOD2 by its agonist MDP. Using intravital videomicroscopy, which allows us to visualize in real-time the ongoing differential cellular responses within the iris vasculature and interstitial tissue *in vivo* (19), we monitored mice out to 16 wks of age (a time when peak arthritis has developed in naïve mice; Fig. 2). As expected at this point in time, wild-type (WT) control mice did not demonstrate any sustained cellular inflammatory responses nor did they develop arthritis (Fig. 2) following systemic MDP exposure. However, the cellular trafficking response was not significantly increased by IL-1Ra-deficiency as we hypothesized (Fig. 1A). The lack of any ocular inflammation in response to MDP in IL-1Ra KO mice was corroborated histologically, which revealed negligible cellular influx within the aqueous humor in the anterior segment of the eye (Fig. 1B, C) and normal anterior segment structure (Fig. 1C) in both MDP-challenged WT and IL-1Ra KO mice. Histological examination also confirmed a lack of any inflammation in the

posterior eye segments of WT versus IL-1Ra KO mice (data not shown). Thus, despite the on-going spontaneous arthritis in the IL-1Ra KO mice they appear to maintain resistance to development of uveitis even upon systemic exposure to MDP.

Since the absence of uveitis in the context of systemically administered MDP could possibly be confounded by the blood-ocular barrier and/or the discordance between ocular and systemic responses, we sought to ascertain whether deficiency in IL-1Ra predisposes to arthritis in mice systemically exposed to MDP. Arthritis was assessed using whole body near-infrared (NIR) fluorescence imaging, a method that allows for detection of *in vivo* protease activity that we have previously demonstrated to be an accurate molecular predictor of arthritis modeled in IL-1Ra KO mice and other arthritis models (16, 20, 22). As expected the IL-1Ra KO mice i.p. injected with saline spontaneously developed arthritis as indicated by the increased NIR signal within the ankles (Fig. 2A,B). However, a prior systemic exposure to MDP did not lead to any worsening of arthritis compared to saline in IL-1Ra KO mice (Fig. 2A–C). The increase in NIR signal intensity corresponds to increased histopathology in ankle joints (Fig. 2C) that show on-going synovial and periarticular infiltration and progressive cartilage damage, which were not further altered by systemic MDP exposure. Negative control WT mice did not develop disease in response to MDP. Thus, despite ongoing systemic disease in the IL-1Ra KO mice, they are not more sensitive to systemic activation of NOD2 by MDP.

IL-1Ra mitigates cellular rolling and adherence, but is dispensable in cell infiltration triggered by intraocular MDP administration

Given the unique microenvironment of the eye and the putative role for IL-1Ra in maintenance of immune privileged state of the eye, we went on to explore the role of IL-1 signaling responses in uveitis triggered locally by intraocular injection of MDP. Consistent with prior reports, intraocular injection of MDP elicited a significant intravascular cellular response within the iris (Fig. 3A), that we have previously demonstrated was NOD2 dependent (23). Deficiency in IL-1Ra significantly increased early cell trafficking responses such as rolling and adherence elicited by MDP; albeit no significant differences in cellular infiltration were observed between IL-1Ra KO mice and WT controls. IL-1Ra deficiency did not result in enhanced cellular influx into the aqueous humor compared to WT controls as assessed histologically (Fig. 3B).

Influence of NOD2 activation and expression on IL-1 production and signaling effects in the eye

Given the enhanced susceptibility of IL-1Ra KO mice to uveitis induced by intraocular injection of LPS (16), we were somewhat surprised by the negligible effect of IL-1Ra on MDP-triggered ocular inflammation. We therefore examined the extent to which locally administered MDP might result in production of the IL-1R agonists, IL-1 α and IL-1 β . We found that in response to MDP, the level of IL-1 β , but not IL-1 α , was significantly increased in eye homogenates in WT mice (Fig. 4A). Baseline levels of IL-1 cytokines were greater in IL-1Ra KO mice but their production was not further increased in response to MDP, indicating that lack of negative regulation of IL-1 signaling does not further perpetuate IL-1

responses as might be expected. Consistent with lack of functionality for IL-1Ra, MDP does not result in excessive production of IL-1Ra within the eye (Fig. 4B).

To test the converse question, we examined whether NOD2 expression influences IL-1Ra production and subsequent IL-1 signaling effects. Ocular levels of IL-1Ra were constitutively high in both naïve WT and NOD2 KO mice (Fig. 4C), although it was noted that the level of IL-1Ra tended to be less in the NOD2 KO mice. This prompted us to further elucidate the extent to which NOD2 expression influences the capacity of IL-1 to trigger ocular inflammation *in vivo*. NOD2 KO mice or WT controls were administered intraocular injections of IL-1 β at doses previously titrated to elicit measurable ocular inflammatory responses (16). In WT mice we observed a significant cellular response from intraocular IL-1 β injection (Fig. 4D) in that cellular rolling and adherence were increased within the first 2 h, which translated to a transient increase in cellular infiltration within the first 5 h; albeit this response was not sustained. Histological evaluation at 24 h following injection corroborated the intravital videomicroscopy observations at this time and pathological signs of uveitis were not observed (data not shown). We find that NOD2 KOs showed comparable cellular responses to IL-1 β as WT mice, indicating that NOD2 deficiency does not impact downstream IL-1 signaling responses.

IL-1Ra plays an important protective role in local inflammatory responses of the joint

In order to evaluate the role of IL-1Ra in regulation of local inflammation within the joint, we examined whether IL-1Ra-deficiency altered MDP-triggered arthritis. In WT mice intra-articular injection of MDP resulted in relatively mild inflammatory arthritis as measured by an increase in NIR-signal intensity (Fig. 5A), which corresponded to mildly inflamed synovium observed histologically (Fig. 5B). We found that deficiency in IL-1Ra markedly exacerbated MDP-triggered arthritis, which corresponds to a significant increase in NIR-signal intensity (Fig. 5A) and histopathological changes, wherein MDP-treated IL-1Ra KO mice exhibited severely inflamed tissue with exacerbated cellular influx of the synovium and worsened articular destruction. This finding underscored the importance of IL-1Ra as local protector of the joint but not the eye upon NOD2 activation by MDP.

DISCUSSION

Despite the resurgence of therapies targeting IL-1 β , very little is understood of the putative biological effects of IL-1 β in ocular inflammatory responses leading to uveitis. Given that the pathogenic mutations in NOD2 fall in an analogous functional domain to that of NLRP3, coupled with some overlapping phenotypic similarities in Blau syndrome and CAPS and other autoinflammatory diseases, it has been hypothesized that Blau syndrome is a disease for which anakinra would be beneficial (24). Yet, the results testing the biological effects of anakinra in patients with Blau syndrome were variable, particularly in relation to ocular disease. While patients treated with anakinra demonstrated improved clinical symptoms in their joints (25), this did not correspond to improved uveitis (25, 26), whilst treatment with canakinumab (anti-IL-1 β blocking antibody) did improve uveitis (27). This is further confounded by recent *in vitro* studies suggesting that IL-1 β is not excessively produced by stimulated peripheral blood mononuclear cells cultured from patients with Blau syndrome

(26); if anything, its production may be diminished, suggesting impaired negative regulation of the IL-1 signaling response in Blau patients (28). The opposing reports for anakinra treatment complicate this question and further illuminate the extent to which IL-1 signaling may be impacted by the target organ such as the eye in patients with Blau syndrome.

Our hypothesis was that mice would show increased susceptibility to inflammatory effects of NOD2 activation by MDP in the context of unimpeded IL-1 signaling (i.e. IL-1Ra KO mice). Contrary to our hypothesis however, we found that systemic exposure to MDP did not result in enhanced arthritis in IL-1Ra KO mice. Indeed, there was little effect (i.e. either exacerbation or reduction) on the onset and/or severity of arthritis or uveitis. This observation suggests that NOD2 activation is secondary to the role for TLR4 in induction of deleterious inflammatory responses relevant to arthritis modeled in IL-1Ra KO mice, thereby distinguishing a role for TLR4 vs NOD2 in microbial recognition and induction of disease. The extent to which innate immune receptors shape arthritis induction in IL-1Ra KO mice is not well understood, and it is possible that there are both positive and negative influences. TLR2 is considered a protective factor, as its deficiency predisposes to worsened spontaneous arthritis in IL-1Ra KO mice (15). We and others have demonstrated a cooperative effect between TLR2 and NOD2 with respect to promotion of PGN-triggered arthritis (20, 29). In our own studies we have examined whether IL-1Ra KO mice are susceptible to systemic exposure of up to 500 µg PGN, but have not observed any significant changes in arthritis onset or severity (i.e. protection or worsening) in IL-1Ra KO mice examined at 16 weeks of age (data not shown). This observation coupled with experiments here involving MDP supports the non-essential or redundant role for the NOD2 pathway in being negatively regulated by IL-1Ra in the systemic induction of arthritis or uveitis.

Whilst IL-1Ra KO mice maintained their resistance to systemic MDP exposure, the protective response generated by IL-1Ra was highly effective in suppression of NOD2-triggered acute inflammatory arthritis in the context of locally administered MDP. Deficiency in IL-1Ra resulted in enhanced protease activation (an *in vivo* molecular marker of joint inflammation) in response to intra-articular injection of MDP, which was coupled with cellular influx into the synovial membrane, periarticular inflammation, and exacerbated articular destruction. Thus, excessive IL-1 signaling due to IL-1Ra deficiency is deleterious during local NOD2 activation, resulting in worsened joint-specific inflammation. This observation may be a clue into the importance of NOD2 in locally-induced responses of the joints versus systemically-induced inflammatory responses.

Various experimental studies using BALB/c mice lacking IL-1Ra have examined the consequences of unfettered IL-1 signaling on arthritis as well as other manifestations such as psoriasis-like skin inflammation and vascular disease (30, 31), yet very little is known of how IL-1Ra regulates uveitis. Our findings indicate that unimpeded IL-1 signaling clearly affects NOD2 activation locally within the joint, yet deficiency in IL-1Ra does not appear to be a critical predisposing factor for MDP-triggered uveitis. We did observe that IL-1Ra controls early steps in cell trafficking responses within the ocular microvasculature such as cell rolling and adherence. However, subsequent cellular infiltration was unaffected, indicating the blood-ocular barrier was not altered despite the lack of IL-1Ra. Moreover, ocular IL-1Ra levels were not altered by intraocular MDP injection and IL-1Ra deficiency

did not affect production of IL-1R agonists IL-1 α and IL-1 β , as is often observed during dysregulation of IL-1 signaling and the ensuing feed-forward consequences. While excessive production of IL-1 β itself may not be an underlying mechanism in Blau syndrome (26, 28), it has been postulated that dysregulation of negative signaling of IL-1 may occur in Blau syndrome (28). Our findings indicate that despite the somewhat reduced baseline level of IL-1Ra in the eyes of NOD2 KO mice, this did not coincide with hypersensitivity in response to exogenously administered IL-1 β , as ocular inflammation in IL-1 β -injected WT and NOD2 KO mice was similar. Overall, the observations derived from the eye indicate that IL-1 β production and/or signaling effects are not likely direct mechanisms by which NOD2 triggers uveitis.

Our reductionist approach employed here with the use of IL-1Ra KO mice highlights how different molecular pathways operate within the joint versus eye that regulate local inflammation involving NOD2 activation. This is interesting conceptually, as one might assume that in a monogenetic disease such as Blau syndrome wherein mutated *NOD2* results in both uveitis and arthritis that similar pathogenic mechanisms function in each target organ. There certainly may be many commonalities, but our studies suggest that the ability of IL-1Ra to regulate NOD2-mediated inflammation is site-specific, i.e. IL-1Ra is an important aspect of restraint for joint inflammation, yet seems dispensable for NOD2-triggered ocular inflammation. This may suggest that in the context of uveitis, other aspects of the innate immune system apart from IL-1Ra are likely to be protective. Such differences may be related to the fact that the eye and joint differ with respect to their cellular composition and structural organization. Maintenance of immune homeostasis is especially important for an immune-privileged organ such as the eye, in which bystander tissue damage resulting from inflammation could pose a potentially serious threat to vision. Thus, a certain threshold of tolerance must be maintained, presumably with IL-1Ra in addition to other anti-inflammatory factors, that together operate in a highly complex and redundant system.

These results exemplify how pathways critical for proper control, through induction and/or repression of inflammation, may differ in the eye versus joints despite them both being targets in the very same disease. These data may help explain in part why some patients with Blau syndrome treated with anakinra benefit from improved arthritis but do not show any improvement in their ocular disease (25, 26). Our ability to successfully understand the means by which NOD2 influences local responses of the eyes and/or joints is critical towards facilitating therapies for patients with Blau syndrome. These concepts may be important for translational purposes beyond patients with Blau syndrome, and could quite possibly relate to polygenetic rheumatic diseases that frequently include uveitis as an extra-articular manifestation.

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Figure 1.

IL-1Ra deficiency does not predispose mice to uveitis after systemic administration of MDP. Ocular inflammation was assessed out to 16 wks of age in mice i.p. injected with MDP or saline at 7 wks of age (i.e. 9 weeks following MDP injection). (A) The differential leukocyte trafficking responses within the iris were evaluated using intravital videomicroscopy and the number of rolling, adherent, and infiltrating cells was quantified. Data shown are at 16 wks. (B, C) Quantification of and representative histological images of the anterior eye segment from MDP-treated WT or IL-1Ra KO mice at 16 wks of age. H&E stained sections, original magnification at 200X, scale bar = 200 μ m. C: cornea; I: iris; L: lens. N=10 mice/ experimental group for 2 experiments.

Figure 2.

Deficiency in IL-1Ra does not predispose mice to joint inflammation in response to systemic administration of MDP. Severity of ankle inflammation was assessed in WT and IL-1Ra KO mice at 16 wks of age (i.e. 9 wks after i.p. injection of MDP or control saline). (A) Representative NIR images of the ankle. (B) Quantification of NIR-fluorescence signal intensity in the ankle joints. (C) Ankle joint histopathology was evaluated and representative histological images of H&E-stained sections from MDP-treated WT and IL-1Ra mice (original magnification at 200X, scale bar = 500 μ m). $\psi p < 0.05$ compared to WT. There was no significant difference between saline and MDP treatments within the IL-1Ra KO mice. N=8 mice/experimental group for 2 experiments.

Figure 3.

IL-1Ra mitigates cellular rolling and adherence, but is dispensable in cell infiltration triggered by intraocular MDP administration. Onset and severity of ocular inflammation were monitored over a 24 h period after administration of an intravitreal injection of MDP or control saline, which was administered in the contralateral eye in 8 week-old mice. (A) The differential leukocyte trafficking responses within the iris was recorded by intravital videomicroscopy and the number of rolling, adherent, and infiltrating cells was quantified. (B) At 24 h following MDP injection uveitis was assessed histologically, wherein the number of infiltrating cells within the aqueous of the anterior segment was enumerated. * $p < 0.05$ comparison between MDP and saline within a genotype, $\psi p < 0.05$ comparison between MDP-treated WT and MDP-treated IL-1Ra KO mice. N=10–12 mice/experimental group for 2 experiments.

Figure 4.

Effects of NOD2 activation and expression on IL-1 production and signaling effects in the eye. (A) Levels of IL-1α and IL-1β or levels of IL-1Ra (B) in eye tissue homogenates of WT versus IL-1Ra KO mice were measured by ELISA at 5 h following intravitreally administered MDP or saline. (C) Levels of IL-1Ra in eye tissue homogenates of naïve WT and NOD2 KO mice were quantified by ELISA. (D) Onset and severity of ocular inflammation were monitored in WT vs. NOD2 KO mice over a 24 h period after intravitreal administration of IL-1β or control saline. The differential cellular responses were quantified by intravital videomicroscopy. * $p < 0.05$ comparison between saline and treatment (i.e. MDP or IL-1β) within a genotype. We did not observe a significant genotypic effect in any scenario. N=11–12 mice/experimental group for 2 experiments.

Figure 5.

IL-1Ra plays an important protective role in local inflammatory responses of the joint. WT and IL-1Ra KO mice (8 weeks of age) were administered an intra-articular injection of MDP or control saline in the contralateral knee and assessed 24 h later for arthritis. (A) Quantification of knee joint inflammation was assessed by NIR-imaging. (B) Representative histological images of knee joint tissue sections from WT and IL-1Ra KO mice injected with MDP, H&E stain (top panels: original magnification 200X; bottom panels: original magnification at 400X), scale bar = 200 μ m. * $p < 0.05$ comparison between MDP and saline within a genotype, $\psi p < 0.05$ comparison between MDP-treated WT and MDP-treated IL-1Ra KO mice. N=8 mice/experimental group for 2 experiments.