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Temporal expression and tissue distribution of interleukin-1β in two strains of guinea pigs with varying propensity for spontaneous knee osteoarthritis

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SUMMARY

Objective: To provide a comprehensive immunohistochemical (IHC) map of the temporal expression and tissue distribution of interleukin-1β (IL-1β) through progression of osteoarthritis (OA) in two strains of guinea pigs with varying propensity for spontaneous knee joint disease.
Methods: OA-prone Hartley and OA-resistant Strain 13 guinea pigs were collected at 60, 120, 180, 240, 360, and 480 days of age ($N = 4$ animals per strain per date). IHC was performed on whole joint preparations; the distribution of IL-1β expression on coronal sections was mapped, semi-quantitatively scored, and correlated to OA grade using Mankin criteria with guinea pig-specific modifications. OA and IHC indices were compared among times and between strains using the Kruskal–Wallis one-way analysis of variance by ranks followed by Dunn's post test.
Results: OA indices for both strains increased from 60 to 480 days of age; a statistically higher score ($P \leq 0.01$) was found in Hartley animals at 180, 240, 360, and 480 days. At 60 days of age, IL-1β expression was detected in cartilage, menisci, synovium, and subchondral bone in both strains. Persistent and statistically increased ($P < 0.05$) IL-1β expression was found in these same tissues in Hartley animals at 120 and 180 days, while Strain 13 animals demonstrated a significant reduction in positive immunostaining. Statistical differences in IHC indices between strains beyond 240 days of age were restricted to synovium (days 240 and 480) and subchondral bone (days 360 and 480).
Conclusions: As expected, histologic OA proceeded in an accelerated manner in Hartley animals relative to Strain 13 animals. The OA-prone strain did not demonstrate reduced IL-1β expression during adult maturity as occurred in the OA-resistant strain, and this persistent expression may have corresponded to early incidence of OA. Future interventional studies are warranted to explore whether dysregulation of IL-1β expression may contribute to premature onset of spontaneous disease in the Hartley guinea pig.

Introduction

Interleukin-1β (IL-1β), a key regulator of innate host immune responses, is repeatedly cited as one of the most prominent cytokines involved in osteoarthritis (OA)-related joint degeneration[1]. It has been well-established in cell culture that IL-1β inhibits the production of type II collagen and aggrecan and potently induces chondrocyte-mediated degradation of extracellular matrix components by stimulating production of matrix metalloproteinases[2,3]. In addition, by promoting prostaglandin- and nitrous oxide-mediated pathways in joint tissue, IL-1β is recognized as a global mediator of inflammation and pain in joints affected by OA[4].

The benefits of an IL-1β competitive antagonist, IL-1 receptor antagonist protein (IL-1Ra), in animal models of surgically-induced OA supports the role of IL-1β in OA pathogenesis[4–6]. A local increase in exogenous IL-1Ra in OA joints by administration of recombinant human (rh)IL-1Ra protein[7], intra-articular injection of rhIL-1Ra transduced cells[8], or adenoviral delivery of IL-1Ra using *in vivo* and *ex vivo* techniques[9,10] has been shown to reduce the progression of experimentally created lesions.

While these studies provide strong evidence that IL-1β is implicit for the onset and development of secondary OA, ambiguity to its ultimate role in primary disease was initially provided by the finding that IL-1β knock-out mice showed accelerated development of OA lesions in surgically and non-surgically altered knees[11].

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Further, intra-articular rhIL-1Ra injections to treat symptomatic knee OA in people found no statistical improvement over *placebo* at 1 month[12] and trials with diacerein, a compound that inhibits IL-1β production from synovium and cartilage[13], did not show clinical, radiographic, or structure-modifying effects as expected[14–16]. Thus, the molecular interactions to explain the relationship between IL-1β and maintenance of healthy articular cartilage have been proposed but are not yet definitively established[17], and characterization of the function of IL-1β in a spontaneous, *in vivo* model remains elusive[11,18].

Reports exist on the presence of the IL-1β transcript[19] and protein[20,21] in OA, but these are generally reported in control vs end-stage OA cartilage, synovium, or synovial fluid[22] and lack documentation of IL-1β expression relative to the development of OA lesions. The aim of this study was to provide a comprehensive analysis of the temporal expression and tissue distribution of IL-1β using immunohistochemistry (IHC) throughout initiation and progression of OA in a naturally-occurring animal model. By providing direct comparisons between two guinea pig strains with varying propensity for the disease, this work provides a foundation upon which mechanistic data regarding the contribution of IL-1β to spontaneous and premature OA can be hypothesized and substantiated.

Materials & methods

This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All protocols were approved by the Institutional Laboratory Animal Care and Use Committee at The Ohio State University. Male Hartley guinea pigs (24 animals total) were obtained from Charles River Laboratories (Wilmington, MA) and male Strain 13 (24 animals total) from the USA Army Medical Research Institute of Infectious Diseases (Fort Detrick, MD) for data collection at 60, 120, 180, 240, 360, and 480 days of age ($N = 4$ animals per time point per strain). The majority of animals was available directly from the suppliers at the required ages and euthanized by exposure to carbon dioxide within 24 h of arrival to the facilities. Hartley and Strain 13 animals intended for harvest at 180 and 240 days of age, however, were raised at approved university laboratory animal facilities until the appropriate time point was reached. Animals were housed individually in solid bottom cages and allowed *ad libitum* water and guinea-pig chow (Harlan Teklad 7006) containing Vitamin C (800 mg/kg) and Vitamin D3 (2.4 IU/g) until euthanasia was performed. Weight (grams) at time of harvest was recorded.

Joint tissue processing & analysis

Both knees from each animal were evaluated using the scoring systems described below. Whole knee joints were fixed in 10% neutral buffered formalin and prepared for histological analysis, as previously described[23–25], with the following modifications: after a monitored period of decalcification in 8% formic acid/hydrochloric acid, joints were cut in half on a coronal plane and further decalcified in 8% formic acid/acetic acid. Decalcification was standardized within harvest age to ensure that adequate processing was attained with minimal exposure to acidic conditions (Table IA). Paraffin sections (5 μm) were taken from the center of the medial tibial plateau in each joint and stained with toluidine blue or subjected to immunostaining, as described below.

Three independent, blinded observers (KSS, SEW, ALB) performed histological grading of serial coronal sections of each knee, using adapted Mankin criteria based upon characteristic features of OA in this species[24,25]. Histological evidence of chondropathy incorporated: (1) grading of articular cartilage structure from 0 to 8; and (2) grading of proteoglycan loss, as determined by loss of toluidine blue staining intensity, from 0 to 6 (Table IB). Chondropathy was scored for the medial and lateral tibial plateaus. The total score for each compartment ranged from 0 (normal) to 14 (severe structural damage and complete loss of toluidine blue staining), providing a total possible tibial index ranging from 0 to 28. Intra- and inter-observer variability was negligible (within one

Table IA
Decalcification protocol for Hartley and Strain 13 guinea pigs collected at specified ages

Harvest age	Number of days exposed to respective decalcification solutions	
	8% formic acid/hydrochloric acid	8% formic acid/acetic acid
60	1	2
120	2	3
180	2	3
240	3	5
360	5	5
480	5	5

Table IB
Semi-quantitative histologic grading scheme for knee joints of the guinea pig[24,25]

	Grade	Descriptions
Articular cartilage structure	0	Normal, smooth uninterrupted
	1	Mild surface irregularities, no clefts
	2	Irregular surface, 1–3 superficial clefts
	3	>Three clefts and/or loss of cartilage to superficial zone
	4	1–3 clefts extending into middle zone
	5	>Three clefts and/or loss of cartilage into middle zone
	6	1–3 clefts extending into deep zone
	7	>Three clefts extending into calcified cartilage zone
	8	Clefts extending to calcified cartilage zone
Toluidine blue staining	0	Uniform staining
	1	Loss of staining in superficial zone for <half the length of the plateau
	2	Loss of staining in superficial zone for >half the length of the plateau
	3	Loss of staining in superficial + middle zone for <half the length of the plateau
	4	Loss of staining in superficial + middle zone for >half the length of the plateau
	5	Loss of staining in all three zones <half the length of the plateau
	6	Loss of staining in all three zones >half the length of the plateau

Table IC
Semi-quantitative immunostaining grading system for knee joints of the guinea pig[27], with modifications

	Score	Qualifier	Description
Percentage of positive cells	0	(−)	No visible immunostaining
	1	Rare	<5% of cells and/or matrix positive
	2	Occasional	6–24% of cells and/or matrix positive
	3	Several	25–49% of cells and/or matrix positive
	4	Frequent	50–75% of cells and/or matrix positive
	5	Extensive	>75% of cells and/or matrix positive
Intensity of immunostaining	0	(−)	No visible immunostaining
	1	+	Minimal visible immunostaining
	2	++	Moderate visible immunostaining
	3	+++	Marked visible immunostaining